EVOLUTION OF THE AROMATIC POTENTIAL DURING RIPENING OF SYRAH GRAPES EXPOSED TO DIFFERENT IRRIGATION STRATEGIES

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ABSTRACT

The aromatic compounds coming from grapes play a decisive role on the quality and regional character of wines. The varietal aroma consists of free aroma compounds and bound aromas or precursors. The different types and quantities of aromatic precursors in grapes are the main source of aromas which differentiate the diverse varieties. It is known that these precursors are synthesized during grape ripening. However, the effect of the irrigation techniques during this process and the optimal moment for harvest are not accurately known.

In this work, the changes in the concentration of glycosidic precursors of Syrah grapes exposed to different irrigation strategies were monitored during ripening in two consecutive years. It has been found that ripening has a significant effect ($p<0.05$) on the concentration of 17-23 compounds out of 64 analyzed, while the irrigation affects the concentration of 4-26 compounds, depending on the year.

RESUMEN

Los compuestos aromáticos procedentes de las uvas juegan un papel decisivo en la calidad y el carácter regional de los vinos. El aroma varietal esta formado por aromas libres y por precursores. Los diferentes tipos y cantidades de precursores aromáticos en la uva son la mayor fuente de los aromas que distinguen las distintas variedades. Estos precursores se van sintetizando durante la maduración. El efecto del riego durante este proceso y cual es el momento óptimo de la vendimia para conseguir una mayor concentración de estos compuestos no se conoce con exactitud.

En este trabajo se han estudiado los cambios producidos en la concentración de precursores glicosídicos en uvas de la variedad Shiraz sometidas a distintos aportes hídricos durante su maduración en dos añadas consecutivas. La maduración afecta significativamente ($p<0.05$) a la concentración de 17-33 compuestos de 64 analizados, mientras el riego lo hace a la concentración de 4-26 compuestos, en función de la añada.

1. INTRODUCTION

The characteristic aroma of a wine is the result of the simultaneous action of a certain number of compounds present in a combination of concentrations relatively specific of the grape variety from which the wine has made.

It can be differentiate between aromatic varieties, such as Muscat, Riesling or Gewürztraminer, whose musts present a characteristic aroma similar to that found in wines;

and non-aromatic or non-floral varieties, whose musts do not present a characteristic aroma. In these last varieties, the varietal aroma is present as odorless precursors. Therefore, varietal aroma consists of (*1*): odorous molecules or free aromas and precursors, which themselves can be divided into non-volatile precursors (odorless) and volatile precursors. The different types and quantities of aromatic precursors in grapes are the main source of aromas which differentiate the diverse varieties.

Glycosidic precursors have been extensively studied after their discovery in the sixties. These compounds are mainly monosacharides or disaccharides, and in all cases the aromatic molecule is linked to a glucose molecule through a β-O-glycoside bound. Among the aglycones, important wine aroma compounds have been identified, including terpenes, norisoprenoids, C6 alcohols, benzenes, volatile phenols, vanillin derivatives, and lactones (*2-4*). These aromas can be released by acid or enzymatic hydrolysis of the β-O-glycoside bound.

It is known that glycosidic precursors accumulate during grape ripening. Nevertheless, the optimal moment for harvest and the effect of the irrigation to achieve a higher concentration of these compounds are not accurately known. The information available in literature about the evolution of the aroma compounds and their precursors during the developing of grapes only concerns the changes in norisoprenoids, terpenes, and methoxypyrazines. A little information is found about the evolution of the precursors of vanillin compounds, volatile phenols, or lactones.

The aims of this work are to study the changes in the glycosidic precursor composition of some of the most important wine aromas during the ripening of Syrah grapes, as well as, to study the effect of the irrigation on the synthesis of these compounds. With this work, it is expected to determine with more accuracy the moment of the highest aromatic quality of grapes to carry out harvest.

2. METHODS

Preparation and analysis of the precursor extract

Glycosidic precursors were extracted from Syrah grapes collected at three different ripening times and one at over-ripening during 2008 vintage, and at two ripening times and one at over-ripening during 2009 vintage. Sampling has carried out in a vineyard split in two parts; each one of them underwent a different irrigation strategy ("up" and "down"). "Down" samples had a higher water supply (50-60 mm), since they were submitted to an additional irrigation process at mid-veraison.

Three replicates of 200 g were collected in each part at every time. Grapes were stored frozen in the winery until their analysis. 100 g of grapes from each sample were destemmed and homogenised, and then juice and skins were separated by centrifugation and filtration. The precursors coming from must were extracted using LiChrolut EN resins. After percolating the sample, resins were first washed with water to remove highly polar compounds and then with dichloromethane to remove free aroma compounds.

After drying the cartridge, the precursors were eluted with ethyl acetate. The ethyl acetate extract was evaporated under vacuum to dryness, then reconstituted in the hydrolysis tampon following the procedure reported by Loscos et al. (*5*). The released aroma compounds were extracted by SPE using LiChrolut EN and determined by GC-MS (*5*). Data were treated using

analysis of the variance (ANOVA) to determine the existence of significant differences between samples. Factors were ripening and irrigation procedure.

3. RESULTS AND DISCUSSION

In both years, the amount of rainwater collected during ripening was very similar, less than 25 mm (data from the National Institute of Meteorology). So, the hydric conditions were similar in both years. On the other hand, the average temperatures were significantly different. In 2008, the average temperature was 26.36ºC, while in 2009 the average has higher, 29.6ºC. This fact involved a higher over-ripening in 2009, even with a loss in the berry weight.

In 2008, the ºBrix values (average of 3 replicates) were the following: "up" (23.2, 23.8, and 25) and "down" (21.6, 21.6, and 25.2). In 2009, the values were: "up" (24.5, 26.4, and 28.1) and "down" (23, 26.2, and 28.3). In 2008, 11 days passed between the first and the last sampling, while in 2009, the difference was only 6 days. The fast accumulation of sugar in 2009 forced to harvest 20 days before than in 2008.

Regarding the glycosidic precursors, as can be seen in Table 1, the behavior is quite different depending on the year. In 2008, only 17 compounds presented significant differences for the ripening factor, while in 2009 differences were found in the double of compounds, 34. Moreover, only 10 compounds had significant differences in both years. Differences are even higher if the irrigation factor is considered.

Table 1. Significant differences ($p<0.05$) of the aroma compounds released from precursors coming from Syrah grapes harvest in 2008 and 2009, depending on the factors: ripening, irrigation, and their interaction.

	2008			2009		
	Ripening	**Irrigation**	**Interaction**	**Ripening**	**Irrigation**	**Interaction**
Terpenes						
α-terpinolene				0,0007	0,0017	
(Z)-linalool oxide				<0.0001	<0.0001	
(E)-linalool oxide				0,0002	0,0002	
Linalool	0.0307		0.0006			
α-Terpineol				0,0006	0,0496	0,0451
β-citronellol	0.0501					
Geraniol	0.0006					
Linalool acetate*	0.0001		0.0061	0,0001	0,0018	
Terpinen-4-ol*				0,0004	0,0248	
δ-terpineol*				0,0002	0,0059	
Neric acid*				0,0004	0,0001	
Nerol oxide*	0.0358					
Norisoprenoids						
β-damascenone					0,0004	<0.0001
β-ionone	<0.0001			0,0005	0,0402	
Vitispirane A*	0.0364	0.0304		0,0468	0,0121	
Vitispirane B*		0.0462		0,0467	0,0151	
Riesling acetal*						
TDN*					0,0199	
TPB*				0,0005		<0.0001
3-Oxo-β-ionone*	0.050			<0.0001	0,0001	<0.0001
Actinidols*				<0.0001	0,0001	<0.0001

Norisoprenoid 1*	0.0023			0,0008		<0.0001
Benzenes						
Benzaldehyde			0.0388	0,0124		
Phenylacetaldehyde	<0.0001			0,0319		
Benzyl alcohol				<0.0001	<0.0001	0,0099
β-phenylethanol				0,0005	<0.0001	0,0079
2-phenoxyethanol	0.055			0,0001		
Benzoic acid						
Phenylacetic acid				0,0277	0,0031	
Volatile phenols						
Guaiacol				<0.0001	<0.0001	
4-ethylguaiacol		0,01	0,01			
eugenol				0,0014		
4-vinylguaiacol						0,0001
2,6-dimethoxyphenol						0,0134
(E)-isoeugenol				0,0071	0,0013	0,0003
4-vinylphenol				0,0373	0,0085	0,0019
4-allyl-2,6-dimethoxyphenol				0,0002	0,0229	
Dihydromethyl-eugenol*						
Vanillin compounds						
Vanillin						
Methyl vanillate		0.0149		0,0001		
Ethyl vanillate				0,0003	0,0109	
Acetovanillone						0,0002
Zingerone						0,0001
Syringaldehyde						0,0413
Acetosyringone				0,0684	0,0471	0,0001
Homovanillyl alcohol*			0,02			
Miscellaneus						
(Z)-3-Hexen-1-ol	<0.0001		0.0106	0,0089		
(E)-2-Hexen-1-ol				0,0009		0,0135
Ethyl decanoate	0.0342		0.0396			
3-methylbutyric acid	0.0185					0,0103
2-methylbutyric acid	0.0357			0,0134		<0.0001
2ethylhexanoic acid	<0.0001	0.0217		0,0386		
Pantolactone	0.0049				0,0336	0,0005

*Data are the relative areas.

In 2008, only 5 compounds presented p values < 0.05, while in 2009 the number of compounds was 25, of which only Vitispiranes A and B presented significant differences in both years.

The evolutions of the impact compounds or the sum of the main groups of compounds in table 1 depending on the irrigation during ripening are plotted in figures 1, 2, 3, 4, and 5 for both years.

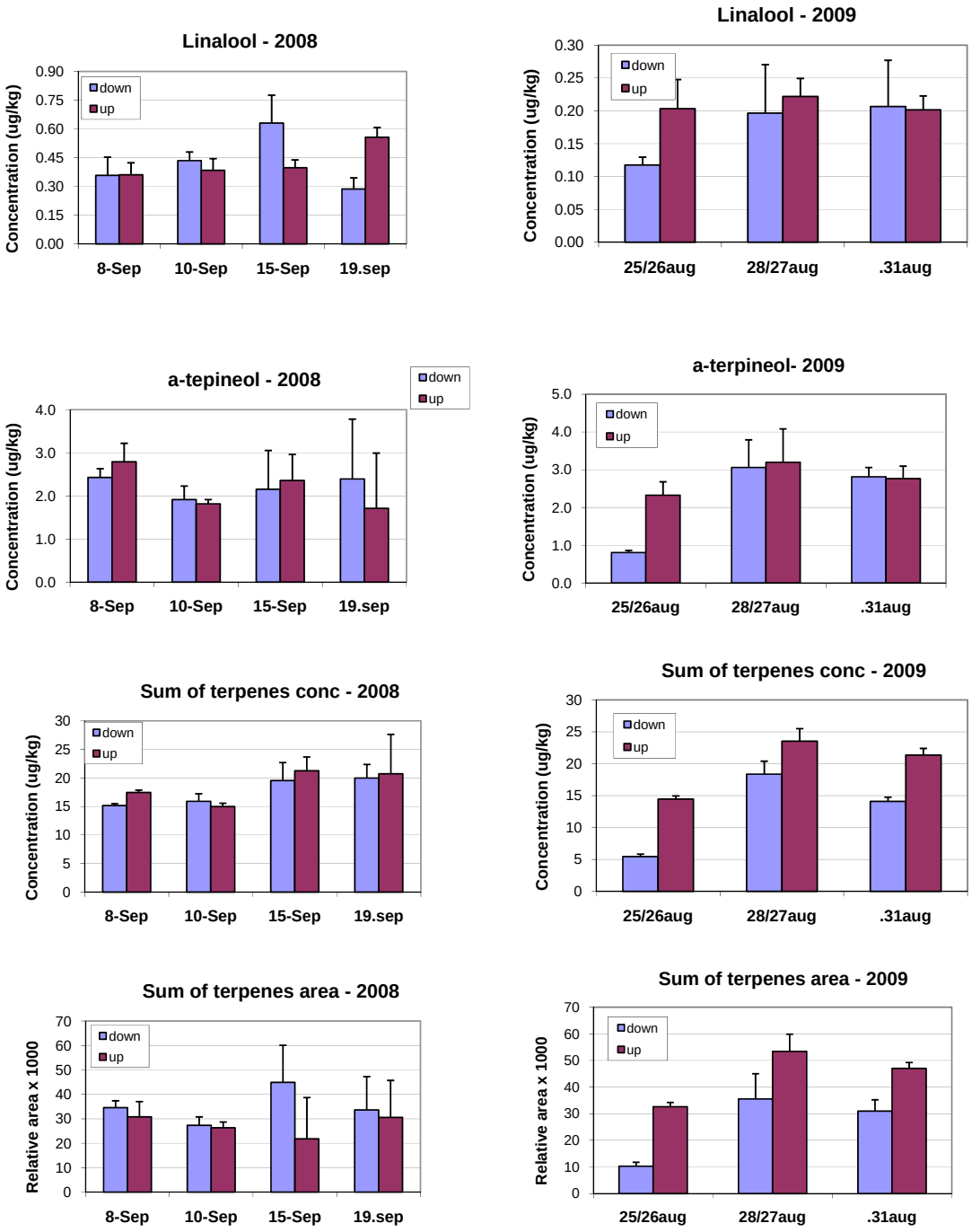

Figure 1. Evolution of the released terpenes during ripening of the Syrah grapes harvest in 2008 and 2009 with two different irrigation strategies ("down" more irrigation than "up")

As the figure 1 shows, the behavior is quite different depending on the year and even the evolution of the amount of terpenes is different depending on the irrigation strategy in the same year. In 2008, linalool increased significantly during ripening and decreased during over-ripening for the most irrigated grapes, while it remained more or less constant during ripening and increased during over-ripening for the less irrigated grapes. On the other hand, the variations between both types of irrigation were lower in 2009, increasing the concentration of this compound during ripening in both cases. The sum of terpenes in

concentration (α-terpinolene, linalool oxides, linalool, α-terpineol, β-citronellol, and geraniol) increased during ripening in both years and the higher values were found with less irrigation. In 2009, the sum of terpenes in area (linalool acetate, terpinen-4-ol, δ-terpineol, neric acid, and nerol oxide) followed the same evolution, but in 2008 a higher concentration of terpenes was found with more irrigation.

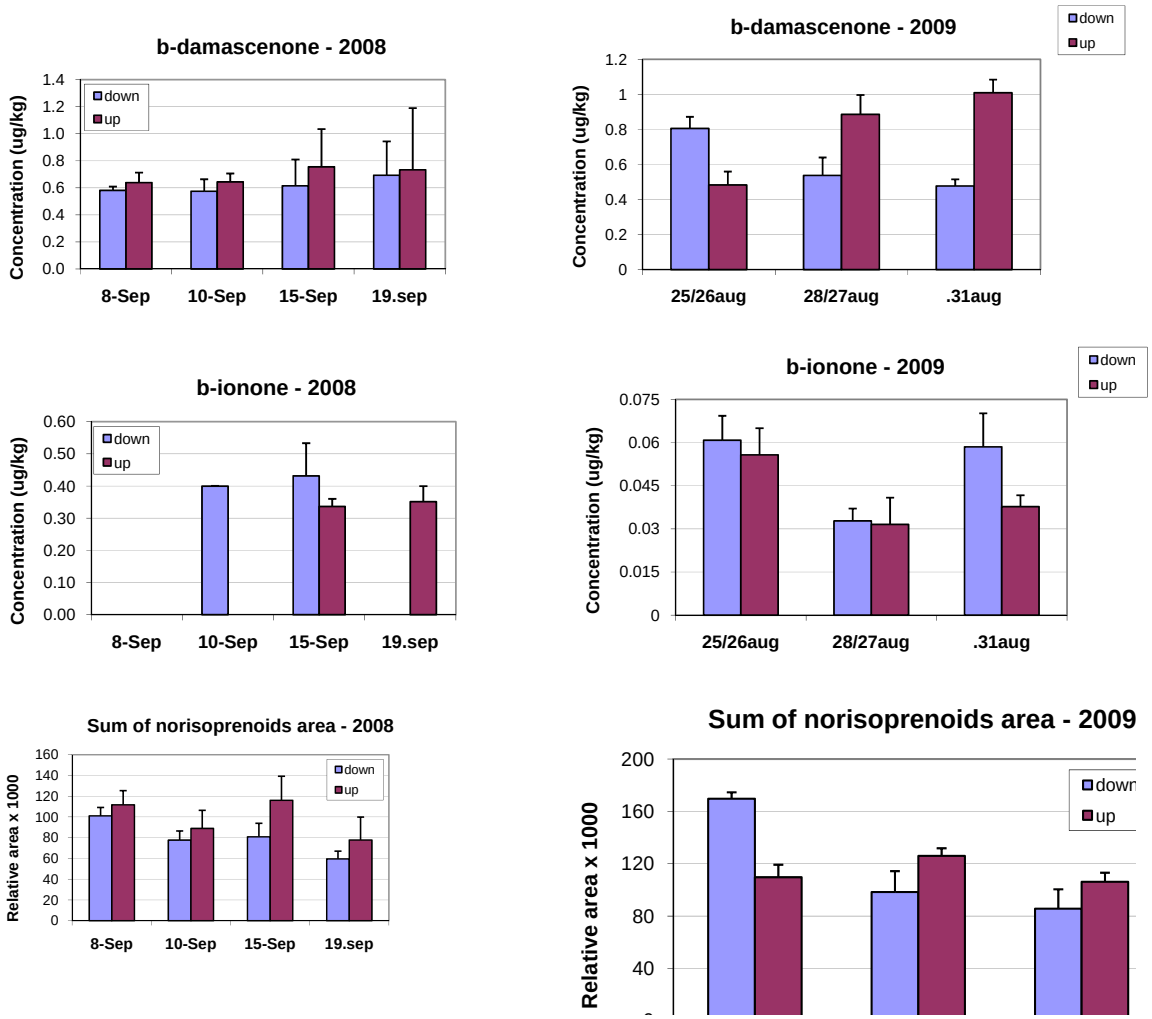

Figure 2. Evolution of the released norisoprenoids during ripening of the Syrah grapes harvest in 2008 and 2009 with two different irrigation strategies ("down" more irrigation than "up")

The norisoprenoids evolution can be seen in figure 2. The concentration of β-damascenone is higher with less irrigation in both years, but the evolution is different depending on the irrigation strategy, especially in 2009, when the concentration decreased during ripening with more irrigation and increased with less irrigation. Regarding β-ionone, the results are more inconsistent from year to year. As can be seen in figure 2, the sum of norisoprenoids in area (all norisoprenoids except β-ionone and β-damascenone) decreases at first, then increases, and after that decreases again. The lot with less irrigation accumulated higher concentration.

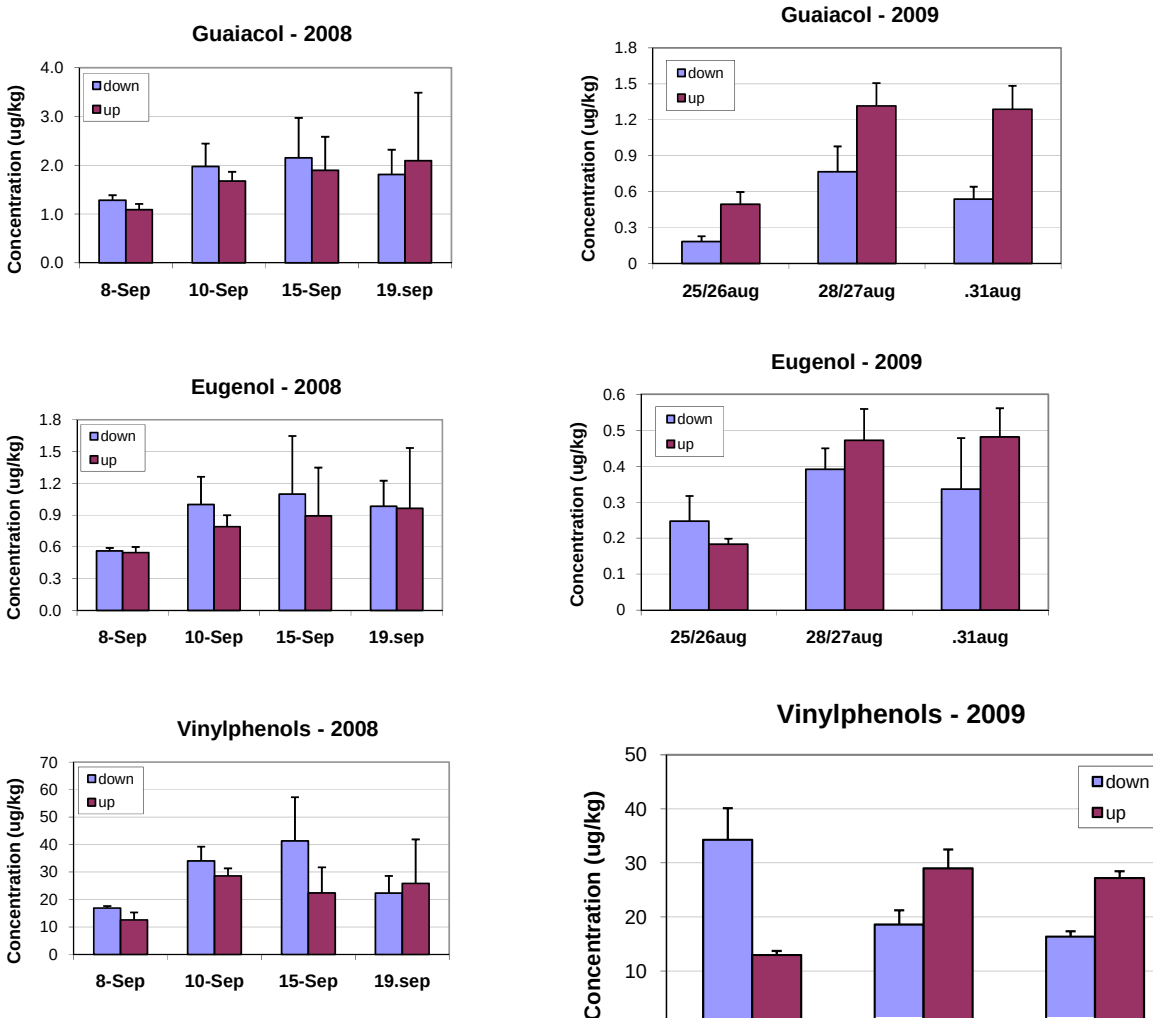

Figure 3. Evolution of the released volatile phenols during ripening of the Syrah grapes harvest in 2008 and 2009 with two different irrigation strategies ("down" more irrigation than "up")

As for the volatile phenols, as shown in figure 3, they tend to increase during ripening, being more abundant with more irrigation in 2008 and the opposite in 2009. In general, they tend to decrease during over-ripening.

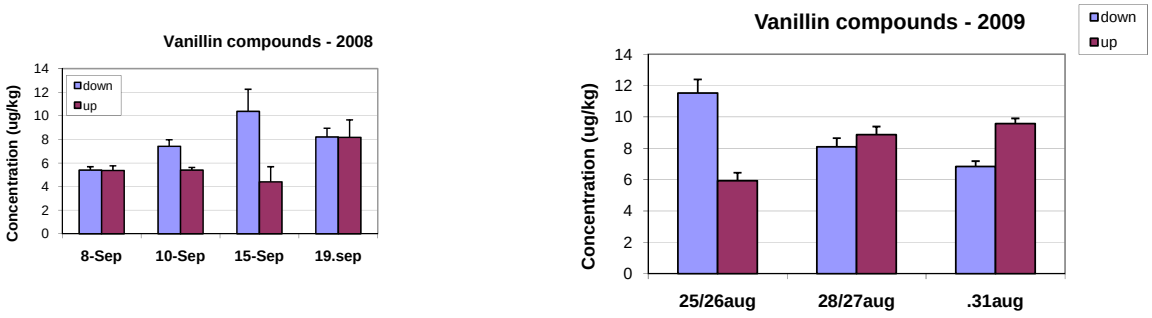

Figure 4. Evolution of the released vanillin compounds during ripening of the Syrah grapes harvest in 2008 and 2009 with two different irrigation strategies ("down" more irrigation than "up")

Figure 4 shows the different evolution of the sum of vanillin compounds depending on the irrigation and the year.

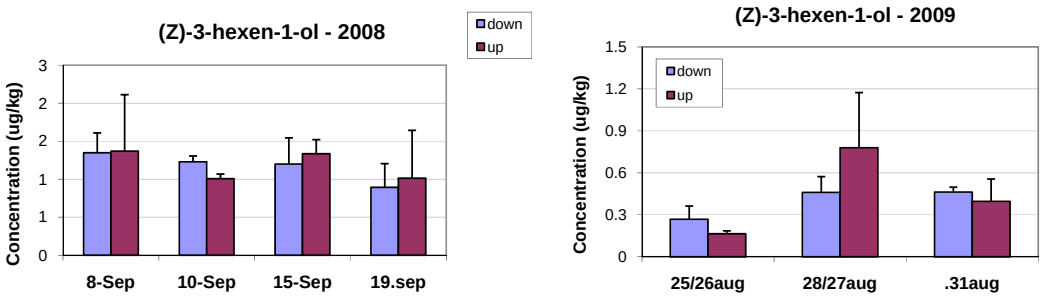

Figure 5. Evolution of the released hexenols during ripening of the Syrah grapes harvest in 2008 and 2009 with two different irrigation strategies ("down" more irrigation than "up")

Z-3-hexen-ol (figure 5) decreases during ripening in 2008 and increases in 2008, but decreases during over-ripening in both years. With respect to the irrigation, nothing can be affirmed. The concentration reached for this compound in 2008 was two times higher than in 2009.

4. CONCLUSIONS

The results of this study suggest continuing monitoring the concentration of the glycosidic precursors during the next seasons to be able to determine the influence of the different factors in the synthesis of these compounds, since it has been found that the evolution during ripening varies very differently depending on the year and the amount of irrigation water.

5. REFERENCES

1. Ribéreau-Gayon, P., Dubourdieu, D., Donèche, B., Lonvaud, A., Varietal aroma. In *Handbook of enology. Vol 2: The Chemistry of Wine. Stabilization and Treatments*, Ribéreau-Gayon, P., Ed. Jonh Wiley & Sons: Chichester, England, **2000;** Vol. 2, pp 205-230.
2. Williams, P. J.; Strauss, C. R.; Wilson, B.; Massywestropp, R. A., Use of C_{18} reversed-phase liquid-chromatography for the isolation of monoterpene glycosides and nor-isoprenoid precursors from grape juice and wines. *Journal Of Chromatography* **1982,** 235, (2), 471-480.
3. Wirth, J.; Guo, W. F.; Baumes, R.; Gunata, Z., Volatile compounds released by enzymatic hydrolysis of glycoconjugates of leaves and grape berries from *Vitis vinifera* Muscat of Alexandria and Shiraz cultivars. *Journal Of Agricultural And Food Chemistry* **2001,** 49, (6), 2917-2923.
4. Schneider, R.; Razungles, A.; Augier, C.; Baumes, R., Monoterpenic and norisoprenoidic glycoconjugates of *Vitis vinifera* L. cv. Melon B. as precursors of odorants in Muscadet wines. *Journal Of Chromatography A* **2001,** 936, (1-2), 145-157.
5. Loscos, N.; Hernandez-Orte, P.; Cacho, J.; Ferreira, V., Release and formation of varietal aroma compounds during alcoholic fermentation from nonfloral grape odorless flavor precursors fractions. *J. Agric. Food Chem.* **2007,** 55, (16), 6674-6684.

TRADTIONAL GRAPE RPODUCTS OF THRACIAN REGION AND LOCAL PRODUCTION FORM IN TURKEY

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ABSTRACT

Grapes harvested from Thracian Vineyards are utilized by marketing to vine factories, table grape varieties by transporting to İstanbul market and rest in family as fresh berry or processed products such as pekmez (grape molasses), bulama (solid molasses), hardaliye (grape juice flavored and protected with mustard) and salamura yaprak (brined leaf). Despite lowest protein level, the main traditional food Pekmez, is a good diet food with its high carbonhydrate and mineral content. "Bulama" is a solid molasses produced by bleaching the settling juice and foam added likid molasses with airing. "Hardaliye" is grape juice flavored with mustard is an alcohol free beverage produced by lactic acid fermentation of grinded mustard seed added grape must. Brined leaf is the main ingredient of Turkish traditional meal ''Sarma''. Yapıncak grape variety leaves are most used as brined leaf in Thracian Region.

Molasses, Bulama, Hardaliye and Brined leaf of Thracian Region and production technics is surveyed in this study.

INTRODUCTION

Viticulture is one of the most important activity among other agricultural activities. This agricultural activity affects directly social and commercial life of the area. So it is a culture. Grapes can usually be classified as table grape, wine/must grape and dried grape. Becides these common classification styles, local traditional products have also been developed. These products, known in different names and they were earlier as viticulture and have been consumed by people for centuries. Particularly in late years according to the demand for natural products increase of interest on traditional products has been seen (Gülcü et al. 2009).

Thrace, gateway of Turkey to Europe, also has an important position with its agricultural potential. Vineyards of the local cities (Tekirdağ, Edirne and Kırklareli) constitutes % 0,87 of the total cultivated agricultural lands. According to the data of the Local Agricultural Departments, vineyard are of the region in 2006 was 9321 ha and grape production in the same year was 80.000 - 100.000 tons.(Semerci 2006, Kiracı et al 2007)

Wine grape production is common in the Tekirdağ and these grapes are marketed to wine factories. On the other hand grapes produced in small family enterprises (<2000 m2 vineyard) are used to supply the consumption of the family as form of fresh grape, pekmez and bulama. Grape production of Kırklareli and Edirne consists of table grape growth and produced grapes are marketed in the local markets or used for the production fresh grape, molasses and hardaliye (Durgut and Arın 2005, Kiracı et al 2007).

In this study the production methods of the traditional grape products of Thrace Region including pekmez (grape molasses), bulama (solid molasses), hardaliye (grape juice flavored with mustard) and salamura yaprak (brined leaf) were examined.

TRADTIONAL GRAPE RPODUCTS AND OF THRACIAN REGION

Pekmez (Grape molasses)

Although containing lower level of protein pekmez is a good diet food that can be consumed by person at any age with its higher carbonhydrate and mineral content. The sugar in the pekmez are in the form of glucose and fruktose and they don't need to be decomposed in the digestive system. These sugars can diffused into blood easly without consuming energy and thus produces energy for human body rapidly (Taneli 1990). All fruit containing lower level of sugar can be used to produce pekmez but today the production of pekmez from grape is common (Batu 2006).

Pekmez in Thrace region is produced generally from grape but production from sugar cane is also seen in Kırklareli. Home made molasses produced by using local production methods is consumed by family members or marketed in the local markets.

Classical method is also used in the production of pekmez. First of all grapes were crashed by human power to obtain must. This must is boiled in the oven for a while and then by adding pekmez earth "kestirme" process (acid reduction) is applied. This earthl is collected from different locations of the region, crumbled, sieved and used by appliying to grapes before squeezing or after boiling the must in the oven. Pekmez earth contains 50-90% $CaCO_3$ and the amount added to must varies due to acidity level of must. 1-5 kg pekmez earth is generally used for 100 liter must. After kestirme process must is kept for one night to cool and deposition of residues. The liquid must is absorbed with tube after the duration period. This liquid must is boiled in open boilers in the higher temperatured oven for a while and thickened. During boiling this must is mixed and foams were taken out by spoon. While boiling continues must and foams starts to redden. Colour becomes darker and bubbling decreases. When a small amount of pekmez is taken by spoon and dropped on to plate and stayed like bead or when last drops dropped by the two sided of the spoon on to plate it can be understood that pekmez is ready and no need to be boiled. Due to boiling in the oper boilers these molasses are darker.

Bulama (Solid molasses)

Bulama (solid molasses) varies from region to region in the point of production method and variety of production materials. Bulama is a solid molasses colouring light yellow to light brown. This product, produced in Thrace for years, is nowadays produced by grape growers at home conditions to sell on the local market. Tekirdağ is the main city on region that producing bulama mostly. Bulama is commonly produced from grape and grape molasses but sugar cane bulama is also available on the market. Çöğen (*Gypsophila L.*) roots were boiled and this çöğen juice is added to bulama to open the colour and hardening of molasses.

Bulama is produced in Tekirdağ in this way; firstly pekmez earth was added to must and boiled in 70-80 ºC for for acid reduction. Çöğen roots and 20 lt water is boiled for 10-15 min in another boiler. This water is throw away because it is bitter. 30-40 lt water is added to çöğen and boiled till half of the water evaporate. This process repeated 3 times. Then 30 lt must, 15 lt çöven, 2 kg sucrose are boiled till it becomes like pekmez. Çöğen foams was prepared in another pot and added to this pekmez. Pekmez is mixed with wood spoon and

the addition of foam is continued. At this point the temperature of the oven must be decreased. After 30 min mixture the colour of pekmez becomes lighter and after becoming thicker the the addition of foam stops and boiler is removed from cooker (Gülcü and Demirci 2009)

Hardaliye (Grape juice flavored and protected with mustard)

Hardaliye is a traditional product of Thrace produced from dark coloured aromatic grapes through lactic acid fermentation (Çoşkun 2001). Crushed mustard seed used fort he production of hardaliye contains isothiochytanate volatile oil and this chemical prevents the formation of product to wine by prohibiting fermentation with its antimicrobial content. Hardaliye is known as vitamine source, blood pressure and immune system regulator, papazkarası grape variety is generally used in the production of the hardaliye (Anonim 2009). Hardaliye has been produced in Trace cities such as Kırklareli, Edirne, Tekirdağ and Gelibolu since 1839.

Grapes are washed, cruched and placing into barrel, having a tap. 10 cm from bottom. One time crushed grape (1 kg), one time crushed black mustard seeds + potassium benzoat (2+1 g) mixture and cherry leaf was filled. Mustard seed and potassium benzoat benzoat is used to prevent alcohol fermentation through prohibiting yeast activity. Also isothiochytanates oil prevents formation of some microorganisms and helps for the storage of product. Aroma is given with addition on black mustard seed and cherry leaf. The barrels are closed and incubated at room temperature for 15-20 days. After incubation, the mixture is filtered and stored at cold place (Coşkun at al 2009, Anonymous 2010).

Yaprak salamura (Brined leaf)

The storage and protection of leaves by making salamura after collection of leaves on spring is a common method used for years. This method is consists of the storage after fermenting. Carbonhydrates, proteins and other organic matter is processed to biochemical differentiation by microorganisms and lactic acid bacterias.

Yapıncak grape variety is preferred production of salamura yaprak in Thrace, because it have thin, hairless and entire leaves. Leaves are collected washed and seperated. in spring Then filled to plastic or glass pots. Brine (salamura) is added to this pots and kept 4-5 weeks for fermentation. İf salamura level decreases salamura is added to pots.

CONCLUSIONS

Grapes and grape products are very important among the Thrace region eating and drinking culture and traditional products because of the region which is centrally located in vineyards. Although traditional foods have been forgotten now, in recent years, to revive and to keep alive these products is very important for the regions's socio-economic development and local culture presentation if it is considered that consumer demand for natural and functional traditional foods.

BIBLIOGRAPHY

Anonymous, 2009. "Hardaliye'yi Millileştirelim". Kırklareli Hareketi Derneği Haber Bülteni, Şubat 2009, sayı: 4 p. 3 (In Turkish)

Anonymous, 2010. Kırklareli Üzüm Yetiştiricileri Birliği, Kırklareli, Turkey (In Turkish)

Batu A., 2006. Klasik Ve Modern Yönteme Göre Sıvı Ve Beyaz Katı Üzüm Pekmezi (Zile Pekmezi) Üretimi. Gıda Teknolojileri Elektronik Dergisi 2006 (2) p. 9-26. www.teknolojikarastirmalar.org (In Turkish)

Coşkun F. 2001. Hardaliye Üretim Teknolojisi Üzerinde Bir Araştırma. Doktora Tezi, Trakya Üniversitesi Fen Bilimleri Enstitüsü, Tekirdağ. (In Turkish)

Coşkun F., Arıcı, M., Gülcü, M., Çelikyurt, G. 2009. Farklı Yöntemler Kullanılarak Üretilen Hardaliyelerin Bazı Fizikokimyasal, Mikrobiyolojik ve Duyusal Özellikleri. II. Geleneksel Gıdalar Sempozyumu Bildiriler Kitabı, Van, Turkey, p. 665-669 (In Turkish)

Durgut M.R. and Arın S., 2005. Trakya Yöresi Bağcılığının Mekanizasyon Düzeyi ve Sorunları. Tekirdağ Ziraat Fakültesi Dergisi 2005 (2) 3, p. 287-297 (In Turkish)

Gülcü M., Taşeri L., Boz Y., 2009. Trakya Bağcılığı ve Alternatif Üzüm Değerlendirme Şekilleri. TASİAD (Tekirdağ Aktif Sanayici İşadamları Derneği) Dergisi yıl: 3 Sayı: 3, p. 24-26 (In Turkish)

Gülcü M. and Demirci A.Ş., 2009. Tekirdağ Yöresinde Üretilen Katı Pekmez "Bulama" Üzerine Bir Araştırma. II. Geleneksel Gıdalar Sempozyumu Bildiriler Kitabı, Van, Turkey, p. 86-91 (In Turkish)

Kiracı M.A., Özer C., Yayla F., Uysal T., Özdemir G., 2007. Trakya Bölgesinde Bağcılığın Mevcut Durumu ve Üreticilerin Örgütlenme Olanaklarının Belirlenmesi. Sonuç Raporu, Tekirdağ Bağcılık Araştırma Enstitüsü, 58 p. (In Turkish)

Semerci, A. 2006. Trakyada Tarımsal Yapı Verimlilik ve Gelişmişlik Düzeyi. Tarım ve Mühendislik, p: 76-77. (In Turkish)

Taneli B., 1990. Bebek Beslenmesinde Incir ve Üzümün Önemi. "Sağlıklı Beslenmede Kuru Incir ve Çekirdeksiz Kuru Üzümün Önemi" Semineri. İzmir Ticaret Odası. Tarişbank Genel Müdürlüğü Yayın No 1990/2 İzmir, Turkey p. 23-32 (In Turkish)

INFLUENCIA DE PORTAINJETOS EN EL INDICE RELATIVO DE CLOROFILA Y EN LOS TENORES DE NITRATO Y POTASIO DE LA SAVIA DEL PECIOLO DE NIAGARA ROSADA*

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RESUMEN

Evaluaron la influencia de los portainjertos 'IAC 766', 'IAC 572', 'IAC 31'3 y 'IAC 571-6' en el índice relativo de clorofila (IRC) y en los tenores de nitrato y potasio en la savia de los pecíolos de la vid Niagara Rosada. El experimento estaba ubicado en Votuporanga, 20º15'S. y 50º30'W., Estado de São Paulo, Brasil. La poda de las vides fue en 18/08/2009/, y el muestreo de las hojas cuando las vides estaban en pleno florecimiento al 20/09/2009. Efectuose las mediciones del IRC de las hojas empleando el clorofilómetro. A seguir extrajeron la savia de los pecíolos para leer los tenores de NO_3^- e de K^+, con el medidor portátil Cardy Meter. Injertada en el patrón IAC 766, la vid presentó mayores valores del IRC en las hojas (43) y en los tenores de NO_3^- en el pecíolo (2710ppm), difiriendo de manera significativa de los demás patrones. Los menores valores fueran obtenidos con las vides injertadas en el portainjerto IAC 571-6 con IRC de 38,7 y tenor de NO_3^- de 1108ppm. Injertada en los patrones IAC 572 y IAC 313 las vides presentaron tenores de K^+ en la savia del peciolo de, respectivamente, 3080 y 2790 ppm.

ABSTRACT

This study evaluated the influence of the rootstocks IAC 766, IAC 572, IAC 313 and IAC 571-6 in the relative chlorophyll index (IRC) and contents of the nitrate (NO_3^-) and potassium (K^+) in the sap of petioles of the grapevine Niagara Rosada. The experimental area is located in Votuporanga, 20º15'S. y 50º30'W, São Paulo State, Brazil. The pruning of the vineyards was performed in 18/08/2009, being the sampling of leaves accomplished in the full bloom of the grapevine, in 20/09/2009. The measurement of IRC in the leaves was performed being used the chlorophyll meter. Later, in the removed petioles of the leaves, the sap was extracted for the reading of the contents of NO_3^- and of K^+, being used the portable meters Cardy Meter. The rootstock IAC 766 provided larger values in IRC in the leaves (43) and in the contents of NO_3^- in the petiole (2710ppm), differing significantly of the other rootstocks. The rootstocks IAC 572 and IAC 313 presented contents of K^+ in the sap of the petiole, of, respectively, 3080 and 2790ppm.

INTRODUCCIÓN

En Brasil, el Estado de São Paulo sobresale como el mayor productor nacional de uvas para mesa, con 33,8 millones de pies y producción de 189,7 mil toneladas, con la variedad Niagara Rosada correspondiendo por 48,2% de la producción (Instituto de Economia Agrícola, 2010). La región noroeste tiene actualmente 200ha, o sea 2,0% de la producción de esta variedad, con fuerte tendencia de ampliación en la superficie de cultivo debido al menor costo de producción cuando comparado con el cultivo de *Vitis vinifera*. Además, la cosecha es efectuada entre los meses de agosto hasta octubre, período entre las cosechas de las mas importantes regiones viticolas ubicadas en la parte sureste del Estado.

Como en otras regiones vitícolas, el uso de patrones es obligatorio, teniendo como objetivos principales el control de las plagas de suelo, como filoxera (*Daktulosphaira vitifoliae* Fitch, 1856), la perla de la tierra (*Eurhizococcus brasiliensis,* Hempel, 1922) y nematodos; adaptación los diversos tipos de suelo; la resistencia a la sequia y/o el exceso de la humedad; aumento en vigor, precocidad de la producción y productividad de la vid. Aunque haya disponibilidad de innumerables variedades de portainjertos, cada cual tiene su limitación, y solamente la experimentación regional podrá determinarse cuál se ajusta según cada condición de la cultura. Braga (1988) sugirió para la región de Jundiaí el uso del patrón 106-8Mgt y de 'IAC 766', pero actualmente la preferencia es por el 'IAC 766'.

Algunos trabajos habían sido llevados a cabo con objetivo de evaluar el comportamiento de la vid 'Niagara' injertada en distintos portainjertos, demostrando la influencia en el crecimiento vegetativo, la productividad, la fenologia y las características de los racimos y de las bayas. Cuánto a los aspectos nutricionales, el método tradicional para la evaluación del estado nutricional de las plantas es la análisis foliar. Trabajos en la literatura retrataran la variación en los tenores de nutrientes en función del patrón (Albuquerque, Dechen, 2000; Tecchio *et al*., 2007; Csikász-krizsics, Diófási; 2008; Miele *et al*., 2009). Tecchio *et al*. (2007), en examen sobre la nutrición de la vid 'Niagara Rosada', han evidenciado que, con el portainjerto 'IAC 766', los tenores foliares de N y K fueran mayores cuando comparados con el patrón 106-8Mgt. Miele et al. (2009) evaluaron el efecto de portainjertos en los tenores de nutrientes en tejidos de la vid "Cabernet Sauvignon"; obtuvieran variaciones de la cantidad de N, P, K, CA y Mg en el limbo, pecíolo, escobajo y baya de la vid 'Cabernet-Sauvignon', siendo que tal efecto varió en función del órgano y nutriente en estudio. De acuerdo con Wolpert (2005), los ensayos con asociación entre nutrición mineral y patrones son importantes para obtener informaciones consistentes debido a las interacciones complejas entre las combinaciones copa y portainjertos, el tipo de suelo, el tipo de clima y la tecnología de la producción.

Sin embargo, otros recursos se pueden emplear para ayudar a la evaluación del estado nutricional de las vides, sobresaliendo el clorofilômetro SPAD-502 para la determinación del índice relativo de clorofila (IRC) y el medidor portátil Cardy Meter para la lectura de los tenores de NO_3^- y de K^+ de la savia de los pecíolos.

El clorofilômetro (SPAD-502, Minolta) es un aparato portátil con que se puede obtener el índice relativo de la clorofila en la hoja (IRC), siendo basado en la intensidad de la coloración verde de las hojas, que hace la correlación de la clorofila con el nitrógeno de la hoja. Es un aparato rápido, sencillo, con ventaja de ser un método no destructivo (Godoy *et al*., 2008). Los valores obtenidos por el clorofilómetro son calculados por la lectura que distingue de la cantidad de luz transmitida por la hoja en dos regiones de longitud de onda (650 nanómetro y 940 nanómetro), y la absorción de luz por la clorofila, que ocurre en la primera longitud de onda (Swiader, Moore, 2002). En los cultivos de tomate (Guimarães *et al*., 1999), de frijol

(Carvalho *et al.,* 2003) y de café (Godoy *et al*., 2008), hubieron correlación positiva y significativa entre el IRC y la concentración del nitrógeno de las hojas. En la cultura de la vid hay pocos trabajos en la literatura empleándose los medidores portátil (Porro *et al*., 2001; Amarante *et al*., 2009). Porro *et al.* (2001) lograron correlaciones significativas entre el índice relativo de clorofila con los tenores en las hojas de N y P en la cultura de la vid y, con los tenores en las hojas de N, Ca, K y Mg en el cultivo del manzanero, probando la posibilidad del uso de este equipo en optimizar el uso del abono.

En relación a los medidores portátiles de NO_3^- y de K^+ en la savia de los pecíolos, Nagarajah (1999) obtuve correlación significativa entre los tenores de nitrógeno y potasio obtenidos por el análisis químico convencional.

Para el cv Niagara Rosada, no hay datos en la literatura usándose los medidores portatiles del IRC y de los tenores de NO_3^- y de K^+ en la savia de los pecíolos.

Como hay grandes posibilidades de la región de Jales llegar a ser un importante sitio productor de la vid 'Niagara Rosada' y de la importancia en se obtener los métodos alternativos para la evaluación del estado nutricional de las vides, este trabajo tuvo el propósito de evaluar el efecto del portainjerto en el índice relativo del clorofila (IRC) y en los tenores del nitrato y del potasio de la savia del pecíolo de la vid 'Niagara Rosada'. Otro propósito es también correlacionar el IRC de la hoja con los tenores del nitrato de la savia del pecíolo.

MATERIAL Y MÉTODOS

Este experimento fue realizado en un viñedo ubicado en Votuporanga, a 20º15'S. y 50º30'W., precipitación pluvial de 1312mm, temperatura media mensual de 23,6ºC, noroeste del Estado de São Paulo, Brasil.. Suelo tipo Argisolo Vermelho Amarelo (Embrapa, 1999), y clima tipo Aw (Koëppen).

El viñedo estaba sostenido en sistema de parral alto, las vides plantadas en un espacio de 2,0m x 2,0m. Los tratamientos fueran una combinación del cv. Niagara Rosada injertada en los portainjertos 'IAC 766', 'IAC 572', 'IAC 313' e 'IAC 571-6'. La análisis estadística fue en bloques enteramente al azar, con 4 tratamientos, representados por los portainjertos y 10 bloques.

Las vides fueran podadas con 6 a 8 yemas en 18 de agosto de 2009. El muestreo de las hojas fue llevado a cabo en la plena floración de la vid, en 20/09/2009, recogiendo 16 hojas por parcela experimental. Fue medida el IRC de las hojas usándose el clorofilómetro (SPAD-502, Minolta). Más adelante, en los pecíolos quitados de las hojas, extrajeron la savia para la lectura de los tenores textos de NO_3^- y de K^+, usándose el dosificador portátil Cardy Meter (Horiba, Inc.) equipado con microelectrodos sensibles al nitrato y al potasio, respectivamente.

Con los datos obtenidos, la prueba de normalidad fue verificada con el paquete estadístico MINITAB, para averiguar si los datos presentaban distribución normal. Las medias se compararon por la prueba de Tukey ($P<0,05$).

RESULTADOS Y DISCUSIÓN

Hubo efecto significativo de los patrones en el IRC y en los tenores de nitrato y de potasio en la savia del pecíolo de la vid 'Niagara Rosada' (Cuadro 1). La vid injertada en el portainjerto 'IAC 766' tuvo mayor IRC en las hojas con índice 43 y en los tenores de NO_3^- en el pecíolo con 2710ppm, diferenciando de modo significativo con los demás portainjertos

(Cuadro 2). Los valores menores fueron obtenidos cuando injertada sobre el portainjerto 'IAC 571-6', siendo el IRC 38.7 y el e o tenor de NO_3^- de 1108ppm.

Injertadas sobre los portainjertos 'IAC 572' e 'IAC 313' presentaron tenores de K^+ en la savia del pecíolo, de respectivamente 3080 y 2790ppm, siendo superior a los valores obtenidos con los demás patrones.

Trabajos relacionados con las variaciones en el IRC y los tenores de NO_3^- de K^+ en la savia del pecíolo de una variedad en función de la combinación con el no se encuentran en literatura. Sin embargo, se deduce que las variaciones encontradas pueden ser debidas a la absorción diferenciada de los nutrientes por los portainjertos. De acuerdo con Iannini (1984) los portainjertos indujeron gran variación en el vigor, en consecuencia de los diversos requisitos nutricionales y capacidad de la absorción de agua y nutrientes; por lo tanto sus raíces en función de las variedades presentan una selectividad en la absorción de los íons de la solución del suelo. Albuquerque, Dechen (2000), Tecchio *et al*. (2007), Csikász-krizsics, Diófási (2008) e Miele *et al*. (2009) obtuvieran variaciones significativas en los tenores foliares de nutrientes en función de los portainjertos. Más allá del vigor del patrón, Vercesi (1987) caracterizó la capacidad de la absorción mineral del portainjerto en función del origen genético del material vegetativo usado.

Cuadro 1 Resultado del test F de análisis de varianza del índice relativo de clorofila (IRC), del tenor de nitrato y de potásio en la savia del pecíolo de hojas amostrados en pleno florecimiento de la vid 'Niagara Rosada' injertada en diferentes portainjertos. Votuporanga, SP, 2009.

Fuentes de Variación	IRC	Tenor de Nitrato (ppm)	Tenor de Potásio (ppm)
Bloque	1,46 NS	0,56 NS	1,26 NS
Portainjerto	45,3**	17,8**	13,1**

NS – no significativo; * - significativo al 5% por el test F; ** - significativo al 1%; por el test F.

Cuadro 2 Resultados promedios del índice relativo de clorofila (IRC), del tenor de nitrato y de potásio en la savia del pecíolo de hojas amostrados en pleno florecimiento de la vid 'Niagara Rosada' injertada en diferentes portainjertos. Votuporanga, SP, 2009.

Portainjerto	IRC	Tenor de Nitrato (ppm)	Tenor de Potásio (ppm)
'IAC 313'	41,0 B	2230 AB	2790 A
'IAC 572'	38,2 C	2047 B	3080 A
'IAC 571-6'	37,8 C	1108 C	2230 B
'IAC 766'	43,0 A	2710 A	2250 B
CV (%)	2,90	24,84	14,11
DMS	1,42	615,5	447,05

Médias con la misma letra, en la columna, no presentan diferencia significativa entre si (Tukey $\leq$ 0,05).

Hubo correlación positiva y significativa entre el IRC de la hoja con el tenor de NO_3^- en la savia del pecíolo de la vid "Niagara rosada", con coeficiente de correlación 0.61 ** (Figura 1), evidenciando la relación entre el IRC y el nitrógeno foliar. Estos resultados están de acuerdo con los obtenidos por Guimarães et al. (1999), Carvalho et al. (2003) y Godoy y et al. (2008) que también obtuvieron una correlación positiva entre el IRC y la concentración de N foliar en las culturas del tomate, del frijol y del cafe, respectivamente.

Esta correlación se debe a la participación del N en la composición estructural de la molécula de clorofila, que, de acuerdo con Taiz, Zeiger (1991) y Marschner (1995), está presente en la porción del porfirina de anillos de los tetrapirrólicos.

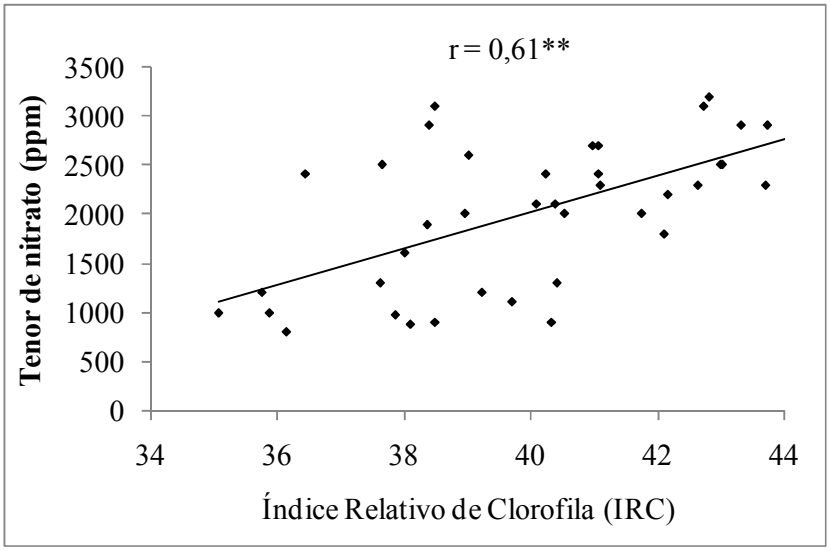

Fig. 1 Correlacion entre el índice relativo de Clorofila (IRC) con el tenor del nitrato de la savia del pecíolo de las hojas muestreadas en la plena floración de la vid 'Niagara Rosada' injertada en diferentes portainjertos. Votuporanga, SP, 2009

CONCLUSIONES

Fue evidenciado un comportamiento nutricional de la vid 'Niagara Rosada' en función del portainjerto y la posibilidad del uso de estos equipos para asistir al diagnostico nutricional de la vid. Sin embargo, hay necesidad de experimentaciones que tengan por objetivo la calibración del clorofilômetro y de los medidores portatiles de NO_3^- e K^+ de la savia del pecíolo para el cultivo de la vid.

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REFERENCIAS BIBLIOGRÁFICAS

ALBUQUERQUE, T.C.S., 1996. Uva para exportação: aspectos técnicos da produção. 1 ed. Brasília: Centro Nacional de Pesquisa de Mandioca e Fruticultura/Embrapa. 53 p.

ALBUQUERQUE, T.C.S., DECHEN, A.R, 2000. Absorção de macronutrientes por porta-enxertos e cultivares de videira em hidroponia. *Scientia Agrícola*, v.57, n.1, p. 135-139.

AMARANTE, C.V.T., ZANARDI, O.Z., MIQUELOTO, A., STEFFENS, C.A., ERHART, J., ALMEIDA, J.A, 2009. Quantificação da área e do teor de clorofilas em folhas de plantas jovens de videira 'Cabernet Sauvignon' mediante métodos não destrutivos. *Revista Brasileira de Fruticultura*, v. 31, n. 5, p. 680-686.

BRAGA, F. G, 1988. Cultura da uva Niagara Rosada. 1 ed. São Paulo: Nobel. 66 p.

CARVALHO, M.A.C., FURLANI JÚNIOR, E., ARF F., SÁ, M.E., PAULINO, H.B., BUZETTI, S., 2003. Doses e épocas de aplicação de nitrogênio e teores foliares deste nutriente e de clorofila em feijoeiro. *Revista Brasileira de Ciência do Solo*, v. 27, n.3, p. 445-450.

CSIKÁSZ-KRIZSICS, A., DIÓFÁSI, L., 2008. Effects of rootstock-scion combinations on macroelements availability of the vines- alany-nemesfajta kombinációk hatása a szőlő makroelem felvételére. *Journal Central European Agricultures*, v. 9, n. 3, p. 495-504.

GODOY, L.J.G., SANTOS, T.S.,VILLAS BOAS, R. L., LEITE JÚNIOR, J.B. 2008. Índice relativo de clorofila e o estado nutricional em nitrogênio durante o ciclo do cafeeiro fertirrigado. *Revista Brasileira de Ciência do Solo*, v. 52, p. 217-226.

GUIMARÃES, T.G., FONTES, P.C.R., PEREIRA, P.R.G., ALVAREZ, V.H., MONERAT, P.H., 1999. Teores de clorofila determinados por medidor portátil e sua relação com formas de nitrogênio em folhas de tomateiro cultivados em dois tipos de solo. *Bragantia*, v. 58, n. 1, p. 209-216.

IANNINI, B., 1984. Importanza e funzioni del portinnesto nella viticoltura moderna. *Revista di Viticoltura e di Enologia*, v. 37, n.7/8, p. 394-419.

INSTITUTO DE ECONOMIA AGRÍCOLA. Área cultivada e produção. Disponível em: <http:// www.iea.sp.gov.br.>. Acesso em: 16 de março 2010.

MARSCHNER, H., 1995. Mineral nutrition of higher plants. 2. ed. Berna: International Potash Institute. 680 p.

MIELE, A., RIZZON, L.A., GIOVANNINI, E., 2009. Efeito do porta-enxerto no teor de nutrientes em tecidos da videira 'Cabernet Sauvignon'. *Revista Brasileira de Fruticultura*, v. 33, n. 4, p. 1141-1149.

NAGARAJAH, S., 1999. A petiole sap test for nitrate and potassium in Sultana grapevines. *Australian Journal of Grape and Wine Research*, v. 5, 56–60.

PORRO, D., DORIGATTI, C., STEFANINI, M., CESCHINI, A., 2001. Use of SPAD meter in diagnosis of nutritional status in apple and grapevine. *Acta Horticulturae*, n.564, p.243-252.

VERCESI, A., 1987. Gli assorbimenti radicali della vite: meccanismi e fattori influenti, *Vignevini*, v. 4, p.47-55.

SOUSA, J.S.I., 1996. Uvas para o Brasil. 2. ed. Piracicaba: FEALQ. 791 p.

SWIADER, J.M., MOORE, A., 2002. SPAD - chlorophyll response to nitrogen fertilization and evaluation of nitrogen status in dryland and irrigated pumpkins. *Journal of Plant Nutrition*, v. 25, p.1089-1100.

TAIZ, L., ZEIGER, E., 1991. Plant physiology. 1 ed. California. 565p.

TECCHIO, M.A., PIRES, E.J.P., TERRA, M.M., MOURA, M.F., 2007. Produtividade e teores de nutrientes da videira Niagara Rosada em vinhedos nos municípios de Louveira e Jundiaí. *Bioscience Journal*, v. 23, n.1, p. 48-58.

WOLPERT, J.A., SMART, D.R., ANDERSON, M., 2005. Lower Petiole Potassium Concentration at Bloom in Rootstocks with *Vitis berlandieri* Genetic Backgrounds. *American Journal of Enology and Viticulture*, v. 56, n. 2, p. 163-169.

LA PROTEÓMICA COMO HERRAMIENTA DE ESTUDIO Y CARACTERIZACIÓN DEL HONGO FITOPATÓGENO DE LA VID *Botrytis cinerea*

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RESUMEN

La proteómica se ha revelado como una importante tecnología emergente, no obstante, aun son escasas las aportaciones de estas técnicas a la biología de los hongos fitopatógenos. Entre estos organismos cabe destacar *Botrytis cinerea*, patógeno responsable de la podredumbre gris en el cultivo de vid. Nuestro grupo viene desarrollando distintas aproximaciones proteómicas para dilucidar las proteínas implicadas en el ciclo infectivo de *B. cinerea*. Se ha determinado el proteoma en distintas condiciones de inducción de patogenicidad, así como su secretoma. Estos estudios han aportado pruebas de la validez de la proteómica para determinar nuevos factores de patogenicidad. Los resultados obtenidos han puesto de manifiesto la utilidad de esta técnica para estudiar la biología básica del hongo, así como determinar los mecanismos de patogenicidad empleados por estos organismos para causar enfermedades en los cultivos.

PROTEOMICS APPROACHES AS A TOOL TO STUDY AND CHARACTERIZE THE GRAPEVINE PHYTOPATHOGENIC FUNGUS *Botrytis cinerea*

SUMMARY

Proteomics techniques have revealed as an emerging technology in molecular biology. However, at present, the number of papers applying these technologies to the biology of fungal phytopathogen is still low. Among these organisms, should be empathized *Botrytis cinerea*. This phytopathogenic fungus is the responsible of grape putrefaction in the grapevine cultivation. Our group has been developing several proteomics approaches to this fungus trying to elucidate the proteins implicated in the infective cycle of *B cinerea*. We had followed the changes in the 2-DE profiles varying the culture conditions to induce the pathogenic response. Moreover, we had followed the variations in fungal secretome. These studies have contributed to establish the validity of proteomics approaches to determine new pathogenicity factors. The obtained results have confirm too, the utility of this techniques to study the basic biology of the fungi, as well as determining the mechanisms of pathogenicity used by these organisms to cause diseases grape crops.

INTRODUCCIÓN

La agricultura es históricamente una de las principales actividades del hombre. La comunidad autónoma andaluza es la primera región agrícola de España gracias a su clima favorable, a la fertilidad de sus tierras, así como al continuo esfuerzo de mejora llevado a cabo en los últimos años por todas las partes implicadas en el sector, incluyendo a las administraciones central, autonómica y sobre todo a los agricultores y empresarios agrícolas, cada vez más orientados a la innovación en el sector. En Andalucía, esta innovación se ha puesto de manifiesto con la aparición de polos agrícolas de desarrollo, tales como el sector fresero de Huelva, las destacadas zonas vitivinícolas andaluzas, el cultivo de tropicales en la costa mediterránea así como, el desarrollo del cultivo bajo plástico en Almería. Se ha conseguido con ello evitar el abandono del campo por la ciudad, así como una innegable producción de riqueza. No obstante, las reformas de la política agraria común de la Unión Europea (UE), el descenso de los precios de comercialización, así como, un aumento en los gastos de producción, está poniendo en peligro el hasta ahora boyante sector.

Este enorme desarrollo agrícola está siendo continuamente amenazado por distintas enfermedades, especialmente aquellos cultivos desarrollados en intensivo y en agricultura sostenible. Entre los patógenos más importantes está *Botrytis cinerea*. Este patógeno es el responsable de la "podredumbre gris" en viñedos, fresa y tomate, siendo capaz de atacar a cualquier órgano de la planta, en cualquiera de sus fases de desarrollo, y tanto "*in planta*" como durante el almacenaje y distribución del fruto. El coste anual de los tratamientos contra este patógeno asciende a 540 millones de euros, lo que constituye un 10% del mercado mundial de fungicidas (UIPP, 2002). En España, como media anual, el consumo en fungicidas contra *Botrytis*, es alrededor de 3 millones de euros en tomate, 2 millones en fresa y 3 millones en viña (BASF, comunicación personal). Por otro lado, *Colletotrichum* spp. es el responsable de la antracnosis en vid, fresa y tomate. Se estima que las pérdidas en los cultivos producidas por este hongo oscilan en torno a los 100 millones de euros anuales, a lo que habría que sumar el gasto que estos realizan en fungicidas. Según los últimos datos, procedentes de la Red de Alerta e Información Fitosanitaria (www.juntadeandalucia.es/agriculturaypesca), en este momento, *Botrytis* afecta al 8'2% de los racimos de la zona de Sanlúcar de Barrameda. En la zona fresera de Huelva, se mantienen unos niveles de incidencia leve-moderado. Y en Almería, *Botrytis* afecta al 8'5% de los invernaderos que están en plena recolección.

En los últimos años, con el desarrollo de las técnicas de biología molecular, ha sido posible profundizar en el conocimiento de los mecanismos moleculares empleados por estos organismos durante su ciclo infectivo. El estudio del Proteoma se ha convertido en una técnica extremadamente útil para desentrañar la biología de un impresionante número de plantas, tejidos y microorganismos. Pero a pesar de estos avances, aún son pocos los estudios sobre distintos hongos fitopatógenos de interés en la agricultura. Además, la proteómica como herramienta para la descripción de procesos biológicos, metabólicos, fisiológicos o patológicos, está alcanzando un gran desarrollo en los últimos años. Algunos autores describen el periodo actual como "la era postgenómica" (Fernández-Acero *et al.*, 2007a). Los trabajos realizados con estos organismos se refieren fundamentalmente a especies de interés industrial o agrícola. Para arrojar luz sobre este campo, se realizó una aproximación al proteoma del hongo fitopatógeno *Botrytis cinerea*. Este hongo posee un amplio y complicado arsenal de

factores de patogenicidad y virulencia. Descubrir estos factores y establecer su implicación en los procesos de infección permitirá entender el funcionamiento del proceso infectivo, así como proporcionar nuevas dianas terapéuticas que posibiliten el diseño de fungicidas racionales para el control del fitopatógeno.

Como un primer objetivo de esta estrategia de estudio, nos propusimos la optimización del protocolo para la obtención y estudio del proteoma de *B. cinerea*. El diseño experimental planteado en nuestro grupo tiene como objetivos determinar un perfil proteómico de referencia que nos permita (i) caracterizar las proteínas mayoritarias presentes en el hongo en condiciones de cultivo estándar, (ii) determinar si alguna de estas proteínas están relacionadas con los procesos de patogenicidad y (iii) buscar entre estas proteínas aquéllas susceptibles de convertirse en dianas terapéuticas para el diseño de nuevos fungicidas. Existen otros estudios en los que se intentan buscar aquellos factores implicados en los procesos infectivos de distintos organismos. En la mayoría de éstos se realiza una criba previa, en la que se elige un gen candidato y se ve su influencia en dichos procesos, mediante el estudio de su expresión o mediante mutagénesis dirigida. A diferencia de estos estudios, un acercamiento proteómico implica la no existencia de genes candidatos *a priori*, estudiándose la totalidad de las proteínas expresadas bajo unas condiciones determinadas.

Con este horizonte, nuestro grupo de investigación ha venido realizando distintas aproximaciones al proteoma de *B. cinerea*. Este hongo es capaz de infectar a más de 200 plantas distintas, entre las que se incluyen, el tomate, la fresa y la vid, cultivos de una gran importancia económica en nuestra comunidad andaluza. En el 2006 realizamos la primera aproximación al proteoma del hongo mediante la aplicación de la electroforesis bidimensional (Fernández-Acero *et al.*, 2006), dicho estudio se ha continuado con el estudio diferencial entre proteomas de cepas de *B. cinerea* con distinta virulencia (Fernández-Acero *et al.*, 2007b) y la realización del primer estudio en profundidad que permitiera el establecimiento del primer mapa proteómico de *B. cinerea*, con la identificación de más de 300 proteínas(Fernández-Acero *et al.*, 2009).

B. cinerea utiliza un amplio arsenal para completar su ciclo infectivo. Entre los recursos de los que dispone el hongo se incluyen la producción de toxinas, las cuales parecen modular la distinta virulencia entre cepas; y la producción de especies activas de oxígeno. Pero el principal mecanismo de infección utilizado por el hongo consiste en la producción y secreción de un complejo conjunto de proteínas (secretoma) capaz de transformar la biomasa vegetal en biomasa fúngica, jugando un papel crucial durante la penetración y maceración de los tejidos vegetales.

Distintos estudios moleculares han estudiado estas proteínas. Es conocido que *B. cinerea* expresa un conjunto de genes que codifican para distintas enzimas degradadoras de la pared celular (cell wall degrading enzymes, CWDE). La inactivación de algunos de estos genes, mediante mutagénesis dirigida, parece reducir la virulencia de los mutantes, por ejemplo polygalacturonase *BcPG*1, pectin methyl-esterase *Bcpme1*, endo-β–1,4-xylanase *Xyn11A* (Van Kan, 2006). Sin embargo, la inactivación de otros genes que codifican para las mismas "CWDE" parecen no afectar la virulencia de los mutantes, por ejemplo *cel5A* que codifica para una endo-β–1,4-glucanasa, cutinasa A o lipasa (Van Kan, 2006). Estos datos demuestran que no es posible establecer una relación directa entre gene codificantes para CWDE y factores de patogenicidad/virulencia, ya que la mayoría de estos genes están codificados por

familias multigénicas con funciones redundantes, que explicarían los diferentes fenotipos mutantes. El presente trabajo describe el primer estudio, basado en el uso combinado de 2-DE/ MALDI TOF/TOF, sobre el secretoma de *B. cinerea*. Los distintos perfiles electroforéticos has sido obtenidos mediante el uso de glucosa, pectina, almidón, celulosa y paredes celulares de tomate desproteinizadas como fuentes de carbono para inducir el mecanismo de infección.

MATERIAL Y MÉTODOS

2.1.- Cultivo y extracción de proteínas de *B. cinerea*. Electroforesis bidimensional (2-DE)

La cepa *B. cinerea* 2100 fue obtenida de la Colección Española De Cultivos Tipo (www.cect.org). El procedimiento de cultivo se realizó según Fernández-Acero *et al.*(Fernández-Acero *et al.*, 2009). En resumen, se preparó medio mínimo salino (MSM) que fue suplementado con 1% de la fuente de carbono a ensayar glucosa, pectina, almidón, celulosa (CMC) y paredes celulares de tomate (TCW) desproteinizadas, en tres replicas independientes. Después de 5 días de cultivo (22ºC, 180 rpm), el caldo fue filtrado y centrifugado para retirar el micelio. El extracto proteico fue obtenido mediante un método mejorado de precipitación con TCA/DOC, seguido de otra precipitación con fenol. La separación mediante 2-DE se realizó según Fernández-Acero et al. (Fernández-Acero *et al.*, 2009; Fernández-Acero *et al.*, 2010).

2.2.- Análisis e identificación de las proteínas

Después de la electroforesis, los geles se tiñeron con SYPRO Ruby (Invitrogen, Karlsruhe, Germany) y fueron visualizados mediante un Fuji FLA 3000 Fluorescence Laser Scanner (Fuji, Photofilm Co. Ltd., Tokyo, Japan). Los spots de interés fueron digeridos e identificados según Fernández-Acero et al.(Fernández-Acero *et al.*, 2009).

RESULTADOS Y DISCUSIÓN

Desde el 2004 el grupo de Microbiología de la Universidad de Cádiz ha venido realizando aportaciones en el campo de la proteómica fúngica, trabajando con *B. cinerea*. Se realizó y publico la primera descripción del proteoma de *B. cinerea*. La naturaleza inédita del trabajo hizo necesaria la optimización de todo el proceso, desde la extracción de las proteínas hasta la identificación de las mismas (Fernández-Acero *et al.*, 2006). Poco tiempo después, se realizó el primer estudio de proteómica diferencial con *B. cinerea* (Fernández-Acero *et al.*, 2007a). En dicho estudio se planteó la comparación de los proteomas de dos cepas de *B. cinerea* con distinta virulencia, una de ellas muy virulenta y productora de toxinas (botridial y dihidrobotridial), mientras que la otra presentaba una menor virulencia, así como una nula producción de dichas toxinas. De esta primera comparación entre ambos proteomas, se seleccionaron aquéllas proteínas específicamente representadas en cada una de las cepas candidatas así como aquellas sobreexpresadas en la cepa de mayor virulencia.

Estos estudios han aportado pruebas de la validez de la proteómica para determinar nuevos factores de patogenicidad. Entre las proteínas que podrían tener un papel relevante durante el ciclo infectivo destacan: (i) la malato dehidrogenasa (MDH)

dependiente de NADPH, presente en tres clusters y sobreexpresada en la cepa más virulenta. La MDH cataliza la transformación de malato a oxalacetato, principal precursor del ácido oxálico, descrito como factor de patogenicidad. Una alta producción de ácido oxálico provoca un descenso del pH de los cultivos, necesario para la producción de las toxinas del hongo (botridial y dihidrobotridial). Todos estos datos indican el nuevo papel que la MDH podría tener en la patogenicidad del hongo. Recientemente, se ha demostrado el papel que estas oxidasas dependientes de NADPH juegan en la diferenciación y patogenicidad de *B. cinerea* (Segmüller et al., 2008) lo que parece coincidir con nuestras observaciones. (ii) La gliceraldehído 3-fosfato deshidrogenasa (GAPDH) encontrada de forma exclusiva en la cepa de mayor virulencia. El papel de esta enzima en la patogenicidad de distintos organismos ha sido ampliamente descrito en bibliografía (Alderete *et al.*, 2001), por lo que aparte de su función metabólica podría tener un papel durante el ciclo infectivo tal y como ha sido descrito recientemente para la hexokinase Hxk1 de *B. cinerea* (Rui and Hahn, 2007). (iii) El regulador transcripcional metE/metH, sobreexpresada o específica de la cepa de mayor virulencia. Esta proteína está implicada en la síntesis de metionina, ruta que ha sido ampliamente utilizada para el diseño de fungicidas. La variabilidad encontrada entre las cepas de *B cinerea* podría ser la base molecular de los distintos fenotipos de resistencia a fungicidas descritos para el fitopatógeno. (iv) La ciclofilina ha sido observada de forma exclusiva en la cepa más virulenta y está relacionada con el ensamblaje y regulación de las proteínas. Su papel como factor de virulencia ha sido descrito en distintos hongos incluido *B. cinerea*, (Viaud *et al.*, 2002; Viaud *et al.*, 2003) relacionado con los procesos de invasión y colonización de los tejidos. Estos datos nos confirman la utilidad de la proteómica para detectar componentes implicados en el mecanismo de infección de los hongos fitopatógenos. Además, esta proteína forma un complejo con la calmodulina, que a su vez está implicada en distintas cascadas de señalización celular, por lo que podría realizar funciones también a este nivel.

Recientemente, en colaboración con el grupo de proteómica (MS Group) del Instituto Max Planck "for plant breeding research" (MPIZ) de Colonia, Alemania se desarrolló el primer mapa proteómico de *B. cinerea* durante la degradación de la celulosa. Se identificaron por primera vez un número significativo de proteínas, realizándose la clasificación funcional (PANTHER: Protein ANalysis THrough Evolutionary Relationships; www.pantherdb.org) de las mismas utilizando dos criterios, el proceso biológico y su función molecular. Debido a que la celulosa es uno de los principales componentes de la pared celular de las plantas, muchas de las proteínas identificadas juegan un papel crucial durante la patogénesis del hongo (Fernández-Acero *et al.*, 2009). Además, también en colaboración con el Instituto Max Planck (MPIZ) de Colonia, se ha desarrollado el análisis del secretoma de *B. cinerea* (Fernández-Acero et al., 2010). En este trabajo, se optimizó la inducción de la patogenicidad fúngica mediante la adición de distintos elicitores vegetales. Esta inducción varió entre la treintena de spots proteicos detectados en estado de reposo (usando glucosa) hasta el centenar en estado de patogenicidad inducida (utilizando extractos vegetales) (Fernández-Acero *et al.*, 2010). En este estudio se identificaron más de 70 proteínas, describiéndose la mayoría de las actividades que para este tipo de proteínas están descritas en la base de datos de factores de virulencia y patogenicidad "phi-pathogen". A partir de este trabajo se han identificado aquéllas proteínas implicadas en los estadios iniciales del ciclo infectivo, las cuales están siendo estudiadas para comprobar su uso como herramienta de diagnóstico y posterior patente.

Los resultados obtenidos han puesto de manifiesto la utilidad de esta técnica para estudiar la biología básica del hongo, así como, determinar los mecanismos de patogenicidad empleados por estos organismos para causar las enfermedades en los cultivos. Un estudio sistemático del proteoma de aquéllos hongos fitopatógenos que más duramente afecten a la agricultura (*B. cinerea, Colletotrichum* spp., etc.), aparte de aclarar su funcionamiento vegetativo, nos permitirá conocer con detalle las proteínas causantes de los síntomas de la enfermedad, conocidas como factores de patogenicidad/virulencia. Aprovechando la experiencia previa del grupo, esta información será de utilidad para diseñar nuevas estrategias de control que permitan la inhibición selectiva de estos factores, impidiendo el desarrollo de la enfermedad. Además, también será de aplicabilidad para el diseño y posterior patente de nuevas estrategias de diagnóstico, que permitan un control exhaustivo del material vegetal utilizado, así como un correcto estudio de la epidemiología, origen y fuentes de inóculo en estos patógenos.

Recientemente nuestro grupo ha comenzado el estudio de los proteomas asociados a las estructuras de resistencia del hongo, conidios y esclerocios (figura 1). Estas estructuras son responsables del mantenimiento del inoculo en el campo entre las campañas agrícolas. Hemos conseguido obtener los perfiles proteicos mediante 2-DE. La mayoría de los spots proteicos se encuentran localizados entre 5 y 8 de pI y entre 14 y 105 kDa de peso molecular. En estos momentos las proteínas están siendo identificadas por MALDI TOF/TOF, donde esperamos encontrar aquellas proteínas implicadas en los estadios iniciales de la infección.

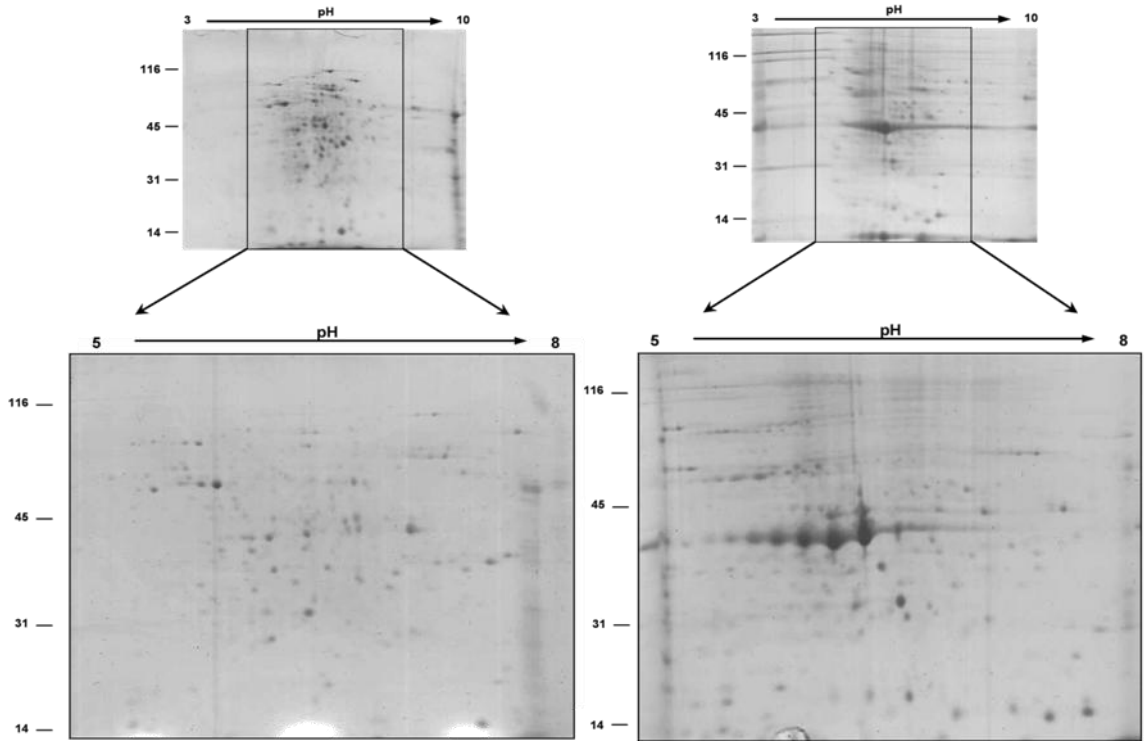

Figura 1: Geles bidimensionales de los conidios (izquierda) y esclerocios (derecha) de *B. cinerea*, mostrando la separación en IPG 3-10 (arriba) y el definitivo IPG 5-8 (abajo).

Una vez separadas las proteínas, aquellos spots presentes en todas y cada una de las tres replicas fueron usadas para el cálculo de la variabilidad analítica del experimento. Ciento ocho spots fueron utilizados obteniendo un promedio de CV% de 42.05%, promedio similar a los obtenidos en estudios previos. El perfil proteico de los esclerocios fue algo más diverso, aunque mostró aproximadamente la misma distribución, sus espectro de pesos moleculares fue algo más amplio (3.1 to 158.8 kDa). Usando 205 spots presentes en todas las replicas se determino su CV% analítico que fue de 44.61% (González-Rodríguez *et* al., 2010). Estos resultados son muy parecidos a los encontrados al estudiar el proteoma de *Sclerotinia sclerotiorum* (Russo *et al.*, 1985) que presentó una proteína mayoritaria de 34-36 kDa y 3 isoformas con un pI de 6.2, 6 y 5.8. Nuestros geles mostraron 6 isoformas con un peso molecular de 41 kDa, y un pI de 5.23, 5.51, 5.65, 5.81, 5.94 and 6.19. A pesar de que tenemos que esperar los resultados de la identificación proteica, podemos presuponer que es la misma proteína, Ssp1, involucrada en el desarrollo de los esclerocios de ambos hongos.

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REFERENCIAS BIBLIOGRÁFICAS

Alderete, J.F., Millsap, K.W., Lehker, M.W., Benchimol, M., 2001. Enzymes on microbial pathogens and *Trichomonas vaginalis*: molecular mimicry and functional diversity. *Cellular Microbiology* 3, 359-370.

Fernández-Acero, F.J., Carbú, M., Garrido, C., Vallejo, I., Cantoral, J.M., 2007a. Proteomic Advances in Phytopathogenic Fungi. *Current Proteomics*, 79-88.

Fernández-Acero, F.J., Colby, T., Harzen, A., Cantoral, J.M., Schmidt, J., 2009. Proteomic analysis of the phytopathogenic fungus *Botrytis cinerea* during cellulose degradation. *Proteomics* 9, 2892-2902.

Fernández-Acero, F.J., Colby, T., Harzen, A., Carbú, M., Weineke, U., Cantoral, J.M., Schmidt, J., 2010. 2-DE proteomic approach to the *Botrytis cinerea* secretome induced with different plant elicitors. *Proteomics* DOI 10.1002/pmic.200900408.

Fernández-Acero, F.J., Jorge, I., Calvo, E., Vallejo, I., Carbú, M., Camafeita, L.E., Garrido, C., López, J.A., Cantoral, J.M., Jorrin, J., 2007b. Proteomic analysis of phytopathogenic fungus *Botrytis cinerea* as a potential tool for identifying pathogenicity factors, therapeutic targets and for basic research. *Archives of Microbiology* 187, 207–215.

Fernández-Acero, F.J., Jorge, I., Calvo, E., Vallejo, I., Carbú, M., Camafeita, L.E., López, J.A., Cantoral, J.M., Jorrin, J., 2006. Two-dimensional electrophoresis protein profile of the phytopathogenic fungus *Botrytis cinerea*. *Proteomics* 6, S88-S96.

González-Rodríguez, V.E., Garrido, C., Carbú, M., Cantoral, J.M., Fernández-Acero, F.J., 2010. Proteomic approach to *Botrytis cinerea* survival structures. *Proteomica* 5, 130-131.

Rui, O., Hahn, M., 2007. The *Botrytis cinerea* hexokinase, Hxk1, but not the glucokinase, Glk1, is required for normal growth and sugar metabolism, and for pathogenicity on fruits. *Microbiology* 153, 2791-2802.

Segmüller, N., Kokkelink, L., Giesbert, S., Odinius, D., van Kan, J., Tudzynski, P., 2008. NADPH Oxidases Are Involved in Differentiation and Pathogenicity in *Botrytis cinerea*. *Molecular Plant-Microbe Interactions* 21, 808-819.

UIPP, 2002. Annual Report. Union des Industries de la Proteccion des Plantes, Boulogne.

Van Kan, J.A.L., 2006. Licensed to kill: the lifestyle of a necrotrophic plant pathogen. TRENDS in Plant Science 11, 247-253.

Viaud, M., Brunet-Simon, A., Brygoo, Y., Pradier, J.-M., Levis, C., 2003. Cyclophilin A and calcineurin functions investigated by gene inactivation, cyclosporin A inhibition and cDNA arrays approaches in the phytopathogenic fungus *Botrytis cinerea*. *Mol Microbiol* 50, 1451-1465.

Viaud, M.C., Balhadere, P.V., Talbot, N.J., 2002. A *Magnaporthe grisea* Cyclophilin Acts as a Virulence Determinant during Plant Infection. *Plant Cell* 14, 917-930.

Russo, G.M., Van Etten, J.L., 1985. Synthesis and localization of a development-specific protein in *Sclerotinia sclerotiorum*. *J. Bacteriology* 163: 696-703.

GENETIC DIVERSITY AND RELATIONSHIPS BETWEEN WILD GRAPEVINE (*VITIS VINIFERA* SUBSP. *SYLVESTRIS*) POPULATIONS AND ABORIGINAL CULTIVARS IN GEORGIA

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ABSTRACT

18 Georgian and 4 West-European cultivars of *V. vinifera*, 31 individuals of wild *V. vinifera* subsp. *sylvestris* from different regions of Georgia and 2 samples of *V. riparia* were genotyped at eight nuclear microsatellite loci to investigate genetic relationships between cultivated and wild grapevines. This study showed strong genetic similarities between cultivated Georgian grapes and wild grapevine. Oldest Georgian cultivars – 'Krikina', 'Saperavi' and 'Uchakhardani' were unified in one cluster with local wild grapes. Very high value of genetic similarity (0.9) was detected between the old Georgian cultivar 'Rkatsiteli' and wild grapevine from the Lekhura gorge in Shida Kartli. Consequently, the admixture found among local Georgian cultivars and wild grapevine indicates the possibility that these cultivars are derived from ancestral domestication events of local wild grapevine.

ZUSAMMENFASSUNG

18 Georgische und 4 westeuropäische Sorten von *Vitis vinifera*, 31 Individen von wildem *V. vinifera* subsp. *sylvestris* aus verschiedenen Gebieten Georgiens und 2 Proben von *V. riparia* wurden an acht nuclearen Mikrosatelliten genotypisiert, um genetische Beziehungen zwischen kultivierten und wilden Weinreben zu untersuchen. Die in dieser Studie durchgeführten Analysen haben starke genetische Ähnlichkeiten zwischen einheimischen Sorten und wilden Weinreben gezeigt. Die ältesten georgischen Sorten, 'Krikina', 'Saperavi' und 'Uchakhardani' zeigten eine starke Ähnlichkeit zu lokalen Populationen wilder Weinreben. Sehr hoch wurde der Wert der genetischen Ähnlichkeit (0.9) zwischen der alten georgischen Sorte Rkatsiteli und wilder Weinrebe von Shida Kartli entdeckt. Folglich zeigen die unter lokalen georgischen Sorten und wilder Weinrebe gefundenen genetischen Ähnlichkeiten die Möglichkeit an, dass diese Sorten aus Domestizierungsereignissen der lokalen wilden Weinrebe abgeleitet worden sind.

INTRODUCTION

The Caucasus region is considered to be the primary centre of origin of cultivated grapevine (*Vitis vinifera* L.), with high relevance for the further distribution of the crop towards the Mediterranean basin and for the development of European modern cultivars (De Candolle, 1855; Negrul, 1946; Damania *et al.,* 1997; Sefc *et al.,* 2003; Constantini, 2004, This *at al.,* 2006; Vouillamoz *et al.,* 2006). Grapevine is among the first fruits to be cultivated in Georgia (Javakhishvili, 1930). Confirmations for long lasting cultivation of grapevine in Georgia are archaeological remains of berries and seeds of domesticated grapes dated ~6000 BC (vicinity of v. Shulaveri, Southeast Georgia; Ramishvili, 1988). Other archaeological evidences of prehistoric winemaking are found in near proximity of the Caucasian region in northern Iran at the Hajji Firuz Tepe site in the northern Zagros Mountains dated to about 5400–5000 BC (McGovern, 2003) and in the Levant where archaeological findings are dated from 4000 BC (Zohary, Hopf, 2000).

Another indicator of a possible origin of cultivated grapevine in the Caucasus region is high genetic and morphological diversity of both wild and cultivated grapes in this area (Grassi, 2006; Ekhvaia, Akhalkatsi, 2010). About 500 names of autochthonous grapevine varieties, including centuries-old cultivars like 'Rkatsiteli', 'Ojaleshi', 'Saperavi' are known from Georgia (Javakhishvili, 1930; Ketskhoveli *et al.,* 1960). They are characterized by a wide range of colours and shapes of berries and pips, which led already Vavilov (1931) to postulate an evolutionary centre of grape in this region. These cultivars showed great ampelometric variability and broad adaptability to different climates and soils (Negrul, 1946; Ketskhoveli *et al.,* 1960; Ramishvili, 1970; Tsertsvadze, 1989). However, only half of these cultivars have been conserved in some national collections, and today only a small number of local varieties are still cultivated (Chkhartishvili, Tsertsvadze, 2004). This causes genetic erosion on this rich ampelographic heritage, involving loss of a valuable gene pool before it could be evaluated.

Furthermore, at present, wild grapevine [*V. vinifera* L. subsp. *sylvestris* (C.C.Gmel.) Hegi] occurs on the territory of Georgia mainly in riparian forests and reaches upper vegetation zones such as oak-hornbeam, beech and spruce forests up to 900 m a.s.l. (Ramishvili, 1988). It is an endangered species in its natural habitat. In recent years, preservation of genetic variability within wild grapevine populations has become a priority, mainly because of increased human activities and the spread of new pests (Grassi *et al.,* 2003). Therefore it is necessary to conserve wild forms of the crop wild relatives for the maintenance of genetic variability and to avoid genetic erosion (Arnold *et al.,* 1998).

Nowadays, attention is paid to elucidate the diversity of the wild grapevine gene pool to identify the place and period of the original domestication and whether secondary independent domestications also occurred (Grassi *et al.,* 2003; Sefc *et al.,* 2003; Arroyo-Garcia *et al*., 2006). The place and time-frame of grapevine domestication is still an open issue, as most investigations focused on West European cultivars and include just few autochthonous varieties from the Caucasus area (Tsvetkov *et al*., 2005; Grassi *et al*., 2006; Imazio *et al.,* 2006; Vouillamoz *et al*., 2006; Walker *et al.,* 2007), most of which are obtained from collections kept abroad in Bulgaria, Russia and Germany and represent commercial modern cultivars. Especially little is known on genetic diversity of wild grape in Georgia. Therefore, it is of high importance to study aboriginal grape varieties in the place of its supposed domestication and to determine genetic relations among native grapevine cultivars and local wild populations.

In this work we use nuclear microsatellite (SSR) markers as a tool for study genetic

relationships among Georgian autochthonous cultivars kept in the national living collections and wild grapevine populations to shed light on the origin of cultivars in this region.

MATERIALS AND METHODS

A total of 102 samples representing 55 accessions were included in this study: 18 Georgian autochthonous and 4 West European reference grapevine cultivars (*Vitis vinifera*), 31 individuals of wild *V. vinifera* subsp. *sylvestris* and two accessions of the American species *V. riparia* Michx. naturalized in Georgia were analyzed at eight microsatellite loci. Wild accessions were collected from nine locations from different regions of Georgia. All individuals within the studied populations of wild grape were identified as dioecious plants with male or female flowers. Names, sources, and distinctive features of the accessions are given in Table 1.

Tab.1 List of cultivated and wild accessions including codes (first two letters of the codes means: CV-cultivar, W-wild grapevine, VRP- *Vitis riparia*, the number belong to accessions and last 4 letters displays name of cultivar or location of wild populations); colour of berries (R-red, W-white), sex of individuals (F-female, M-male), location and source institution name (MSEM-Martvili State Ethnographical Museum collection, Martvili, Georgia; PG-private ground; IHVO- Institute of Horticulture, Viticulture and Oenology Institute, Tbilisi, Georgia; INRA- National Institute of Agricultural Research, Montpellier, France).

Cultivated group				
Code	**Variety and synonymy**	**Color**	**Origin**	**Source**
CV1ALDS	Aladasturi	R	Georgia	IHVO
CV2AVSH	Avshiluri	R	Georgia	IHVO
CV3CHDI	Chodi	R	Georgia	MSEM
CV4CHVT	Chvitiluri	R	Georgia	IHVO
CV5EGRD	Egurdzuli	W	Georgia	MSEM
CV6KCHI	Kachichi	R	Georgia	IHVO
CV7KCHI	Kachichi	R	Unknown	PG
CV8KMRT	Kamuri Tetri	W	Georgia	MSEM
CV9KHJS	Khojishtoli, Kharistvala Kolkhuri	R	Georgia	MSEM
CV10KRKN	Krikina	R	Georgia	PG
CV11OJLS	Ojaleshi	R	Georgia	MSEM
CV12PNSH	Paneshi	R	Georgia	MSEM
CV13RKTS	Rkatsiteli	W	Georgia	IHVO
CV14SPRV	Saperavi	R	Georgia	IHVO
CV15SKHS	Shkhucheshi	W	Georgia	MSEM
CV16SHNR	Shonuri, Svanuri	R	Georgia	MSEM
CV17TVKV	Tavkveri	R	Georgia	IHVO
CV18UCHK	Uchakhardani	R	Georgia	IHVO
CV19MSCT	Muscat Blancs à Petits Grains B	W	Greece	INRA
CV20CHRD	Chardonnay B	W	France	INRA
CV21CBRS	Cabernet-Sauvignon N	R	France	INRA
CV22CBRF	Cabernet Franc N	R	France	INRA

Wild group			
Code	**Population**	**Sex**	**Location**
W1GRDB	Gardabani1	M	Gardabani forest-park, Gardabani distr., Kvemo Kartli, Georgia
W2GRDB	Gardabani2	M	Gardabani forest-park,Gardabani distr., Kvemo Kartli, Georgia
W3GRDB	Gardabani3	M	Gardabani forest-park, Gardabani distr., Kvemo Kartli, Georgia
W4GRDB	Gardabani4	F	Gardabani forest-park, Gardabani distr., Kvemo Kartli, Georgia

W5BRJM	Borjomi1	F	Between vv. Atskuri and Likani, Borjomi distr., Georgia
W6BRJM	Borjomi2	F	Between vv. Atskuri and Likani, Borjomi distr., Georgia
W7BRJM	Borjomi3	F	Between vv. Atskuri and Likani, Borjomi distr., Georgia
W8BRJM	Borjomi4	M	Between vv. Atskuri and Likani, Borjomi distr., Georgia
W9KSPI	Kaspi1	F	Near vil. Sakorintlo, Kaspi distr., Georgia
W10KSPI	Kaspi2	F	Near vil. Sakorintlo, Kaspi distr., Georgia
W11DSHT	Dusheti1	F	Near vil. Zhinvali, Dusheti, Georgia
W12DSHT	Dusheti2	M	Near vil. Ananuri, Dusheti, Georgia
W13DSHT	Dusheti3	F	Near vil. Ananuri, Dusheti, Georgia
W14DSHT	Dusheti4	F	Near vil. Ananuri, Dusheti, Georgia
W15DSHT	Dusheti5	F	Near vil. Meneso, Dusheti, Georgia
W16DSHT	Dusheti6	F	Near vil. Meneso, Dusheti, Georgia
W17TBLS	Tbilisi1	M	Suburb of Tbilisi, Georgia
W18TBLS	Tbilisi2	M	Suburb of Tbilisi, Georgia
W19TBLS	Tbilisi3	M	Suburb of Tbilisi, Georgia
W20IORI	Iori1	M	Iori Nature Preserve, Sagarejo distr., Georgia
W21IORI	Iori2	M	Iori Nature Preserve, Sagarejo distr., Georgia
W22IORI	Iori3	M	Iori Nature Preserve, Sagarejo distr., Georgia
W23JMKR	Jumas Kure1	F	Jumas Kure Nature preserve, Dedoplis Tskaro distr., Georgia
W24JMKR	Jumas Kure2	F	Jumas Kure Nature preserve, Dedoplis Tskaro distr., Georgia
W25AJRA	Ajara1	M	Near vil. Godgadzeebi, Khulo distr., Georgia
W26AJRA	Ajara2	F	Near vil. Godgadzeebi, Khulo distr., Georgia
W27AJRA	Ajara3	F	Near vil. Godgadzeebi, Khulo distr., Georgia
W28AJRA	Ajara4	M	Near vil. Godgadzeebi, Khulo distr., Georgia
W29AJRA	Ajara5	M	Near vil. Godgadzeebi, Khulo distr., Georgia
W30AJRA	Ajara6	M	Near vil. Godgadzeebi, Khulo distr., Georgia
W31SLSA	Slesas Tsikhe	F	Near ruins of Slesas Tsikhe, Akhaltsikhe distr., Georgia
Vitis riparia			
Code		**Sex**	**Location**
VRP1	*Vitis riparia*	F	Near vil. Martvili, Martvili distr., Georgia
VRP2	*Vitis riparia*	F	Near vil. Sakorintlo, Kaspi distr., Georgia

Genomic DNA was extracted with Qiagen DNeasy Plant Mini Kit according to the manual provided by the manufacturer or according to Lodhi *et al.,* (1994) from silica-dried leaves. Eight microsatellite loci, well characterized in previous studies, were used: VVS4 (Thomas, Scott, 1993); VVMD14, VVMD32 (Bowers *et al.,* 1996); scu04vv and scu14vv (Scott *et al.,* 2000) used in studies on *V. vinifera*, and ssrVrZAG21, ssrVrZAG62 and ssrVrZAG83, originally identified in *V. riparia* (Sefc *et al.,* 1999). Some of these markers had been previously selected by the European GENRES consortium as the core set for genotyping grapevine collections. PCR amplification were performed in 10 µl final volume containing about 10 ng template DNA, 200 µM of each dNTP, 0.1 µM of each primer (one primer from each pair was fluorescently labelled), 0.2 mM $MgCl_2$ and 0.2 U TAG DNA polymerase in 1X reaction buffer (QIAGEN). The thermocycler (PE Biosystems, Gene Amp 9700) was programmed for an initial step of 2 min at 94° C followed by 40 cycles at 92° C for 30 s, 50-56° C for 1 min, and 70° C for 2 min, and a final extension step at 72° C for 10 min.

Polymorphisms of the amplified products were detected in an automated DNA sequencer MegaBASE model 1000 (Amersham Biosciences). Fragment lengths were estimated in relation to an internal size standard (Amersham Biosciences). In each run, we have included four reference cultivars, approved by the projects GENRES-081 (2001) and GrapeGen06

(OIV, 2007). They served as standards in order to have consistent allele sizes over all runs and they allowed allele size comparison of our study with other published data.

Genetic structure of studied groups was examined by analysis of molecular variance (AMOVA) using the Arlequin software package (Schneider *et al.,* 2000). The F_{st} coefficient, as a measure of genetic differentiation among samples, was calculated with 1023 random permutations of the distance matrix used to generate a null distribution of correlation coefficients. Data matrix with the results of the analysis was converted in a binary (0 and 1) matrix based on the presence or absence of each specific allele for each studied accession. From this matrix a dendrogram was obtained by applying the UPGMA method with Dice's coefficient in NTSYS (Rohlf, 2000).

RESULTS AND DISCUSSION

Genetic structure of cultivated and wild groups was analyzed using F statistics. The low level of genetic differentiation (F_{st} = 0,05; P <0.0001) indicated between Georgian cultivated and wild grapevines clearly indicating gene flow between wild and Georgian domesticated grapevines and/or that *in situ* domestication of wild germplasm took place within local populations. This means that autochthonous Georgian grape varieties might stem from local wild grapevine. The fact that the three ancient Georgian cultivars 'Krikina', 'Saperavi' and 'Uchakhardani' fall within cluster B (Fig. 1), which mainly contains wild accessions allowed us to suppose that these cultivars were derived from the earliest local domestication events. Moreover, 'Krikina' is considered to be the oldest and an almost half-wild variety, which according to indigenous knowledge of the local population is until today growing near abandoned settlements and from there transplanted to peasants vineyards. 'Saperavi' is a very old Georgian cultivar since, which currently is highly commercialized in Kakheti (Eastern Georgia) for production of the famous red wines Saperavi and Kindzmarauli. 'Uchakhardani' is an old Colchic variety with black berries. The low heterozygosity of aboriginal cultivars obtained in this study might be considered as indication of their close relationship to local wild grapevine. The confirmation of this suggestion might come from three loci showing an observed heterozygosity (H_o=0.77 VVMD14; H_o=0.63 VrZAG83 and H_o=0.63 VVS4) lower than Hardy-Weinberg conditions as expected for closely related cultivars. Similar results were shown in studies of Croatian, Spanish, and Portuguese cultivars (Maletic *et al.,* 1999; Ibáňez *et al.,* 2003; Cunha *et al.,* 2009; Lopes *et al*., 2009).

Another example confirming the genetic linkage between Georgian cultivars and local wild grapevine is placement of the famous Georgian white cultivar 'Rkatsiteli' in cluster C, where it is closely linked to the wild accession WT19KSPI (GS value 0.90; Fig. 1) due to identical alleles at 11 out of 12 alleles. Thus, the hypothesis that this cultivar could have been selected from local wild grape in the Lekhura gorge can be considered.

Cluster C also contains the West European reference cultivars 'Muscat Blancs à Petits Grains B' (CT19MSCT), 'Chardonnay B' (CT20CHRD), 'Cabernet-Sauvignon N' (CT21CBRS), and 'Cabernet Franc N' (CT22CBRF), which clustered with old Georgian cultivated grapes (Fig. 1). This might be considered as indication that these West European and Georgian cultivars had common ancestors, which is consistent with data obtained by Vouillamoz (2006), who showed that some West European cultivars, such as 'Chasselas', 'Nebbiolo', 'Pinot Noir' and 'Syrah' were closely related to a group of exclusively Georgian cultivars. Moreover, the cluster with western European cultivars in this study contains wild grapevine accessions from different regions of Georgia as well, which supports the hypothesis of common ancestry.

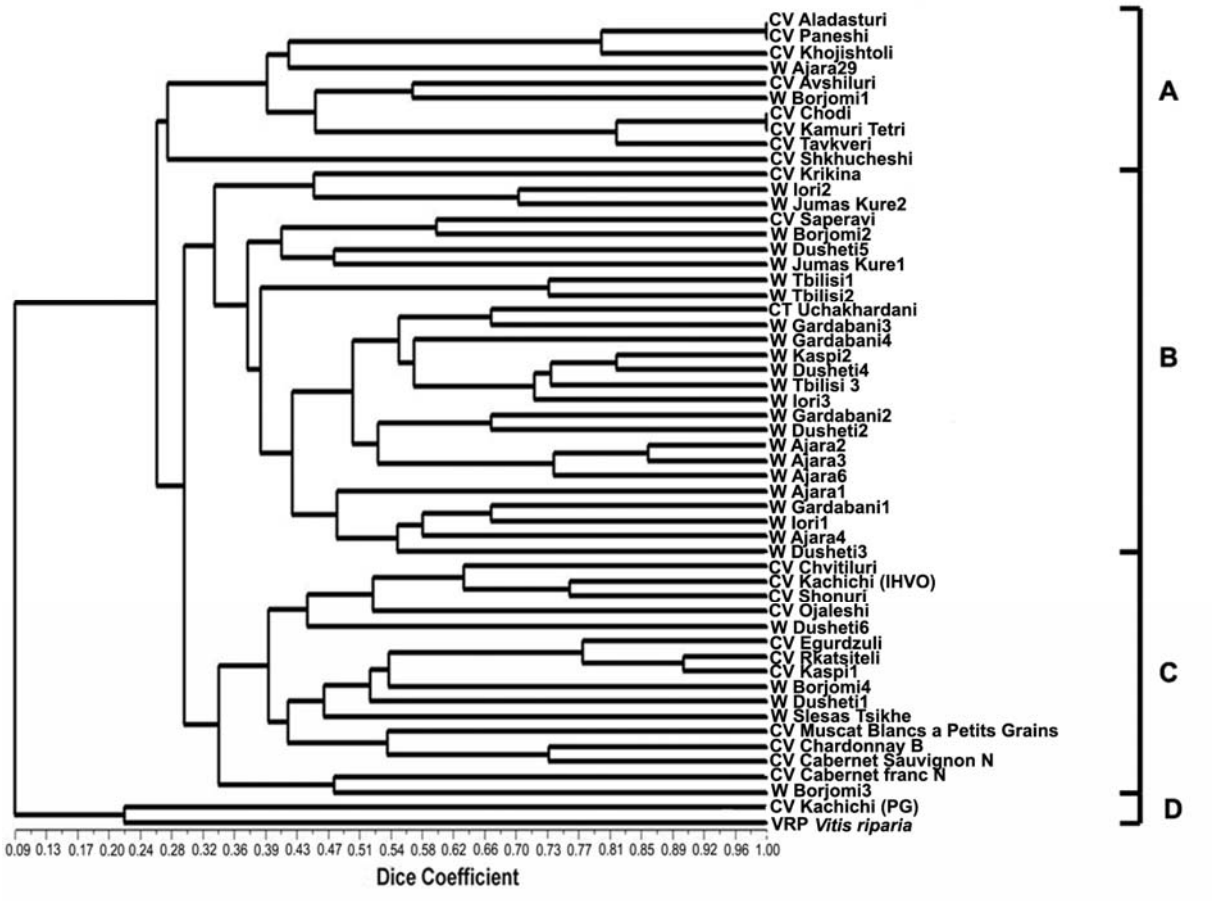

Fig.1 Dendrogram of 18 Georgian and 4 West European cultivars of *V. vinifera*, 31 Georgian wild grapevine accessions and the American species *V. riparia* constructed by Unweighted Pair Group Method with Arithmetic Average cluster analysis based on Dice's coefficient of shared SSR polymorphisms.

CONCLUSION

In conclusion, it should be mentioned that the Georgian cultivated and wild grapevines represent a unique and interesting genetic resource that is characterized by a high similarity level between wild and cultivated grapevines. The admixture found among local Georgian cultivars and wild grapevine indicates the possibility that these cultivars are derived from ancestral domestication of local wild types and/or cross hybridization with native wild populations, thus, either supporting Georgia as one of the oldest centres of domestication of grapevine or at least harbouring valuable genetic resources for grape breeding. However, wild grapevine populations occurring nowadays on the territory of Georgia are threatened by different impacts in their natural habitats and need to be protected. The confirmation of threatened status of the Georgian wild grapevine might be detected low level of heterozygous individuals found for the most of the studied loci, which reflects the isolated status and the reduced number of individuals in the wild populations. Therefore, it is necessary to conserve wild forms and aboriginal cultivars of grape for the maintenance of genetic variability and to avoid genetic erosion of valuable genetic resources for grape breeding in Georgia.

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BIBLIOGRAPHY

Arnold, C., Gillet, F., Cobat, J.M., 1998. Situation de la vigne sauvage (*Vitis vinifera* ssp. *sylvestris)* en Europe. *Vitis* **37**:159-170.

Arroyo-Garcia, R., Ruiz-Garcia, L., Bolling, L., Ocete, R., Lopez, M.A., Arnold, C., Ergul, A., *et al.,* 2006. Multiple origins of cultivated grapevine (*Vitis vinifera* L. ssp. *sativa*) based on chloroplast DNA polymorphisms. *Mol. Ecol.* **15**:3707-3714.

Bowers, J.E., Dangl, G.S., Vignani, R., Meredith, C.P., 1996. Isolation and characterization of new polymorphic simple sequence repeat loci in grape (*Vitis vinifera* L.). *Genome* **39**:628-633.

Chkhartishvili, N., Tsertsvadze, N., 2004. Status of grapevine genetic resources (*Vitis vinifera*) in Georgia. Proc. Int. Meetig "Conservation and use of genetic resources of grapevine in the Caucasus and Northern Black Sea region", 16.10.2003. Tbilisi, Georgia.

Constantini, L., 2004. The archaeobotany of grapevine in the Caucasus and Northern Black Sea region. In: Development of National Programs in Plant Genetic Resources in Southeastern Europe– Conservation of Grapevine in the Caucasus and Northern Black Sea Region». Meeting, Yalta, Ukraine, Institute Vine & Wine Magarach and International Plant Genetic Resources Institute, pp. 73-75.

Cunha, J., Teixeira Santos, M., Carneiro, L.C., Fereiro, P., Eiras-Dias, J., 2009. Portuguese traditional grapevine cultivars and wild vines (*Vitis vinifera* L.) share morphological and genetic traits. *Genet. Res. Crop. Evol.* 56:975-989.

Damania, A.B., Valkoun, J., Willcox, G., Qualset, C.O., 1997. The Origins of Agriculture and Crop Domestication. Proc. Harlan Symp. Aleppo, Syria.

De Candolle, A., 1885. Origine des plantes cultivées. Germer Baillière, Paris.

Ekhvaia, J., Akhalkatsi, M., 2010. Morphological variation and relationships of Georgian populations of *Vitis vinifera* L. ssp. *sylvestris* (C.C. Gmel.) Hegi. *Flora* **205** (in press).

Grassi, F., Labra, M., Imazio, S., Spada, A., Sgorbati, S., Scienza, A., Sala, F., 2003. Evidence of a secondary grapevine domestication centre detected by SSR analysis. *Theor. Appl. Genet.* **107**:1315-1320.

Grassi, F., Labra, M., Imazio, S., Ocete Rubio, R., Failla, O., Scienza, A., Sala, F., 2006. Phylogeographical structure and conservation genetics of wild grapevine. *Cons. Genet.* **7**:837-845.

Ibáñez, J., de Andres, M.T., Molino, A., Borrego, J., 2003. Genetic study of key Spanish grapevine varieties using microsatellite analysis. *Am. J. Enol. Vitic.* 54: 22-30

Imazio, S., Labra, M., Grassi, F., Scienza, A., Failla, O., 2006. Chloroplast microsatellites to investigate the origin of grapevine. *Genet. Res. Crop. Evol.* **53**:1003-1011.

Javakhishvili, I., 1930. Sakartvelos ekonomiuri istoria (Economic History of Georgia). V I, Kartuli Tsigni, Tbilisi (in Georgian).

Ketskhoveli, N., Ramishvili, M., Tabidze, D., 1960. Sakartvelos ampelografia (Ampelography of Georgia). Publish House Acad Sci, Tbilisi (in Georgian).

Lodhi, M.A., Ye, C.N., Weeden, N.F., Reisch, B.I., 1994. A simple and efficient method for DNA extraction from grapevine cultivars and *Vitis* species. *Plant Mol. Biol. Rep.* **12**:6-13.

Lopes, M.S., Mendonça, D., Rodrigues dos Santos, M., Eiras-Dias, J.E., da Cămara Machado, A., 2009. New insights on the genetic basis of Portuguese grapevine and on grapevine domestication. *Genome* 52: 790-800

Maletic, E., Sefc, K.M., Stenkellner, H., Kontic, J.K., Pejic, I., 1999. Genetic characterization of Croatian grapevine cultivars and detection of synonymous cultivars in neighbouring regions. *Vitis* 38: 79-83

McGovern, P.E. 2003. Ancient Wine. Princeton University Press, Princeton.

Negrul, A.M., 1946. Semeistvo Vitaceae Linde. (Family Vitaceae Linde). In: Ampelography of the USSR, v I, Pish. Promish., Moscow, pp. 45-63 (in Russian).

Ramishvili, M., 1970. Ampelography. Ganatleba, Tbilisi (in Georgian).

Ramishvili, R., 1988. Dikorastushii vinograd Zakavkazia (Wild grape of the South Caucasus). Ganatleba, Tbilisi (in Russian).

Rohlf, F.J., 2000. NTSYS-PC Numerical Taxonomy and Multivariate Analysis System, version 2.11a. Exeter Publications, Setauket, New-York.

Sefc, K.M., Steinkellner, H., Glössl, H., Kampfer, S., Regner, F. 1999. Reconstruction of a grapevine pedigree by microsatellite analysis. *Theor. Appl. Genet.* **97**:227-231.

Sefc, K.M., Steinkellner, H., Lefort. F., Botta, R., da Câmara Machado, A., Borrego. J., Maletić, E., Glössl. J., 2003. Evaluation of the genetic contribution of local wild vines to European grapevine cultivars. *Am. J. Enol. Vitic.* **54**:15-21.

Schneider, S., Roessli, D., Excoffier, L., 2000. Arlequin ver. 2.000. A software for population genetic data analysis. Genetics and Biometry Laboratory, University of Geneva, Switzerland.

Scot, K.D., Eggler, P., Seaton, G., Rosseto, M., Ablett, E.M., Lec, L.S., Henry, R.J., 2000. Analysis of SSR derived from grape ESTs. *Theor. Appl. Genet.* **100**:723-726.

This, P., Lacombe, T., Thomas, M.R., 2006. Historical origins and genetic diversity of wine grapes. *Trends Genet*. **22**: 511-519.

Thomas, M.R., Scott, N.S. 1993., Microsatellite repeats in grapevine reveal DNA polymorphisms when analyzed as sequence-tagged sites (STSs). *Theor. Appl. Genet*. **86**:985-990.

Tsertsvadze, N., 1989. Sakartvelos kulturuli vazis klasifikatsia (Classification of Georgian cultivated grapevine). Sabchota Sakartvelo, Tbilisi (in Georgian).

Tsvetkov, J., Atanasov, A., Jankulova, M., Vulchev, V., Todotov, J., Pandeliev, S., Katerov, K., Atanasov, A., Shishmanov, G., Ivanova, N., Laginova, M., 2005. Grapevine plant genetic resources – improvement, preservation and plan biodiversity. *Biotechnol. & Biotechnol. Eq.* **19**:13-21.

Vavilov, N.I., 1931. Dikie rodichi plodovykh dereviev Aziatskoi chasti SSSR i Kavkaza i problema proiskhozhdenia plodovykh dereviev (Wild progenitors of the fruit trees of Turkestan and the Caucasus and the problem of the origin of fruit trees). *Bull. Appl. Bot., Genet., Plant-Breed.* **26**:85-134 (in Russian).

Vouillamoz, J.F., McGovern, P.E., Ergul, A., Söylemezoğlu, G., Tevzadze, G., Meredith, C.P., Stella Grando, M., 2006. Genetic characterization and relationships of traditional grape cultivars from Transcaucasia and Anatolia. *Plant Genet. Res.* **4**:144-158.

Walker, A. R., Lee, E., Bogs, J., McDavid, D.A.J., Thomas, M.R., Robinson, S.P., 2007. White grapes arose through the mutation of two similar and adjacent regulatory genes. *Plant J.* **49**:772-785.

Zohary, D., Hopf, M., 2000. Domestication of Plants in the Old World. Oxford University Press, Oxford, UK.

RICERCHE VOLTE A SALVAGUARDARE IL PROFITTO PER L'IMPRESA VITICOLA PUR CONSERVANDO O AUMENTANDO I LIVELLI OCCUPAZIONALI E L' IMPIEGO DI RISORSE.

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RIASSUNTO

Queste articolate ricerche sono state condotte sui modelli produttivi globali molto espansi (ad esempio:Raggi, Pergole, Tendoni), mediamente espansi (ad esempio:Sylvoz, Casarsa, NiofCasarsa), poco espansi (ad esempio: Guyot , Cordon de Royat, Cordone Speronato di Conegliano) ed in questo lavoro verranno esposti solo i risultati ottenuti sulla Pergola di Soave Storica, sul Sylvoz, sul Guyot e sul Cordon de Royat. Dalle ricerche è emersa la possibilità di salvaguardare o addirittura aumentare il "profitto per l' impresa" pur nel contempo mantenendo o addirittura aumentando i livelli occupazionali e l'impiego di risorse impiegando la "Pergola di Soave Storica" anziché il Sylvoz, il Guyot e soprattutto il Cordon de Royat pur avendo, quest'ultimo dato la più alta qualità enochimica dell'uva ed organolettica classica del vino.Queste ricerche in questo particolare momento storico della realtà locale nazionale europea e soprattutto internazionale sono risultate di grande attualità ed interesse tecnico, socio-economico ed etico.
Parole chiave: viticoltura, sostenibile, solidale, equo, etica

GUARANTEEING FARM PROFIT WHILE MAINTAINING OR INCREASING BOTH PERSONNEL AND RESOURCE USE

SUMMARY

Dhis research was conducted on the training systems that follow: highly expansive (ex. Raggi, Pergole, Tendoni), expansive (Sylvoz, Casarsa, NiofCasarsa) and mildly expansive (Guyot , Cordon de Royat, Cordone Speronato di Conegliano).

We present the results on the trained to Pergola di Soave Storica, Sylvoz, Guyot and Cordon de Royat. The results reveal that is possible to preserve or increase profit while maintaining or increasing personnel and the use of resources by adopting the Pergola di Soave Storica trening system instead of Sylvoz or Guyot or Cordon de Royat, even though the latter yields enologically superior grapes.

Results are interesting from a technical, socio-economic and ethical point of view, especially given the current historical moment at the local, national, European, and international level.
Key word: sustainable, solidarity, fair, ethics, viticultur, wine, grape growing

INTRODUZIONE

Nel lavoro presentato al Convegno GiESCO di Davis- 2009 (California-USA) dal titolo : "Great Chain: urgent necessity the focusing on the "MetaEthical" viticulture or "Great" viticulture: research and various considerations (Cargnello G. 2009), scrivevo che molto, molto tempo fa: 1- sono passato da una "forma mentis" e da un' attività scientifica settoriale, nonché svolta solo per risolvere problemi strettamente tecnici ("PICCOLA" FILIERA) senza collegare queste ricerche agli obiettivi per i quali si fa in questo caso specifico viticoltura ad una indispensabile "forma mentis"

ed attività più interdisciplinare, più interattiva, più innovativa, “più valida” in quanto condotta, (come sempre, alla fine, dovrebbero essere condotta), collegando le ricerche sui mezzi (es. costi, prezzi, quantità unitarie delle produzioni, “qualità” e preferenze varie, mano d’opera, meccanizzazione, studio degli ambienti, “zonazione”, delimitazione delle zone, forme d’allevamento, gestioni: del terroir (clima e terreno) e delle unità di base di territoir (territorio) e non terroir: viticolo, enologico, economico, socio-ambientale, esistenziale, metaetico, del paesaggio, del vigneto, della pianta (radici, fusto, branche, tralci, chioma), della produzione, degli zuccheri dell’uva, dei polifenoli, degli aromi, degli stress, ecc., alcool e salute, vino e salute, vino-acqua e salute, spritz, ecc.), agli obiettivi. Ai veri obiettivi (“Grandi” Obiettivi secondo la “Grande” Filiera (Gruppo Internazionale Etico e “MetaEtico” di Conegliano 2004) della nostra attività, e non solo di questa, i quali dal basso verso l’alto sono rappresentati dalla "qualità" o "profitto": economico, ambientale, sociale, esistenziale, etico in modo sostenibile solidale ed equo per tutto e per tutti: "qualità" o "profitto" “MetaEtico” seguendo la “GRANDE” FILIERA, cioè al di fuori e al di sopra delle varie etiche e quindi al di fuori e al di sopra delle varie inaccettabili realtà settorialmente corporative di “potere” siano esse confessionali e non confessionali, sociali, politiche, partitiche, economiche, ambientali, scientifiche, tecniche, ecc., anche perché nessuno, e neanche l’uomo, ha il diritto di prevaricare sugli altri fattori della “Grande Filiera” (Gruppo Internazionale Etico e “MetaEtico” di Conegliano 2004 lc; Cargnello G. 1986, 1994, 1996, 1999, 2005, 2006a-b, 2007, 2008a-b-c-d, 2009a-b-c, 2010a- b-c; Cargnello G. e col., 1988, 1999, 2009, 2°10a- b; Cargnello G. et Carbonneau A., 2007; Boatto V., e col., 2009; Carbonneau A. et col., 2008, 20010).

E sin dall’inizio su questo modo di lavorare, di fare ricerca, di fare viticoltura, di fare didattica, ecc. illustri personaggi, non solo italiani, relativi al mondo etico, morale, filosofico, politico, amministrativo, sociale, economico, imprenditoriale, scientifico, tecnico, coinvolti da sempre in queste “ricerche” hanno dato pienamente la loro condivisione e collaborazione, ed il nostro contributo in tal senso è ampiamente noto, storico ed intensificato nel tempo (Lc: Gruppo Internazionale Etico e “MetaEtico” di Conegliano 2004; Cargnello G.; Cargnello G. e col.; Cargnello G. et Carbonneau A.; Boatto V., e col.; Carbonneau A. et col.).

Colgo l’occasione di questo importante “Congresso Mondiale OIV”, per onorare l’insistente invito rivoltomi da parte di stimati colleghi di dar seguito, in modo ancor più incisivo rispetto al passato a quanto sto scrivendo da molto tempo (Lc: Cargnello G.; Cargnello G. e col.; Cargnello G. et Carbonneau A.; Boatto V., e col.; Carbonneau A. et col.) ai quali lavori si rimanda nei quali si diceva e si portavano esempi sulla necessità di salvaguardare certamente il “profitto per l’impresa” ma nel contempo di aumentare l’ impiego di risorse, compresa la mano d’opera.

Ed in questo lavoro si vuole esporre i risultati di una ricerca condotta in tal senso.

A tale proposito, in epoche non sospette ed ancor molto prima previste dalla nostra metodologia della “Grande” Filiera, abbiamo condotto ricerche che dimostrano quanto auspicato purtroppo solo recentemente da tutti, compresi famosi economisti, politici e famosi colleghi con le note drammatiche ripercussioni mondiali economiche, sociali ed esistenziali agli occhi di tutti, cioè: 1- sin dal 1986 e successivamente giustamente invertendo subito le finalità delle nostre precedenti ricerche, abbiamo scritto che più che contenere i costi dovevamo e dobbiamo cercare di aumentare il profitto quanto meno d’impresa intervenendo in modo particolare sulla “Produzione Lorda Vendibile” (PLV) agendo, nel caso dei vini e non solo di questi, non sulla qualità sensoriale classica ma su quella economica, e meglio ancora su quella socio-economica-etica (Lc:Cargnello G. 1986, 1994, 1996, 1999, 2005, 2006a-b, 2007, 2008a-b-c-d, 2009a-b-c, 2010a- b-c), 2- sin dal 1986 e successivamente e soprattutto dal 1992 la nostra metodologia di base secondo la “Grande Filiera” prevedeva e prevede: più un modello produttivo impiegava ed impiega risorse, compresa la manodopera, più tale modello produttivo veniva e viene giudicato positivamente, beninteso qualora venga salvaguardato un “equo” profitto per l’impresa, 3- sin dal 1986 e successivamente scrivevamo che “non” dovevamo e “non” dobbiamo puntare ad aumentare la qualità zuccherina dell’uva e quella organolettica classica del vino, ma quanto meno la qualità percepita

dall'acquirente e la cosi detta da noi "Qualità Salutistica" (Salute: dell'uomo, dell'ambiente, del portafoglio, dei punti della patente, ecc., e perché no quella dell'"anima") e meglio ancora quella socio-economica-etica. (Lc:Cargnello G. 1986, 1994, 1996, 1999, 2005, 2006a-b, 2007, 2008a-b-c-d, 2009a-b-c, 2010a- b-c),

E scrivevamo allora che l'aumento dello zucchero dell'uva, della qualità organolettica classica del vino, il contenimento dei costi hanno ragione di essere solo se questi mezzi permettono di migliorare, quanto meno la qualità o il profitto economico, meglio se anche quello sociale, ambientale, esistenziale e soprattutto quello "MetaEtico" come previsto della "Grande Filiera" e pertanto la "diminuzione" dello zucchero dell'uva, della qualità organolettica classica del vino e l'aumento dei costi anche attraverso l'impiego di maggiore manodopera possono addirittura far giudicare positivamente, secondo la "Grande Filiera", il modello produttivo che li determina, e si risottolinea: beninteso qualora venga salvaguardato un "equo" profitto per l'impresa. E si proseguiva illustrando che più della maturità zuccherina dell'uva ci interessava e ci interessa quella salutistica, quella fenolica, quella aromatica, quella acida, ecc.

Inoltre in questo lavoro si vorrebbe apportare un contributo per dimostrare l'urgente necessità: 1- di distinguere adeguatamente i mezzi dagli obiettivi impiegati per raggiungere pienamente i veri obiettivi, ("Grandi Obiettivi" secondo la "Grande Filiera"), della nostra attività, e non solo di questa, 2- di collegare necessariamente i mezzi agli obiettivi, 3- di andare urgentemente e in filiera ("Grande Filiera") oltre la "qualità o profitto" tecnico, economico ed a quello relativo al paesaggio puntando urgentemente e necessariamente su quell'attività che permetta di concretare in tempo utile una VITICOLTURA "METAETICA" o "GRANDE VITICOLTURA" secondo la "Grande Filiera", la quale, come noto, rappresenta il massimo, il meglio, il top, la meta migliore.

MATERIALI E METODI

Queste ricerche inedite sono state condotte nella zona dei vini "Soave" (Verona) (I) per un quindicennio (dal 1973 al 1987), riprese dal 1993 al 1998 ed aggiornate al 2009 su viti di *cv Garganega* in piena produzione ed allevate con i modelli produttivi "Pargola di Soave Storica" (PSS) (Cargnello G., 1978, 1986; Carbonneau A, et Cargnello G., 2003), "Sylvoz", "Guyot" e "Cordon de Royat" (Carbonneau A, et Cargnello G., 2003 lc). Queste ricerche si riferiscono a modelli e a situazioni ordinarie medie così come per i dati riportati i quali sono stati aggiornati al 2009. Per le metodologie e quant'altro, anche per motivi di spazio, si rimanda alla bibliografia dell'autore ed in particolare a Lc: Cargnello G., 1986, 1988, 1996, 1999, 2005, 2006a-b, 2007, 2008a-b-c-d, 2009a-b-c, 2010a- b-c; Cargnello G. et Carbonneau A., 2007.

RISULTATI E DISCUSSIONI

Risultati (Tabella A): rispettivamente nei modelli produttivi "Pergola di Soave Storica" Sylvoz, Guyot e Cordone speronato di Royat: produzione di uva (t/ettaro) 35,7a (1), 24.9b, 16.8c, 8.3d; zucchero contenuto nell'uva (Brix): 15.9d, 16.4c, 17.3b, 19.1a; produzione di zucchero (t/ettaro): 5.676a, 4.084b, 2.906c, 1.585d; analisi sensoriale del vino (max. 100 punti): 74c, 78c, 85b, 96a; mano d'opera impiegata (ore/anno/ettaro): 525, 457, 398, 221; costo totale di produzione dell'uva (€/ettaro): 10160, 8445, 9285, 9086 e €/t di uva prodotta: 284.6, 339.2, 552.7, 1094.7; prezzo dell'uva (€/t): 335, 359, 510, 749; prodotto lordo vendibile (PLV) (€/ettaro): 11960, 8939, 8568, 6217; profitto d'impresa (€/ettaro): +1800, +494, -717, -2869; profitto d'impresa (€/t di uva prodotta): +50.41, +19.48, -42.68, -345.70; "Grande Filiera": "qualità o profitto (max. 100 punti): imprenditoriale 89a, 68b, 49c, 38d; paesistico: 87a, 67c, 79b, 75b; ambientale: 52d, 73c, 91a, 84b; sociale: 78a, 67b, 62c, 60d; esistenziale: 76a, 71b, 68b, 59c; "MetaEtico": 79a, 69b, 62c, 56d.

Table A: 1- PRODUCTION (A- t /104 m² OF GRAPE, B- t /104 m² OF SUGAR), 2- SOLUBLE SOLID (°BRIX), 3- WINE SENSORIAL ANALYSES (MAX. 100), 4- HUMAN LABOR (hours/ years/ 10^4 m²), 5- TOTAL PRODUCTION GRAPE COSTS (A- € /10^4 m², B- € /t of PRODUCED GRAPE), 6- GRAPE PRICE (€ /t), 7- TOTAL OUTPUT (€/10^4 m²), 8- FARM NET/INCOME/ENTREPRISE PROFIT (A- € /10^4 m², B- € /t of PRODUCED GRAPE), 9- "GRANDE FILIERA" ("GREAT CHAIN") ASPECTS: A- ENTREPRENEURIAL, B- LANDSCAPE, C- ENVIRONMENTAL, D- SOCIAL, E- EXISTENTIAL AND F- "METAETHICAL" (MAX. 100) IN FOUR PRODUCTIVE MODELS.

RELIEF / PRODUCTIVE MODELS	PERGOLA DI SOAVE	SYLVOZ	GUYOT	ROYAT SPUR CORDON
1 - PRODUCTION (t/10^4 m^2)				
A - GRAPE	35,7 a*	24,9 b	16,8 c	8,3 d
B - SUGAR	5,676 a	4,084 b	2,906 c	1,585 d
2 - SOLUBLE SOLID (°BRIX)	15,9 d	16,4 c	17,3 b	19,1 a
3 - WINE SENSORIAL ANALYSES (MAX. 100)	74 c	78 c	85 b	96 a
4 - HUMAN LABOR (h/y/10^4 m^2)	525	457	398	221
5 - TOTAL PRODUCTION GRAPE COSTS				
A - € /10^4 m^2	10160	8445	9285	9086
B - € /t OF PRODUCED GRAPE	284,6	339,2	552,7	1094,7
6 - GRAPE PRICE (€ /t)	335	359	510	749
7 - TOTAL OUTPUT (€ /10^4 m^2)	11960	8939	8568	6217
8 - ENTREPRISE PROFIT				
A - € /10^4 m^2	1800	494	-717	-2869
B - € /t OF PRODUCED GRAPE	50,41	19,84	-42,68	-345,70
9 - "GREAT CHAIN" (G.F.) (MAX. 100)				
A - ENTREPRENEURIAL	89 a	68 b	49 c	38 d
B - LANDSCAPE	87 a	67 c	79 b	75 b
C - ENVIRONMENTAL	52 d	73 c	91 a	84 b
D - SOCIAL	78 a	67 b	62 c	60 d
E - EXISTENTIAL	76 a	71 b	68 b	59 c
F - "METAEHTICAL"	79 a	69 b	62 c	56 d

* - Treatments with no letter in common differ significantly at the p= 0,05 significance level. **BY CARGNELLO G. et al. 2010**

Nell'ambito della ricerca essenzialmente emerge nella "Pargola di Soave Storica" (PSS) rispetto al "Sylvoz", al "Guyot" e al "Cordon de Royat": 1- il modello produttivo "Pergola di Soave Storica" è quello che pur impiegando più risorse (+20%, +9%, +12% rispettivamente rispetto al "Sylvoz", al "Guyot" e al "Cordon de Royat") e mano d'opera (+15%, +32%, +138% sempre rispetto al "Sylvoz", al "Guyot" e al "Cordon de Royat") per unità di superficie ha determinato il maggior profitto per l'impresa per ettaro (+73%, +140%, +229% sempre rispetto al "Sylvoz", al "Guyot" e al "Cordon de Royat") e per tonnellata di uva prodotta (+61%, +185%, +786% sempre rispetto al "Sylvoz", al "Guyot" e al "Cordon de Royat"), 2- il modello produttivo Cordon de Royat è quello che pur avendo dato la maggiore qualità zuccherina del mosto (19.1 Brix, cioè +3.2, +2.7, +1.8 gradi Brix di differenza rispetto alla "Pergola di Soave Storica", al Sylvoz e al Guyot) e la migliore qualità sensoriale del vino (sottolineiamo: sono mezzi) (96 punti su 100 rispetto a 74, 78, 85 dalla "Pergola di Soave Storica", del Sylvoz e del Guyot) ha fornito il minor (molto negativo) profitto (sottolineiamo: è l' obiettivo più in basso della "Grande Filiera") per l'impresa ad ettaro (-2869 € rispetto a +1800, +494, -717 dalla "Pergola di Soave Storica", del Sylvoz e del Guyot) e per tonnellata di uva prodotta (-346 € rispetto a +50, +20, -43 dalla "Pergola di Soave Storica", del Sylvoz e del Guyot), 3- secondo la "Grande Filiera" il modello produttivo Pergola di Soave Storica rispetto al Sylvoz, Guyot e Cordon de Royat è quello che ha determinato la minor "qualità o profitto" ambientale (solo 52 punti/100 rispetto a 73, 91 e 84 del Sylvoz, del Guyot e del "Cordon de Royat"), ma ha determinato il maggior "profitto" paesaggistico (87 punti/100 rispetto ai 67, 79 e 75 del Sylvoz, del Guyot e del "Cordon de Royat") imprenditoriale (89 punti/100 rispetto ai 68, 49 e 38 sempre del Sylvoz, del Guyot e del "Cordon de Royat"), sociale (78 punti/100 rispetto ai 67, 62 e 60 sempre del Sylvoz, del Guyot e del "Cordon de Royat"), esistenziale (76 punti/100 rispetto ai 71, 68 e 59 sempre del Sylvoz, del Guyot e del "Cordon de Royat") e soprattutto quello

"MetaEtico" (79 punti/100 rispetto ai 69, 62 e 56 sempre del Sylvoz, del Guyot e del "Cordon de Royat").
Beninteso per l'impresa viticola trasformatrice, ma anche per quella non trasformatrice, questi positivi risultati, e non solo questi, potrebbero addirittura aumentare considerevolmente, ma anche capovolgersi completamente in funzione della realtà operativa dell'impresa e in particolare relativamente, ad esempio, al grado di creatività e soprattutto di valorizzazione delle qualità peculiari specifiche ed aggiuntive a quella tradizionale possedute da tale impresa.
Pertanto per progettare, realizzare, condurre, giudicare, scegliere, valorizzare esaustivamente un modello produttivo, e non solo un modello produttivo ma qualsiasi altra cosa ed attività, compresa la nostra, e per evitare non esaustive e/o addirittura errate impostazioni delle ricerche e/o interpretazione e/o comunicazione dei suoi risultati, con ripercussioni applicative anche catastrofiche, bisogna necessariamente andare certamente oltre, ad esempio, la "qualità o profitto" tecnico (risottolineiamo: rappresenta un mezzo), ma anche oltre alla "qualità o profitto" economico classico (risottolineiamo: rappresenta l'obiettivo più in basso della scala della "Grande Filiera"), ma anche, come lo documentiamo da molto tempo, di considerare tutti gli aspetti socio-ambientali esistenziali etici in modo sostenibile solidale ed equo per tutto e per tutti e quindi in modo "MetaEtico", come esposto nella premessa.
Ed allora per fare **V**iticoltura, (con la V maiuscola), dobbiamo puntare immediatamente e senza indugi e inaccettabili scuse, quanto meno a livello di impostazione mentale e di base, sulla "Metaetica" o "Grande Etica", sulla "Viticoltura Metaetica" o "Grande Viticoltura" secondo la "Grande Filiera".
Quindi si risottolinea che è quanto meno auspicabile non fermarci solo alla qualità o profitto tecnico (mezzo) o a quello dell'imprenditore, del paesaggio, del biologico, ecc., ma dobbiamo necessariamente e urgentemente andare oltre ad essi puntando sulla "VITICOLTURA METAETICA" o "GRANDE VITICOLTURA" secondo la "GRANDE FILIERA".
Questi risultati confermano quelli pubblicati in un' altro lavoro sul modello produttivo "Bellussi" (Cargnello G., 2009) e con gli eventuali distinguo possono essere estesi a tutte le altre similari situazioni e quindi non necessariamente espanse viticolture italiane, impostate ad esempio sui Tendoni, su altre Pergole, su altri modelli a Raggi, sui GDC-Casarsa, sui GDC- NiofCasarsa, sul "Cordone Tridimensionale di Conegliano", sui modelli che sviluppano molti metri di fasce e/o di "cordoni" lineari o meclio cubici per ettaro determinati o da fasce e/o da "cordoni" divisi e/o sovrapposti, da sesti di impianto stretti, ecc. tempo (Lc: Cargnello G.; Cargnello G. e col.; Cargnello G. et Carbonneau A.; Boatto V., e col.; Carbonneau A. et col.).
Pertanto al fine di apportare un contributo per disporre di modelli di viticoltura che possano sommare in se "tutti" i pregi senza i difetti del Bellussi ed in questo caso specifico della Pergola di Soave Storica, del Sylvoz, del Guyot, del Cordon de Royat e di altri "tradizionali" e non "tradizionali" modelli produttivi e quindi nella globalità migliorativi rispetto a tali attuali modelli produttivi e per cercare di avvicinarsi il più possibile alla "Viticoltura MetaEtica" o "Grande Viticoltura", sono in corso interessanti ricerche su promettenti "nuovi" modelli produttivi globali quali il Sylvoz Moderno, il Macon Moderno, il NiofCasarsa , il Cordone Speronato di Conegliano e soprattutto sui "Vertical Tridimensional Minimal Pruning" (VTMP). (Cargnello G. 2007; 2008; 2009; 2010).
(1) I dati contrassegnati con la stessa lettera non sono diversi tra loro, mentre quelli contrassegnati con lettere diverse sono diversi tra loro per $p \leq 0,05$ al test di Duncan.

CONCLUSIONI

Da questo lavoro, le cui radici risalgono a metà degli anni settanta, eseguito nel soavese sulla cv Garganega e condotte utilizzando i modelli produttivi Pergola di Soave Storica, Sylvoz, Guyot e Cordone "speronato" di Royat essenzialmente è emerso: 1- la possibilità di aumentare il profitto per l'impresa pur aumentando l' impiego di risorse, compresa la mano d'opera, 2- il modello produttivo che ha dato la maggiore "qualità" (qualità zuccherina) del mosto ed organolettica del vino (mezzi)

ha fornito il minor (molto negativo) profitto per l'impresa (obiettivo), 3 – il modello produttivo che ha fornito il più positivo profitto d'impresa (molto elevato), ha fatto registrare i più negativo profitto "ambientale", ma ha determinato il maggior "profitto" paesaggistico, imprenditoriale, sociale, esistenziale e soprattutto "MetaEtico" secondo la "Grande Filiera", 4- la necessità inderogabile: a) di adeguati attuali approfondimenti per quanto riguarda la semantica, il vero "senso" ed importanza da attribuire nella scala dei valori, dei "Grandi Valori secondo la"Grande Filiera"", anche in prospettiva ad esempio ai vari mezzi, [es. costi, prezzi, quantità unitarie delle produzioni, qualità e preferenze varie: es. complessiva per il produttore, il tecnico, il ricercatore, l' ambiente, il territorio, l'acquirente, il consumatore ecc., ad es. a: mano d'opera, "consumo" ed "investimento" di suolo, di territorio, di terra, di universo, ad es: meccanizzazione, studio degli ambienti, "zonazione", delimitazione delle zone, forme di allevamento, gestioni: del terroir (o "unità de terroir de base") e delle unità di base di territoir (territorio) e non terroir: viticolo, enologico, economico, socio-ambientale, esistenziale, "MetaEtico", del paesaggio, del vigneto, della pianta (radici, fusto, branche, tralci, chioma), della produzione, degli zuccheri dell'uva, dei polifenoli, degli aromi, dell' acidità, degli stress, ecc., ad es: alcool e salute, vino e salute, vino e acqua e salute, acqua e vino e salute, spritz storico, ecc.], ed agli obiettivi ("Grandi Obiettivi secondo la"Grande Filiera" rappresentati in filiera dall'alto verso il basso dalla qualità o profitto "MetaEtico", da quello esistenziale, da quello socio-ambientale e da quello imprenditoriale), b) di un necessario adeguato collegamento tra gli obiettivi ed i mezzi utilizzati per raggiungere al meglio gli obiettivi ("Grandi Obiettivi), 5 – l'interesse di ricerche su "nuovi-vecchi" modelli produttivi che meglio della Pergola di Soave Storica, del Sylvoz, del Guyot e del Cordon de Royat possano avvicinarsi in modo dinamico al modello ideale relativo alla così detta "Grande Viticoltura", o "Viticoltura MetaEtica" secondo la"Grande Filiera", 6- dulcis in fundo l'urgente necessità di andare oltre alla "qualità o profitto" tecnico, economico, paesaggistico e di considerare anche quello "ambientale", quello sociale, quello esistenziale, quello etico in modo sostenibile, solidale ed equa per tutto e per tutti e quindi in modo "MetaEtico" secondo la "Grande Filiera".

E tutto ciò va inquadrato relativamente alla così detta piramide dell'acquirente, del consumatore, del mercato e non a quella della qualità classica tanto di moda inaccettabile, quindi orientando la produzione in modo diverso rispetto al passato spostandola in modo equilibrato dal prodotto al mercato.

Pertanto relativamente agli aspetti sopra esposti, all' attività di ricerca ed a questa attività, e non solo, per progettare, realizzare, condurre, valutare e valorizzare al meglio ogni cosa, attività e ricerca riteniamo opportuno ed inderogabile, (guadagnando urgentemente il tempo perso), si debba operare in modo "diverso" dal passato mirando sin dall'inizio ed in filiera ("Grande Filiera") al raggiungimento dell'obiettivo massimo, della "meta massima" ed in modo tale da concretare un'attività sostenibile non solo dal punto di vista tecnico (mezzo), ma anche e soprattutto ed in filiera dal punto di vista economico, ambientale, sociale, esistenziale, etico (obiettivi) in modo solidale ed equo per tutto e per tutti ("MetaEtico" secondo la "Grande Filiera") e questo, nel nostro caso specifico, per realizzare pienamente una "Viticoltura MetaEtica" o "Grande Viticoltura".

BIBLIOGRAFIA

CARGNELLO G. (1986): Recherches de nouveaux systèmes de conduite pour une viticulture économique et de qualité dans le Nord de l'Italie. Thèse de Doctorat. ENSAM - Montpellier (France), pp. 1-332.

CARGNELLO G., FERRARIS A., FORNO S. (1988): Costi di produzione e di allevamento della vite. Terra e Vita, 42, pp. 66-72. CARGNELLO G. (1992): Economic and social aspects concerning new training and viticulture systems in "cold" italian environments. Acts 3rd International Cool Climate Symposium, F.A. Geisenheim, Mainz (Germany), 12-14/06, pp 1-6.

CARGNELLO G. (1994): Social and economic aspects concerning new models of an increasingly profitable viticulture in researches on "global reasoned viticulture". XXIV International Horticultural Congress, Kyoto (Japan). pp 1-10.

CARGNELLO G. (1996): La qualité économique, l'économie de la qualité et la qualité économique des préférences: différentes considérations. Compte-rendu n° 9 GESCO, Budapest (Hongrie), 21-23 Août, pp. 379-384.
CARGNELLO G. (1999): Evaluation "globale" de processus productifs, d'aspects techniques et scientifiques divers, etc ... : considérations générales relatives à la grande filière viti-vinicole. GESCO Sicilie, Italie, 6-12 June, pp. 362-371.
CARGNELLO G. avec la collaboration de Luciano Pezza et de Teresa Camatta. (2003): Evaluation globale (technique, scientifique, économique, sociale, existentielle, qualité de la vie...etc.) des modèles productifs: aspects méthodologiques actuels. Compte Rendu n° 13 GESCO Montevideo (Uruguay), 3-8 febrero, pp. 38-40.
ATTI:Gruppo Internazionale Etico e "MetaEtico" di Conegliano
CARGNELLO G. (2004): Modelli vitivinicoli "globali" per produrre in Lazio vini tipici originali innovativi di qualità economica sociale esistenziale nonché di lettura, di promozione e di sviluppo del territorio: aspetti viticoli. Atti del convegno su "La ricerca viticola ed enologica: opportunità di sviluppo e promozione del territorio", Velletri 17 dicembre, pp. 24-3.
CARGNELLO G.. (2005): Les "qualités" et la viticulture éthique équitable soutenable solidaire. Recherches, considérations diverses et applications pratiques. Proceedings Vol. 2 XIVth International Symposium GESCO, Geisenheim, 23-27 August, pp. 787-798.
CARGNELLO G. (2006a): "Ethical Wine e Food e Farming e Living...": ricerche pluriannuali e considerazioni varie. Taste Vin, 2, pp. 15-18.
CARGNELLO G. (2006b): Ricerche e riflessioni varie su quale/i viticoltura/e adottare e su quale/i indice/i impiegare per una sua/loro valutazione settoriale e/o globale tecnica economica sociale esistenziale etica. I° Convegno Nazionale di Viticoltura, Ancona 21-23 Giugno, pp. 1-18.
CARGNELLO G. et CARBONNEAU A. (2007): Méthode de la "Grande Filiére" appliquée au management d'un modéle productif de vignoble. Proceedings XVth International Symposium GESCO Porec - Croatia 20-23 june, pp. 16-33.
CARGNELLO G. con la collaborazione di: Pezza l., Gallo G. E di Forno S., Dalla Torre D., Dassiè M., Da Re G., Spera G., La Torre Anna, Lot S., Lovat L., Casadei G., Celotti Cesarina, Falsetti F., Moretti Simonetta, Garofalo A., Persuric G., Milotic A., Sladonja Barbara, Brscic Kristina, Fiorilo Monica, Tempesta G.F., Rugini E., Biasi Rita, Monarca D. (2008): Mechanization and management of viticulture and of territory: various "innovations" for making universally sustainable the development of viticulture and territory. Proceeding: Seminar 20-22 april, University of Yangling, China, pp. 1-19.
CARGNELLO G. (2008a): L'universatilità "Etica e MetaEtica" secondo la "Grande Filiera" (GF) delle produzioni come elemento equilibratore base tra unicità e uniformità del mosaico paesistico produttivo internazionale e del WTO. XIII Convegno internazionale interdisciplinare: Unicity, Uniformity and Universality in the Identification of the Landscape and Cultural Mosaic, Aquileia (UD) 17-18 settembre pp. 4-9.
CARGNELLO G. (2008b): Nuove innovative filiere produttive: Corta, Super Corta e Grandi. Urgente necessità di concretare la "GRANDE FILIERA SUPER CORTA". TASTE VIN 5 pp. 24-29.
CARBONNEAU A., CARGNELLO G., MURISIER F. (2008): Passerillage sur souche: une tecnique innovative de raisonnement de la maturation au service de la Viticolture Durable. Une alternative à l'enrichissement en sucres. Synthèse d'essai. 31 st World congress of vine an wine 6 th general assembly of the O.I.V. Verona, Italy, 15-20 June. pp. 9-22.
CARGNELLO G. (2008c): L'universatilità "Etica e MetaEtica" secondo la "Grande Filiera" (GF) delle produzioni come elemento equilibratore base tra unicità e uniformità del mosaico paesistico produttivo internazionale e del WTO. XIII Convegno internazionale interdisciplinare: Unicity, Uniformity and Universality in the Identification of the Landscape and Cultural Mosaic, Aquileia (UD) 17-18 settembre pp. 4-6.

CARGNELLO G. (2008d): Nuove innovative filiere produttive: Corta, Super Corta e Grandi. Urgente necessità di concretare la "GRANDE FILIERA SUPER CORTA". Taste-Vin, n° 5 pp. 24-26.
CARGNELLO G. (2009a): Urgente necessità di approfondimenti semantici ed "etici". Innovazione, sostenibilità, mode, emozioni, salute, produzioni, "metaetica" e filiere in viticoltura. Taste-Vin, n° 1 pp. 62-63.
CARGNELLO G. (2009b): Recenti originali contributi per l'innovazione e il progresso tecnico, socio-economico ed etico del settore vitivinicolo. Taste-Vin, n° 2 pp. 63-67.
BOATTO V., GALLETTO L., SCAGGIANTE S., TEO G., BARISAN L., CARGNELLO G., PEZZA L., GALLO G., DI GAETANO R., CAMATTA Teresa, BONGHI C., BIANCHINI Federica, VEILLEUX Lissa, NEGRO V, †CURTOLO C., PANCOT M., CECCHETTO G. (2009): Research in the technical, socio-economical, and ethical aspects for Veneto vitivinicolture (north est Italy). 16 th International Symposium GIESCO 2009 Uc-Davis (USA - CA). pp. 283-287.
CARGNELLO G., BAZZOFFI P., BUCELLI P. (2009): "Rural districts": the productive "units" which determin the products typicity, originality and "metaethical" affirmation. 16 th International Symposium GIESCO 2009 Uc-Davis (USA - CA). pp. 605.
CARGNELLO G. (2009c): "Great chain: urgent necessity the focusing on the "metaethical" viticulture or "great" viticulture: research and various considerations. 16 th International Symposium GIESCO 2009 Uc-Davis (USA - CA). pp. 31-35.
CARGNELLO G., DI GAETANO R., DI GAETANO S., PEZZA L., GALLO G., CARGNELLO Elena, TEO G., VEILLEUX Lissa (2010): Proposte di sviluppo "eticamente" sostenibile ed esempi di successo della viticoltura di montagna e/o in forte pendenza della Sicilia Sud Orientale dal punto di vista tecnico, economico, ambientale, sociale, esistenziale, etico, culturale e "turistico relazionale". CERVIM – III Congresso Internazionale sulla Viticoltura di montagna e in forte pendenza, 12-14 maggio, Castiglione di Sicilia (I). In litteris.
CARBONNEAU A., CARGNELLO G., OJEDA H., TONIETTO J., SCHULTZ H. (2010): Synthesis of the contribution of the GiESCO (Group of international Experts of vitivinicultural Systems for CoOperation) to the study of terroirs. VIII International terroir congress 14-18 June, Soave - (VR) Italy. In litteris.
CARGNELLO G. (2010): Innovative and sustainable winegrowing techniques related to OCM, new challenges, new products and ethics. XXXIII congresso mondiale della vigna e del vino – ottava assemblea generale dell'OIV, 20-27 Giugno, Tbilisi (Georgia). In litteris.
CARGNELLO G. (2010): Contributo di base finale della nostra filiera CONAVI 2006 – 2008 - 2010. Un esempio concreto di attività che va oltre la "qualità - profitto" tecnico ed "economico d'impresa" per una viticoltura "etica" e "metaetica" o "grande" viticoltura. CONAVI – III Convegno Nazionale di Viticoltura 5-9 Luglio S. Michele all'Adige (TN). In litteris.
CARGNELLO G. with the collaboration of: FORNO S., DALLA TORRE D., DASSIE M., DA RE G. , SPERA G., LA TORRE Anna, LOT S., LOVAT L., CASADEI G., CELOTTI Cesarina, FALSETTI F., MORETTI Simonetta, GAROFALO A., PERSURIC G., MILOTIC A., SLADONJA Barbara, BRSCIC Kristina, FIORILO Mónica, TEMPESTA G.F., RUGINI E., BIASI Rita, MONARCA D. (2010): Innovations variées pour rendre «universellement» «soutenable» le développement de la viticulture. Recherches et considérations variées. 8° Simposio de Vitivinicultura do Alentejo 5-7 Majo, Evora (Portugal). In litteris.
CARGNELLO G. (2010): Great Chain: "Great Quality" - "Great Ethica" or "Meta-Ethical Great" - "Ethical Viticulture Great" or "Meta-Ethical Viticulture" or "Great Viticulture". Urgent necessity to go further to or beyond the technical, economical and landscape quality profit focusing on the "Meta-Ethical Viticulture (Great)" or "Great Viticulture". Research and various considerations. 8° Simposio de Vitivinicultura do Alentejo 5-7 Majo, Evora (Portugal). pp 38-47.

FUNGAL GRAPEVINE TRUNK PATHOGENS IN SLOVAKIA

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ABSTRACT

Trunk diseases caused by fungi are considerable diseases in all grape growing regions worldwide. In 2008-2009 a survey of fungal grapevine trunk pathogens was conducted. Totally, 301 samples from grape-growing areas of Slovakia was examined, in 77,7 % of these samples was detected some of fungal trunk pathogen – esca syndrome (from this amount 47,2 % was positive for Petri disease) and 22,3 % samples was negative to pathogenic fungi. Fungal genera reported as pathogenic to grapevine in other countries (*Botryosphaeria, Cryptovalsa, Fusarium*) was observed. It seems that occurence of grapevine pathogens that has not been economically important before is rising. Seeing that these pathogens can spread through the grapevine propagation material, its better controlment is needed.

INTRODUCTION

The expansion of wine-growing territory, and mainly vegetative propagation method led to the spread of viral, bacterial and fungal diseases of grapevines. This can result in a significant economic losses, particularly significant reducing of crop, affecting fruit quality and reducing the viability of the infected plants in vineyards. Currently, trunk diseases caused by fungi are considerable diseases in all grape growing regions worldwide. In the years 2008-2009 was carried out the monitoring of grapevine health status in different areas of Slovakia, focused on quantity and severity of fungal trunk diseases in young plantations and older vineyards.

MATERIAL AND METHODS

Grapevine samples of different cultivars was collected from vineyards at various maturity stages and from various grape-growing areas of Slovakia. From all samples, three wood segments were cutted with a length of approximately 25 mm (from base, middle part and under the graft union), from which the bark was removed and surface sterilized with sodium hypochlorite, then rinsed three times in sterile distilled water. Wood segments were then dried on sterile filter paper, dipped in 96% ethanol, flamed and placed on solidified malt agar in a Petri dish. Cultivation took place 30 days at 25 ± 2 ° C in the dark. Isolated pure cultures of fungi were identified based on morphological and cultural characteristics.

RESULTS AND DISCUSSION

Totally, 301 samples of grapevine was examined, in 77,7 % of these samples was detected some of fungal trunk pathogen – esca syndrome (from this amount 47,2 % was positive for Petri disease) and 22,3 % samples was negative to pathogenic fungi. These pathogenic fungi were observed: *Phaeomoniella chlamydopora, Botryospaheria obtusa, Cryptovalsa ampelina* and *Fusarium* sp. *Phaeomoniella chlamydopora* is main causal agent of Petri disease. It is a

serious disease that reduces sustainability and productivity of the grapevines, increases failure rate of grapevine plants, makes difficulties in establishing new vineyards and causes premature decline and death of young vines. Fungi *Botryospaheria obtusa*, *Cryptovalsa ampelina and Fusarium* sp. belong to a group of fungi causing deterioration (wilting) wood tissues, arms, shoots and cause premature declining of grapevines (esca syndrome). They usually occur on older grapevines, except of *Fusarium* sp. which was also often observed in young plants. It was also recorded the concurrent incidence of pathogenic fungi in tested samples.

CONCLUSIONS

The monitoring of grapevines healh status in Slovakia shows that occurence of grapevine pathogens that has not been economically important before is rising. Above three-quarters of all samples tested were affected by fungal pathohen. As the grapevine trunk diseases are serious diseases that practically can not be cured, prevention remains the most important agrotechnical measures to prevent the spread of diseases in new plantings. Better controlment of grapevine propagation material is needed. Legislative changes are prepared with stricter rules for the marketing of vine propagating material to prevent the sale of infected grafts.

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Maping a health state of vine in Slovakia

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ABSTRACT

The work analysis the stage of virus occurence in grape vines in Slovakia. The vines were obtained directly from vineyards as well from vine reproducers to maping their health state. We were testing five virus diseases, Arabic Mosaic, Grapevine fanleaf virus, Grapevine Leafroll virus I and III. The vines, which were tested on Arabic Mosaic showed shocked results, all vines were positive, vine tested in Grapevine fanleaf virus were positiv in 72,6% in 2008 respectively 50% in 2009. Vine with Grapevine Leafroll virus I was positive in 55,2% and Grapevine Leafroll virus III was figured in 45,7% cases.

Introduction:

Slovakia as a member of Europen union has to follow legislation of this community. It is important to respect legislative acts and amending acts, which was appointed by Europen union. Slovak government will have to ensure building virulencefree glashouse. for preparing plants material without virus. The health state of plans has increasing movements in Slovakia.

A biological material used in Slovakia, nowdays do not have conditions for the European market. Slovakia will have to certify plant material likes a member of Europen union, it is technolgy for preparing virus-free vines. A program for health state of vines needed to test and keep will prepar following European´s Plant Protection Organisation for safe plants method, which is accepting in each state of Europen union.

Materials and Methods:

The analyses were accomplished by complete kits (BIOREBA). 480 complete kits consist of 0,1 ml IgG, 0,1 ml conjugate, 2,5 ml positive control, 2,5 ml negative control, extraction buffer (10*/5*) 250/500ml, coating buffer 1 tablet (0,1 l), conjugate buffer (10*) 10 ml, substrate buffer (5*) 20 ml, washing buffer 1 pouch (5 l), substrate (p NPP) 5 tablets (0,1 l), egg albumin 25 g, urea 150 g, microtiter plates 5*F96 plates.

DAS-ELISA was used as an analytical method to identify 5 viruses, these are Arabic Mosaic, Grapevine fanleaf virus, Grapevine Leafroll virus I and Grapevine Leafroll virus III. It consist of 4 phases, the first is catch a coating, when the specific antibody adsorbed to surface of microtiter wells. Cover plates were placed in a humid box and incubated at 30°C for 4 h or at 4°C overnight. The empty wells were washed 3-4 times with washing buffer.

After that were followed with antigen (incubation of plant extract). Different extraction buffers were added 200µl per well. Cover plates were placed in a humid box and incubated at 4°C overnight. It washed as in the first step.

The third step was added in conjugate (incubation of enzyme – labeled antibody). The conjugate enzyme was diluted 1000 times in conjugate buffer and added 200µl per well. Cover plates were placed in a humid box and incubated at 30°C for 5 h. It washed as in first step.

The substrate was added in the last step and was brought a color reaction indicated infected samples. 1mg/ml of p-nitrophenyl phosphate was dissolved in substrate buffer. This buffer was added 200µl per well. It was incubated at 18-25 °C in the dark. Yellow color reaction could read after 30-120 min visually with photometer at 405 nm.

Results and Discussion:

All the samples were analyzed by bio-analytical method, DAS-ELISA, which is an authentic method for analyze viruses in plants. A biological material used in Slovakia, nowdays do not have conditions for the European market. Slovakia will have to certify

plant material likes a member of Europen union, it is technology for preparing virus-free vines.

Wine grafts were tested on 5 virus diseases, these are Arabic Mosaic, Grapevine fanleaf virus, Grapevine Leafroll virus I and III. Grapevine fanleaf virus is widespread virus disease of grapevine. It spread in all regions. This virus see above grade parts of plants. Grapevine Leafroll virus, consist of four serotyps (I-IV). Symptoms of Grapevine Leafroll virus appear after early autumn. The older leafs roll firs and later this disease attack younger leafs. ArMV infects many plants, for example grapevine, raspberry, strawberry, cucumber and so on. This diseas bring deformation leafs. Testing of vine on virus diseases show, that the number of infected vineyards is increase. More than half of the tested samples were infected by at least one of the mentioned diseases. Fig.1-5 show a number of infected grafts to five tested viruses.

Fig.1 shows a number of infected grafts to Grapevine fanleaf virus, which were tested in 2008. The low positive grafts were represented in 45,3 %, medium positive grafts were represented in 6,8 %, high positive grafts were represented in 20,5% and the number of negative grafts was 27,4 %.

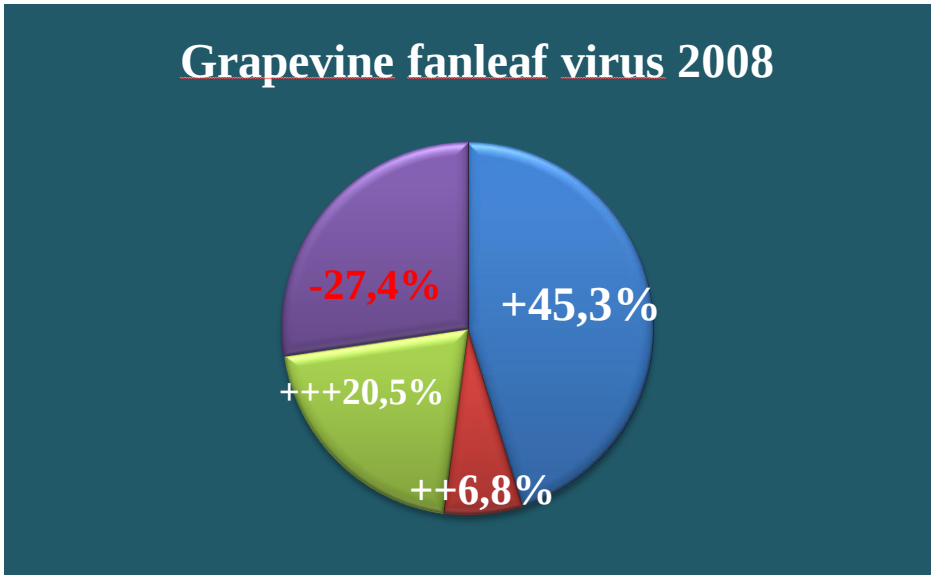

*Fig 1 Number of infected grafts to **Grapevine fanleaf virus** in 2008, + - low infectedness, ++ - medium infectedness, +++ - high infectedness, - graft without viruses*

Fig.2 shows a number of infected grafts to Grapevine Leafroll virus, which were tested in 2008. The low positive grafts were represented in 45,3 %, medium positive grafts were

represented in 6,8 %, high positive grafts were represented in 20,5% and the number of negative grafts was 27,4 %.

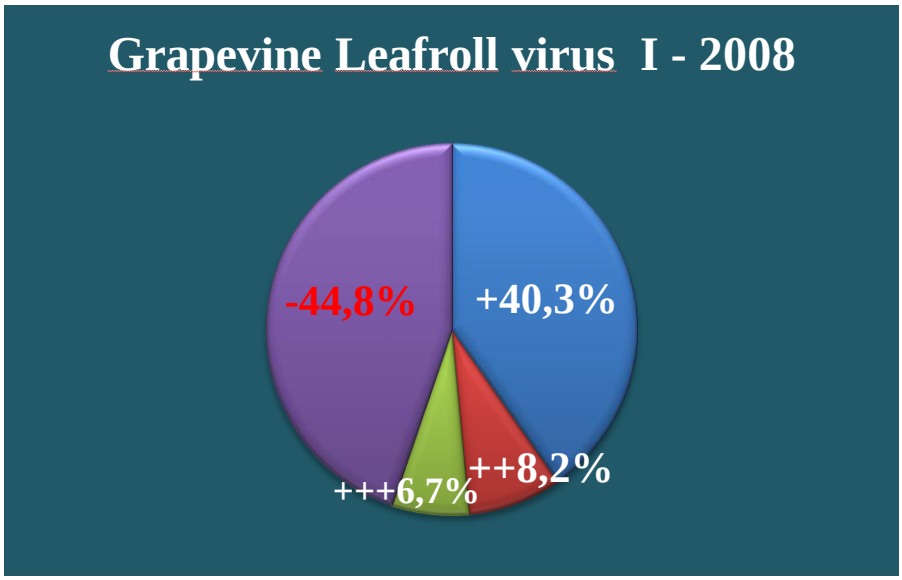

*Fig 2 Number of infected grafts to **Grapevine Leafroll virus** in 2008, + - low infectedness, ++ - medium infectedness, +++ - high infectedness, - graft without*

Fig.3 shows a number of infected grafts to Grapevine fanleaf virus, which were tested in 2008. The low positive grafts were represented in 45,3 %, medium positive grafts were represented in 6,8 %, high positive grafts were represented in 20,5% and the number of negative grafts was 27,4 %.

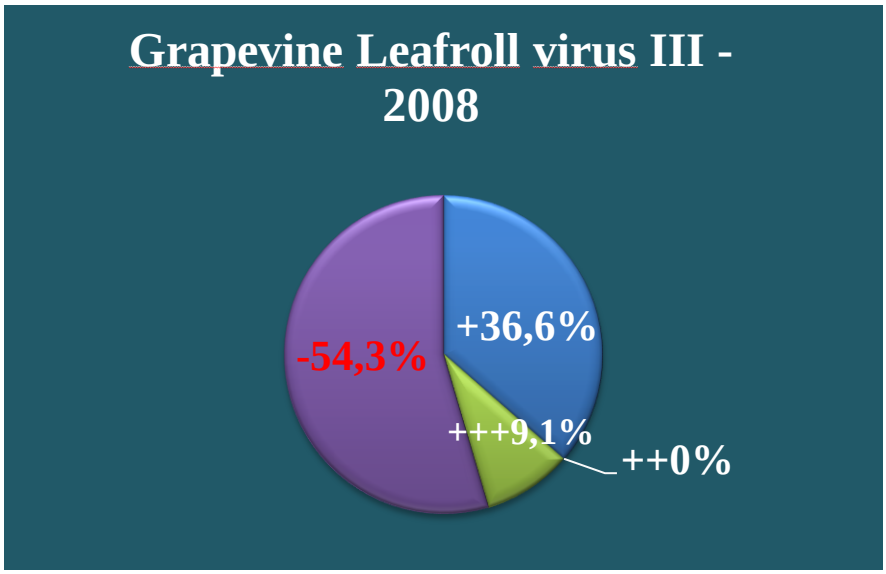

*Fig. 3 Number of infected grafts to **Grapevine Leafroll virus** in 2008, + - low infectedness, ++ - medium infectedness, +++ - high infectedness, - graft without*

Fig.4 shows a number of infected grafts to Grapevine fanleaf virus, which were tested in 2008. The low positive grafts were represented in 45,3 %, medium positive grafts were represented in 6,8 %, high positive grafts were represented in 20,5% and the number of negative grafts was 27,4 %.

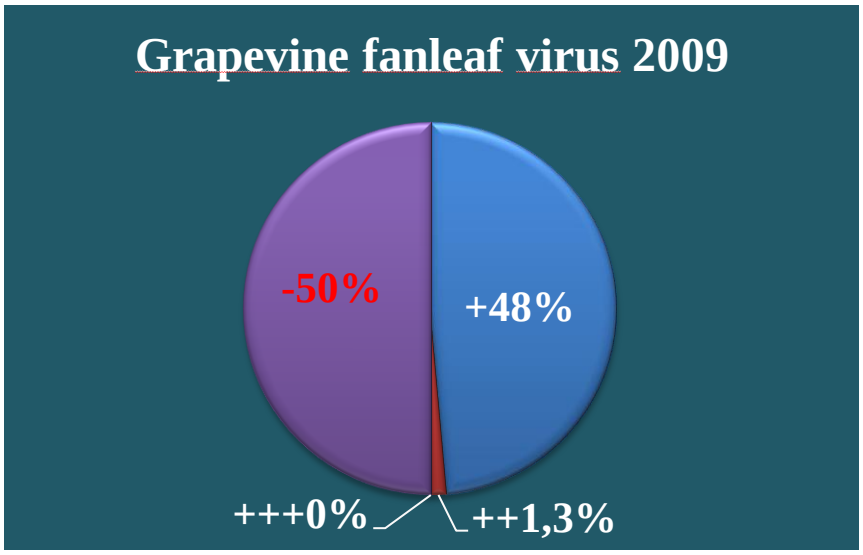

Fig. 4 Number of infected grafts to ***Grapevine fanleaf virus*** *in 2008, + - low infectedness, ++ - medium infectedness, +++ - high infectedness, - graft without*

Fig.5 shows a number of infected grafts to Arabic mosaic, which were tested in 2009. Arabic Mosaic showed shocked results, all vines were positive The differences are just in low, medium and high infectedness. The biggest number is in low positive grafts, which were represented 86,7%, medium positive grafts were represented in 12%, high positive grafts were represented in 1,3%.

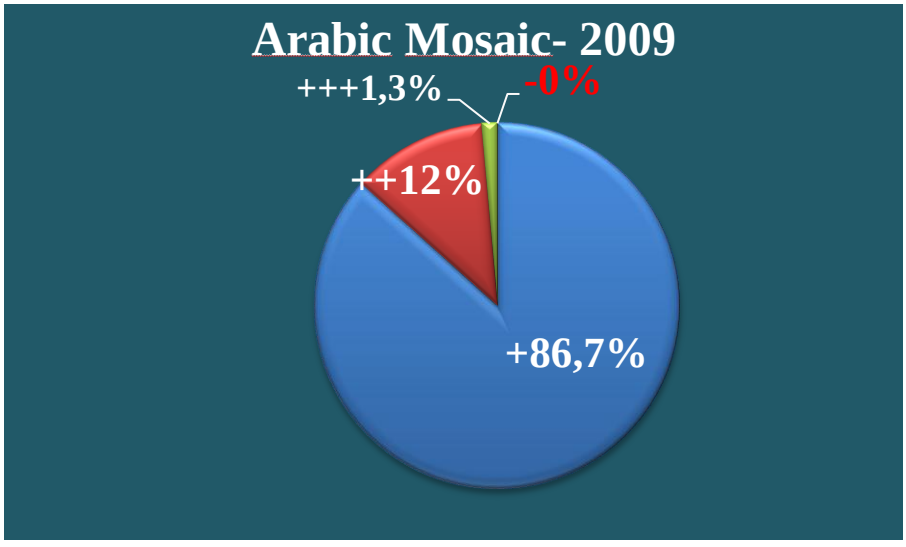

*Fig. 5 Number of infected grafts to **Arabic Mosaic** in 2008, + - low infectedness, ++ - medium infectedness, +++ - high infectedness, - graft without*

Conclusions

The number of infected vineyards in Slovakia is increase. The target for the future is to prevent of speading diseases in new planting and providing for the viniculturism the opportunity of pre-testing of their own plants of vine as well as the possibility of their recovery. The targets of this work were maped the health state of vine in Slovakia, diagnozed virus diseases. We prepared virulencefree glashouse to sanify the vineyards and to prepare certify materials.

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Bibliography

www.uksup.sk/download/oos/20040216_metodika_us_ovo_2003.doc

Vinič 2, Gašpar Vanek a kol. :Vinič 2, ochrana, Príroda a. s. Bratislava, 87 – 91, 1996

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Patrimoine viticole français : conserver, valoriser et innover dans la tradition

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RESUMÉ

L'encépagement historique français est un patrimoine unique par sa richesse et sa diversité. A ce jour, on estime à près de 450 le nombre de cépages autochtones. Les différentes mutations que le vignoble a subies (invasion phylloxérique, maladies, crises sociales et économiques, influences commerciales...) ont généré un appauvrissement de la diversité au sein des régions viticoles françaises. Conscients de ce problème, les organismes techniques chargés de la sélection, l'Institut National de la Recherche Agronomique (INRA) et l'Institut Français de la Vigne et du Vin (IFV), fédérés autour de leurs partenaires par la Commission Technique Nationale de Sélection et de Participation (CTNSP) ont mis en place une démarche organisée et cohérente de sauvegarde et de valorisation de ces variétés autochtones. En effet dans le respect de la Charte Nationale pour la gestion des ressources génétiques de la vigne, les techniciens régionaux, appuyés par les ampélographes de l'INRA et de l'IFV ont prospecté les vieilles parcelles dans la plupart des vignobles français et mis en place des conservatoires. Cette réserve génétique constitue donc un patrimoine considérable, grâce auquel la viticulture française pourra continuer à innover.

ABSTRACT

The French varietal heritage is unique by its richness and diversity. We estimate that approximately 450 grapevine varieties are native of France. These varieties are described and maintained in the main collection, INRA Vassal. Since the middle 18th, the French vineyard went through several crisis such as phylloxera and pests invasions, also social and economical crisis or development of new trends in the wine consumption,… Then, numerous varieties have today almost disappeared.

The French selection organizations led by INRA and IFV, in association with their partners, were sensitive about this loss, considering that preservation is a priority for the scientific community and the wine industry.

They have been developing a partnership (CTNSP) in order to give every vineyard the possibility to maintain, evaluate and develop its specific varieties.

The National Protocol for repository brings harmonization through ampelography expertise, DNA and virus testings,…

Then, winegrowers have now the opportunity to renew their vineyard and develop their local patrimony.

For instance, cultivars such as Trousseau gris, Mollard, Prunelard, Rivairenc are thus rehabilitated and, for some, vinified and marketed. These “niche” products are required by consumers in search of authenticity and of innovation.

Introduction

Le vignoble français, un des plus anciens au monde, recèle une diversité variétale historique parmi les plus importantes. Cette richesse trouve son expression notamment dans le cadre des AOP et des terroirs français. Au fil du temps, l'encépagement a progressivement évolué, au gré des différentes mutations que le vignoble a pu connaitre (crise phylloxérique, crises économiques, évolution du goût des consommateurs...) conduisant à une perte de diversité incontestable. Depuis plusieurs années, les techniciens de la filière, conscients de la situation et organisés dans le cadre de la Commission Technique Nationale de Sélection et de Participation (CTNSP), ont mis en place différents programmes de sauvegarde et de valorisation. Le présent article ne constitue pas un inventaire exhaustif des démarches mais rend compte de certaines initiatives régionales exemplaires.

Rappel du contexte :

400 à 450 cépages peuvent être considérés comme originaires de France (Lacombe, 2002). Seuls 294 variétés (cuve et table) sont inscrites au Catalogue Officiel National (source CTPS, section vigne 2010). Depuis 1958 (cadastre viticole français, IVCC), la part des 20 à 40 cépages majoritaires n'a cessé de croître (figure 1).

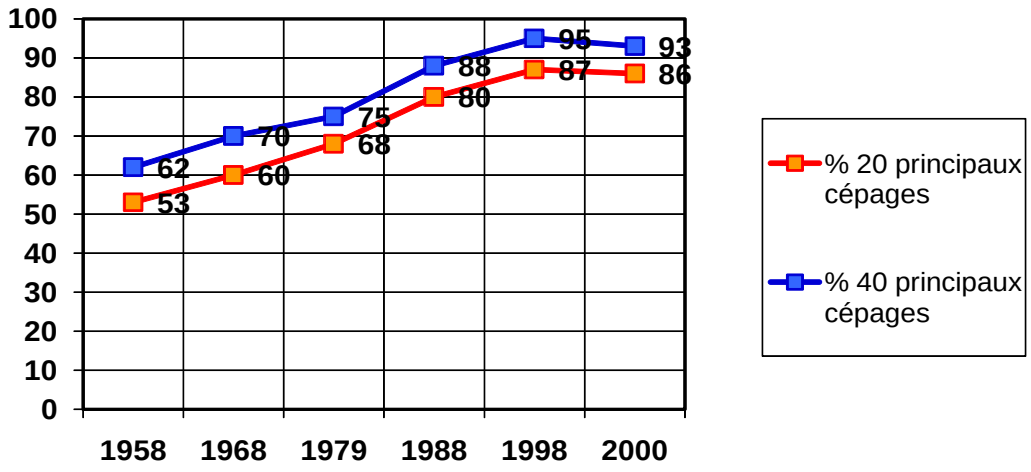

Figure 1 : évolution de la part des cépages majoritaires du vignoble de cuve français de 1958 à 2000.
(Boursiquot, communication personnelle)

Parallèlement à cette perte de diversité variétale, une structuration de la conservation viticole française s'est organisée, et ce, à différents niveaux :

- **Collection internationale des cépages et Vitacées de l'INRA de Vassal** (34340 Marseillan-France), comprenant environ 5 500 accessions de *Vitis vinifera* originaires de 54 pays, ainsi que des porte-greffes, des hybrides interspécifiques et des espèces de Vitacées.

- **Collection de l'Institut Français de la Vigne et du Vin, Pôle National Matériel Végétal au domaine de l'Espiguette** (30240 Le Grau du Roi - France), conservatoire des clones sélectionnés (environ 4 000 clones, dont plus d'un millier agréés et diffusés).

- **Conservatoires génétiques régionaux** (diversité inter et intra-variétale) (figure 2) Ces conservatoires sont gérés par des structures consulaires, des interprofessions ou des associations chargées d'assurer la sauvegarde du patrimoine variétal de leur région.

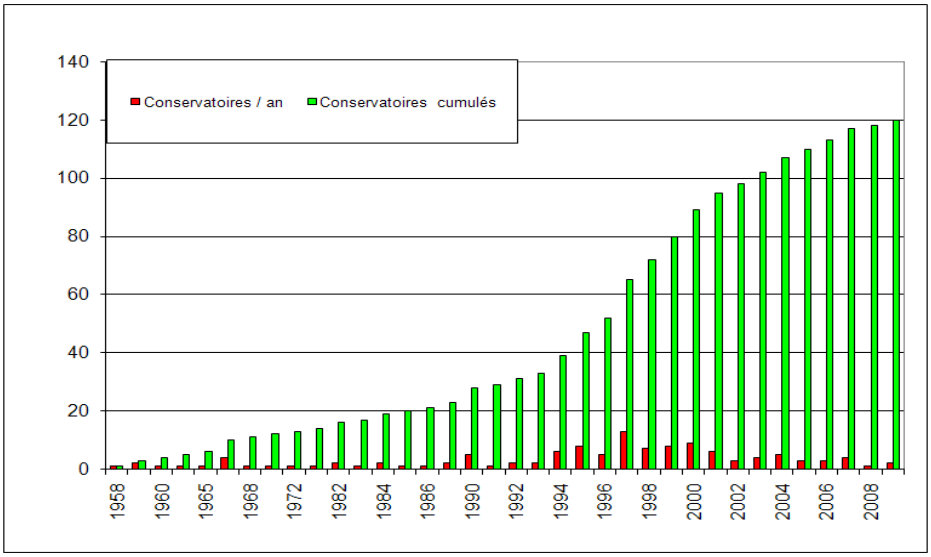

Figure 2 : Effectifs cumulés des conservatoires de clones de vignes en France (IFV, 2010)

Ce réseau de partenaires est fédéré autour de l'IFV et de l'INRA de Montpellier dans le cadre de la Commission Technique Nationale de Sélection et de Participation, qui regroupe 33 structures (figure 3). La base de données de ce réseau est consultable à l'adresse suivante : http://bioweb.ensam.inra.fr/collections_vigne.

Figure 3 : Carte des partenaires de la sélection vigne française

C'est dans ce contexte que l'IFV, l'INRA et leurs partenaires ont élaboré un cadre technique permettant une cohérence et une rigueur dans les différentes actions de conservation de la vigne en France. Ces actions sont réalisées dans le respect de la Charte Nationale pour la gestion des ressources génétiques de la vigne, consultable à l'adresse suivante :
http://bioweb.supagro.inra.fr/collection_vigne/Charte.html.

Ce document (*Méthode d'installation, de gestion et d'étude des conservatoires de clones de vigne*, section Vigne du CTPS, 2005), précise notamment :

- les règles à suivre durant la phase de prospection des ressources génétiques :

* caractériser la variété et sa diversité
* privilégier les parcelles âgées
* privilégier le nombre de parcelles au nombre de souches par parcelle

- l'état sanitaire et les tests à réaliser :

* GFLV et ArMV
* GLRaV-1, 2 et 3

- **les modalités liées à la réalisation des plants de vigne :**
    * traitement à l'eau chaude vis-à-vis des phytoplasmes
    * utilisation de porte-greffe de catégorie base

- **l'implantation du conservatoire :**
    * choix du site
    * dispositif

- **le suivi technique et l'entretien du conservatoire :**
    * remplacements
    * suivi sanitaire

- **l'étude des accessions conservées :**
    * caractéristiques morphologiques
    * caractéristiques agronomiques voire génétiques
    * sensibilités diverses

Sauvegarde de variétés non inscrites au Catalogue Officiel français

Parmi les variétés historiques françaises non inscrites au Catalogue Officiel, plusieurs font l'objet de travaux de sauvegarde en vue de leur éventuelle inscription, nous pouvons citer les travaux sur les cépages suivants (liste non exhaustive) :

- cv. Trousseau gris G : forme grise du Trousseau N du Jura, connu dans les Charentes sous le nom de "Chauché gris", il a été l'un des cépages les plus cultivés dans cette région entre le 13ème et le 18ème siècle. 6 accessions saines (indemnes des virus GFLV, ArMV, GLRaV-1, GLRaV-2 et GLRaV-3) ont été retrouvées dans le cadre de travaux menés par le Conservatoire du Vignoble Charentais. Deux programmes d'essais sont en cours depuis 2005, l'un pour l'élaboration de Pineau des Charentes, l'autre pour la production de vins de pays charentais.

- Cépages autochtones du Gers : suite à des prospections dans les plus anciennes vignes de ce département, une parcelle a été implantée en 2002, qui comprend 39 cépages représentés par 113 clones. 20 de ces variétés ne figurent pas au Catalogue Officiel, et 12 étaient absentes jusqu'alors des collections (dont 9 sans dénomination connue). Des répétitions et des cépages témoins permettent d'exploiter cette parcelle dans le cadre de travaux d'évaluation, fruits d'un partenariat entre les Producteurs de Plaimont (union de coopératives, initiatrice du projet) et l'IFV Sud-Ouest. Deux années de mesures et de vinifications ont été effectuées à ce jour, et des résultats prometteurs pourraient déboucher sur une expérimentation plus large centrée sur deux ou trois variétés qualitatives.

- Cépages autochtones du nord Aveyron : plusieurs années de recherches bibliographiques et de prospections dans ce petit vignoble, autrefois beaucoup plus développé, ont permis l'implantation de 6 conservatoires (Fer N, Négret de Banhars N, Mouyssaguès N, Chenin B, Saint-Côme B, Fel B), et l'inscription de deux variétés au Catalogue Officiel (Négret de Banhars N et Saint-Côme B). Actuellement, les travaux d'évaluation se poursuivent sur le cépage Fel B, et deux parcelles vont être installées en vue d'une inscription dans un futur proche.

- Cépages autochtones des Pyrénées-Atlantiques : La Chambre d'Agriculture des Pyrénées-Atlantiques réalise depuis plusieurs années des travaux de prospection et de sauvegarde des vieilles variétés pyrénéennes. Ces prospections ont conduit à l'implantation d'un conservatoire de 21 variétés blanches en 2010 et un conservatoire de 14 variétés noires est prévu pour l'année 2011.

- Cépages autochtones de la région Corse : Le CIVAM de la région Corse mène depuis plus de 20 ans un travail sur le patrimoine ampélographique de l'île, avec comme objectif la valorisation des cépages anciens. Ce travail a permis la redécouverte de 23 variétés qui ont fait l'objet d'une mise en conservatoire et en évaluation pour 154 clones. La part des cépages corses atteint désormais plus de la moitié de l'encépagement local. 6 variétés anciennes ont été introduites dans les décrets AOP en plus des 3 cépages principaux. 14 variétés insulaires sont inscrites au Catalogue Officiel et 3 font l'objet d'une demande d'inscription (Cualtacciu B, Brustianu B, Rossula bianca B)

- cv. Mornen N : ce cépage rencontré en Savoie, dans la Vallée du Rhône a été décrit vers la fin du 19ème siècle par Mas et Pulliat (Boursiquot et Lacombe, 2009). Cultivé à l'état de traces dans le vignoble de la Vallée du Gier, il a récemment été reconsidéré par les vignerons locaux, et l'Association pour le développement des Coteaux du Gier a initié une démarche afin de l'inscrire au Catalogue Officiel. Ces travaux s'inscrivent dans une volonté d'identification d'un vignoble local et de développement de ses variétés les plus anciennes.

- Cépages autochtones de Franche-Comté : depuis 2004, la Société de Viticulture du Jura et la Chambre d'Agriculture du Jura ont mené des prospections dans le vignoble jurassien. Ce travail important de sauvegarde va permettre dès 2011 la réalisation d'un conservatoire sur la commune du Vernois (39210 - France). 130 accessions représentant 55 variétés seront regroupées dont certaines considérées comme très rares dans le vignoble (Peurion B, Béclan N, Mezi N, Gueuche noir....)

Au delà de ces actions menées dans le cadre du réseau de partenaires de la CTNSP, on peut également citer :

- la sauvegarde des cépages auvergnats (dont notamment le Noir Fleurien N, le Limberger N, ...) par la Fédération Viticole du Puy-de-Dôme et la Sicarex du Beaujolais,
- les travaux de conservation menés par le Centre d'Ampélographie Alpine pour les cépages savoyards à Villard d'Héry (73800 - France) en complément des travaux initiés par la Chambre d'Agriculture de Savoie sur les cépages autochtones majeurs (Mondeuse N, Jacquère N...).

Valorisation de variétés inscrites au Catalogue Officiel français

Plusieurs cépages secondaires, pourtant déjà inscrits au Catalogue Officiel français, ne font l'objet d'une valorisation, notamment par la sélection clonale, que depuis peu de temps :

- cv. Claverie B : originaire du bassin de l'Adour, le Claverie B n'est plus cultivé dans son vignoble d'origine : les Landes. Un conservatoire de 32 accessions a été mis en place en 2003 sur la commune d'Eugénie-les-Bains par la Chambre d'Agriculture des Landes. A ce jour, il n'y a pas encore de clone agréé.

- cv. Durif N : Connu aux Etats-Unis sous le nom erroné de "Petite Syrah", il était répandu dans le sud-ouest de la France au 19ème siècle. Actuellement, ce cépage a pratiquement disparu du territoire national, avec une surface cultivée inférieure à un hectare (IFV, 2007). L'IFV Sud-Ouest a mené des travaux qui ont récemment permis l'agrément du clone 1130.

- cv. Knipperlé B : non reconnu en AOP Alsace, il est pourtant très ancien dans cette région où sa surface cultivée a pu atteindre les 5 000 ha (Schneider, communication personnelle). Il s'agit d'un cépage issu d'un croisement de Gouais B x Pinot. A ce jour, il n'y a pas de clones agréés de ce cépage, mais un conservatoire de 65 accessions a été implanté en 2003 suite aux travaux menés par le CIVA et l'INRA.

- cv. Mollard N : originaire des Hautes-Alpes, il serait probablement un des nombreux descendants du Gouais B. Suite à des prospections menées dans la haute vallée de la Durance à la fin des années 80, une vingtaine d'accessions sont conservées au domaine de l'Espiguette et 2 clones ont été agréés en 2003 (clones 993 et 996). Considéré comme un cépage de « niche », le Mollard gagne en popularité, permet l'élaboration de vins rosés de bonne qualité et fait désormais l'objet d'un intérêt particulier dans la communauté viticole locale.

- cv. Prunelard N : cet ancien cépage du sud-ouest, dont une étude récente a démontré qu'il était le père du Cot N ou Malbec (Boursiquot *et al*, 2008), l'autre parent étant la Magdeleine noire des Charentes, n'était plus cultivé que de façon anecdotique à Gaillac. Après l'implantation d'un conservatoire riche de 20 origines et plusieurs années d'évaluations agronomiques et œnologiques, le cépage a intégré deux AOP (Gaillac et Marcillac). Actuellement, des travaux de sélection clonale sont en cours, et le cépage suscite un réel engouement (20 ha de nouvelles plantations à Gaillac ces trois dernières années).

- cv. Rivairenc : cépage très ancien du Languedoc, dénommé "Aspiran" jusqu'en 2006, le Rivairenc N est un cépage rustique qui existe dans le vignoble à l'état de souches éparses. Une forme blanche et une forme grise existent également. Des travaux de prospections ont été menés dans le Minervois à l'initiative de la Chambre d'Agriculture de l'Aude. A ce jour, il n'y pas encore de clone agréé.

- cv. Romorantin B : originaire du Centre de la France et issu d'un croisement entre Pinot teinturier N x Gouais B, le Romorantin B possède déjà 4 clones agréés (clones 466, 873, 928 et 929). Des travaux menés par l'IFV Val-de-Loire et la Chambre d'Agriculture du Loir-et-Cher ont permis la réalisation d'un conservatoire d'une cinquantaine de clones en 2007 dans le Loir-et-Cher.

- cv. Savagnin rose Rs: il s'agit de la forme rose non aromatique du Savagnin blanc B. Il était cultivé en Alsace avant 1900 où il est nommé "Klevener de Heiligenstein". Des prospections dans les vignobles d'Alsace et du Jura ont permis la création d'un conservatoire d'une quinzaine d'accessions implanté en Alsace. A ce jour, il existe un clone agréé de Savagnin rose Rs (clone 763).

Conclusion

Face à l'érosion variétale mesurée depuis 1958, les partenaires de la sélection viticole française (CTNSP) ont réagi de manière cohérente et structurée. A ce jour, il existe 120 conservatoires implantés dans les régions viticoles, maintenant la diversité des principaux cépages français, mais aussi de certaines variétés secondaires. Cependant, même si un travail considérable a déjà été réalisé, de nombreux cépages restent encore sans conservatoires ni clones agréés. Des actions urgentes de prospection et de sauvegarde doivent donc être poursuivies afin de conserver dans les meilleures conditions possibles le patrimoine viticole français. La valorisation du matériel végétal reste un des leviers majeurs d'une viticulture dynamique et ouverte à l'innovation.

Remerciements à l'ensemble des partenaires de la CTNSP.

Bibliographie:

Bordenave Louis *et al.,* 2007, *Etude historique, génétique et ampélographique des cépages Pyrénéo Atlantiques*, Bulletin de l'OIV, n°920-922, p. 553-586.

Boursiquot Jean-Michel., 2002, *Les grandes tendances de l'évolution de l'encépagement en France et dans le Monde,* SIVAL Angers.

Boursiquot Jean-Michel *et al*., 2008 : *Parentage of Merlot and related winegrape cultivars of southwestern France: discovery of the missing link,* Australian Journal of Grape and Wine Research.

ENTAV-INRA, 2005, *Méthode d'installation, de gestion et d'étude des conservatoires de clones de vigne.*

Galet Pierre., 1990, *Cépages et vignobles de France. Vol. II,* Montpellier. Imprimerie Dehan.

Institut Français de la Vigne et du Vin., 2007, *Catalogue des variétés et clones de vigne cultivés en France.*

Lacombe Thierry, 2002, *Les cépages de cuve secondaires en France, Convegno Internazionale - Valore e funzione dei vitigni auctoni e tradizionali*, Firenze, Italie.

Lacombe Thierry, 2008, *Status of the French Vitis National Collection*, pp 73-74, in: Maul E, J.E. Eiras Dias, H. Kaserer, T. Lacombe, J.M. Ortiz, A. Schneider, L. Maggioni and E. Lipman, compilers, Report of a Working Group on *Vitis*, First meeting, 12-14 june 2003, Palic, Serbia and Montenegro. 184 p.

Genetic diversity of Georgian varieties of *Vitis vinifera* subsp. *sylvestris*

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ABSTRACT

Genetic diversity geographically diverse group of Georgian wild grape samples *Vitis vinifera* subsp. *sylvestris* was studied. Sample analysis at four polymorphic microsatellite loci (VVMD7, VVMD27, VVS2, ZAG62) revealed high level of polymorphism, detecting at the same time their individual, unique allelic profile. Genetic polymorphism in wild grape population is slightly higher from what has been observed in cultural varieties. The obtained results confirm strong evolutionary links between the wild and cultivated grapes.

Es wurde die genetische Vielfalt geographisch unterschiedlicher Gruppen von georgischen Traubesorten *Vitis Vinifera* subsp. *sylvestris* studiert. Durch die Untersuchung von vier polymorphischen Mikrosatelite Orten (VVMD7, VVMD27, VVS2, ZAG62) wurde entdeckt ein hohes Niveau von Polymorphie, das auf die individuelle, unikale alelische Gestalt von der erforschten Exemplaren hinweist. Genetisches Polymorphismus der wilden Traubesorten ist etwa höher, als das Niveau der Variation der schon beobachteten kulturellen Sorten. Daher die erzielten Ergebnisse hinweisen auf den engeren evolutionaeren Zusammenhang zwischen den wilden und kulturellen Traubensorten.

INTRODUCTION

The Eurasian grape (*Vitis vinifera L.*) is one of the most widely cultivated and economically important agricultural crop in the world. The cultivated subspecies, *Vitis vinifera* subsp. *vinifera* includes thousands of cultivars (5000 to 7000) and have been domesticated from the wild subspecies - *Vitis vinifera* subsp. *sylvestris*, which is widespread Eurasian species, occurring as a climbing vine in forests from Spain to Turkmenistan (Fig.1) [Alleweldt; Dettweiler,1994, Levadoux, 1956, Zohary; Hopf, 2000]. Subspecies *sylvestris* is dioecious, with female and male flowers occurring in roughly the same proportion in populations and exhibit small, acidic berries relative to cultivated grapes. According to the most researchers [Negrul, 1946, Zhukovski, 1971, Zohary; Speigel-Roy, 1975, Jackson, 1994, Zohary; Hopf, 2000, Sauer, 1993], the South Caucasus is the area where grapes were most likely first domesticated.

Fig. 1. Current distribution of *Vitis vinifera* subsp. *sylvestris* (adapted from Zohary; Hopf 2000). Isolated occurences to the east (Tajikistan, Turkmenistan) do not appear in this map.

Wild varieties of *Vitis vinifera* subsp. *sylvestris* are very abundant in the South Caucasus, where many intermediate wild forms were observed, including those with characters associated with cultivated forms such as white fruits, hermaphroditic flowers, and larger sized seeds [Zohary; Hopf, 2000, Zohary; Spiegel-Roy, 1975]. Specific climatic conditions in this area were favorable for the diversification of the wild varieties from which cultivated grapes could be chosen for domestication [Jackson, 1994].

This report represents the first attempt to investigate genetic diversity of Georgian wild grape samples.

MATERIALS AND METHODS

The wild grape samples were collected from different geographic zones of Georgia. Table 1 represents list of investigated wild grapevine samples, with the indication of their geographic locations.

Tab. 1. List of studied wild grapevine samples, with the indication of their geographic locations.

Vitis vinifera subsp. ***sylvestris*** specimens	Geographic location of ***Vitis vinifera*** subsp. ***sylvestris*** specimens	
G-6	Gardabani reserve, Gardabani district	41^{o} 22. 554'N, 45^{o} 03.70'E
Q-l	Village Qsovrisi, Mtskheta district	41^{0} 59.17'N, 44^{0} 30.56'E
B-1	Likani, Borjomi district	41^{0} 47.51,5'N, 43^{0} 20.462'E
B-3	Chobiskhevi, Borjomi district	41^{0}47.441'N, 43^{0}18.149'E
S-8	Korugi forest reserve, Sagaredjo district	41^{0}37.48'N, 45^{0}27.11'E
Qiz.-1	Vashlovani reserve, Dedoplistskaro district	-
Seva	Village Ruispiri, Telavi district	-

Trueness to wild accessions were based on ampelographical analysis of collected grape leaves, which were confirmed by comparing with the morphological descriptions of typical *Vitis vinifera* subsp. *sylvestris*. In most cases the flowers were also investigated.

Total genomic DNA was isolated from young grape leaves. The leaves were ground in liquid nitrogen. For DNA isolation the CTAB based extraction procedure was used [Lodhi *et al.*;1994]. When necessary, extracted DNAs were purified with GenElute columns (Sigma-Aldrich, St. Louis, MO). In the case of silica dried leaves DNA was isolated by Plant genomic DNA extraction miniprep system (VIOGENE,USA). DNA were analyzed at four: ssrVrZAG62, VVMD7, VVMD27 and VVS2 loci.

For the microsatellite analysis three step PCR was performed in 25 µl of reaction volumes using Promega PCR Master Mix System: PCR Master Mix solution containing Taq DNA Polymerase, dNTPs, $MgCl_2$ and reaction buffers at optimal concentrations for efficient amplification of DNA template by PCR. 20 pmol primers were used in each reaction (Primers were synthesized by Sigma Genosys Company). Amplification were carried out by using Techne PCR amplifier. Three step PCR protocol included 7 min denaturing at 95°C, 30 cycles of 94°C denaturing during 45 seconds, 50°C annealing (30 second) and 72°C extension (90 seconds), followed by a final extension process at 72°C (7 minutes). For the electrophoresis 2,5 µl of each PCR product was loaded on a 8% polyacrylamide/urea sequencing gel, electrophoresed by 2117 Multiphor II LKB Electrophoretic system and visualized by silver staining technique according to Promega Manual. Allele sizes were determined using defined size markers. As a control, DNA of two French cultivars (Cabernet Sauvignon and Pinot Noire) and our earlier-studied two Georgian cultivars (Rkatsiteli and Saperavi) were used [This *et al.*; 2004, Tabidze *et al.*; 2006].

RESULTS AND DISCUSSION

For the genetic diversity studies of *Vitis vinifera* subsp. *sylvestris* nuclear DNA microsatellite analysis at four VVMD7, VVMD27, VVS2, ZAG62 polymorphic microsatellite loci were used. Microsatellite profiles of these specimens are given in Table 2.

Tab. 2. Genetic profiles of Georgian wild grapevine populations at 4 microsatellite loci. Allele sizes are given in base pairs (bp).

Vitis vinifera subsp. ***sylvestris*** specimens	ZAG 62	VVMD 27	VVS 2	VVMD 7
G-6	196 –192	189 – 177	139-131	254 - 235
Q-l	190 –190	227 – 201	145 - 129	259 - 247
B-1	206 – 202	191 – 191	139 - 135	249 - 243
B-3	198 –198	227 – 217	141 - 135	252 - 240
S-8	204 –198	187 – 181	143 - 133	245 - 235
Qiz.-1	200 – 188	195 –175	139 - 137	246 - 240
Seva	202 –196	185 –179	143 - 141	-

Seven studied grape samples at four VVMD7, VVMD27, VVS2, ZAG62 polymorphic microsatellite loci generates 39 alleles and observed heterozygosity (Ho) are ranged from 0.857 to 1.0 with mean value 0.897. Number of alleles varied between 9-12, with a mean value 9.75 and an expected heterozygosity is also high: ranged from 0.856 at locus VVMD7 to 0.906 at VVMD27 loci, with mean value 0.874 (Table 3).

Tab. 3. Genetic variability of Georgian wild grapevine populations at 4 microsatellite loci.

	ZAG 62	VVMD 27	VVS 2	VVMD 7	Mean
Number of alleles	9	12	9	9	9.75
Observed heterozygosity (Ho)	0.857	0.875	1.0	0.857	0.897
Expected heterozygosity (He)	0.867	0.906	0.867	0.856	0.874

Mean value of genetic variability of Georgian wild grape is much higher compared to wild grape samples from Tunisia and France [Aradhya *et al.*; 2003]. The most heterogeneous locus for Georgian wild grapes is VVMD27. It contains 12 alleles ranged from 175 to 227 bp. 227 bp long allele was outside the size range represented in the literature: In our experiments two such alleles in specimens Q-1 and B-3 were detected [This *et al.*; 2004].

Our previous study revealed, that Georgian grape cultivars are characterized with high level of genetic variability: seven studied varieties at six microsatellite loci generates 50 alleles, with 0.833 average observed heterozygosity, which is much higher than already available data of analyzed *Vitis vinifera* accessions of European countries [Tabidze *et al.*; 2006, Vouillamoz *et al.*; 2006]. Comparison of genetic variability of cultivated and wild grape revealed high level of genetic diversity in both group of plant. Extent of genetic polymorphism in wild accessions is only slightly higher, than in cultural varieties, which confirms the idea, that the wild subspecies *sylvestris* has easily crossed with the cultivated subspecies *vinifera*, producing an array of intermediate forms [Negrul, 1946]. Obviously, specific climatically conditions for this region were more favorable for the natural selection of wild varieties forms, which gave rise to multitude of new grape varieties adapted to these specific area, where they have been selected by the human activities.

CONCLUSION

Genetic diversity analysis of wild grape samples *Vitis vinifera* subsp. sylvestris from different geographic locations of Georgia at four polymorphic microsatellite loci (VVMD7, VVMD27, VVS2, ZAG62) revealed high level of polymorphism, detecting at the same time their individual, unique allelic profile. Genetic polymorphism in wild grape population is slightly higher from what has been observed in cultural varieties. The obtained results confirm strong relationships between the wild and cultivated grapes, also detecting the same evolutionary links between them. Genetic diversity study of wild grape samples is very important from an ethno-botanical standpoint. It helps understanding evolutionary origin of grapevine, geographical origin among wild relatives and process of its diversification. Identification of ancestral grape wild populations can be further applied for the selection and improvement of grapevine varieties.

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BIBLIOGRAPHY

Aradhya M, et al. (2003) Genetic structure and differentiation in cultivated grape, *Vitis vinifera* L. Genet. Res. Camb. 81, 179-192.

Jackson R (1994) Wine Science Principles and Application. Academic Press. New York.

Levadoux L (1956) Les populations sauvages et cultivées de Vitis vinifera. Annales de l'amélioration des Plantes, 6, 59-118.

Lodhi M, et al. (1994) A simple and efficient method for DNA extraction from grapevine cultivars, *vitis* species and *ampelopsis.* Plant Mol. Biol. Reports, 12, 6- 13.

Negrul AM (1946) Ampelography of USSR, v. I, Pishepromizdat, (Frolov-Bagreev A., ed.) Moscow (In Russian).

Sauer JD 1993. Historical Geography of Crop Plants. CRC Press, Boca Raton USA.

Tabidze V, et al. (2006) Microsatellite analysis of *Vitis vinifera* L. Georgian cultivars. Bulletin of the Georgian Academy of Sciences. 174, 333-336.

This P, et al. (2004) Development of a standard set of microsatellite reference alleles for identification of grape cultivars. Theoret. Appl. Genet., 109, 1448-1458.

Vouillamoz JF, et al. (2006) Genetic characterization and relationships of traditional grape cultivars from Transcaucasia and Anatolia. Plant Genetic Resources, 4, 144-158.

Zohary D, Spiegel-Roy P (1975) Beginnings of the fruit growing in the Old World. Science, 187, 319-327.

Zohary D, Hopf M (2000) Domestication of Plants in the Old World. Oxford University Press, Oxford UK.

Zhukowski P (1971) Cultured Plants and their wild relatives. Kolos Edition, Leningrad (In Russian).

RESPUESTA CUALITATIVA Y PRODUCTIVA DEL cv. TEMPRANILLO CULTIVADO EN VASO, EN LAS CONDICIONES SEMIÁRIDAS DEL VALLE DEL DUERO, AL RETRASO EN LA APLICACIÓN DE RIEGO MODERADO

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ABSTRACT

The study aims to analyze the effect of delaying the irrigation over the cycle on grape quality of cv. Tempranillo, head trained, as well as the productive response, in the Duero river Valley. The trial was carried out in 2005-2008 with the following irrigation experimental treatments: P20 (20% ETo, from the vegetative growth stopping) and E20 (20% ETo, from veraison), both irrigated until the week before harvest. The delay of irrigation apply until veraison slightly reduced vegetative growth, while significantly reduced yield (27%) through out its components. The advancement of irrigation apply has favoured total acidity and reduced soluble solids content (ºBrix). The different vine water stress slightly modified berry size, but hardly affected the ratio Fresh skin weight/Berry weight. However, skin of P20 was more hydrated, i.e., had a higher proportion of water. The delay of the application of irrigation has reduced the phenolic substances contained in skins, except of proanthocyanidins, but their effects assessed in relation to each kg of fresh grape, have favoured the amount of phenolic substances tested, due to dilution in berry of the treatment more irrigated.

RESUMEN

El objetivo del trabajo es analizar el efecto del retraso de la época de riego en la calidad de la uva del cv. Tempranillo, así como en la respuesta productiva, en cepas conducidas en vaso en el valle del río Duero. El ensayo se ha llevado a cabo en 2005-2008, con los tratamientos experimentales de época de riego: P20 (20% ETo, a partir de la parada de crecimiento) y E20 (20% ETo, a partir del envero), en ambos casos hasta la semana anterior a la vendimia. El retraso de la aplicación del riego hasta el envero apenas ha reducido el desarrollo vegetativo, mientras que ha reducido apreciablemente el rendimiento a través de sus diversos componentes. El adelanto del riego ha favorecido la acidez total y ha reducido el contenido en sólidos solubles (ºBrix) del mosto. El diferente grado de estrés hídrico de las cepas modificó ligeramente el tamaño de baya, pero apenas incidió en la relación peso de hollejo fresco/peso de baya. Sin embargo, el hollejo de P20 estuvo más hidratado, es decir, mostró mayor proporción de agua. El retraso en la aplicación del riego ha reducido las sustancias fenólicas contenidas en el hollejo, excepto de las proantocianidinas, pero sus efectos, estimados en relación a cada kg de uva fresca, han favorecido la cantidad de las sustancias fenólicas analizadas, debido a la dilución en la baya del tratamiento más regado.

INTRODUCCIÓN

La calidad de la uva tinta viene determinada en gran medida por los compuestos fenólicos existentes en el hollejo y las semillas de la baya, ya que estas sustancias son las responsables del color, en el caso de los antocianos, y la astringencia, el amargor y el

cuerpo, en el caso de las catequinas y las proantocianidinas, de los vinos. Tanto la cantidad global como la distribución de estas sustancias se ven modificadas por una gran variedad de factores edafoclimáticos y de cultivo (Downey et al. 2006). Entre estos factores, la disponibilidad de agua en los diferentes estados fenológicos de la viña ocupa un lugar primordial (García Escudero 1991; Nadal y Arola 1995; Dry et al. 2000; Ojeda et al. 2002; Roby et al. 2004).

Los efectos de la carga de cosecha en la calidad del vino resultan controvertidos, pues parecen estar más relacionados con la capacidad del viñedo (Yuste et al. 2002) que con la propia carga de uva (Bravdo 1984, en Mendez et al. 2009). En relación con este aspecto, lo que es evidente es que el riego favorece, en mayor o menor medida, la respuesta fructífera del viñedo (Prichard 2007, en Mendez et al. 2009), a través de los distintos procesos y componentes del mismo, que pueden verse alterados en función de la fase del ciclo vegetativo en que se aplique el riego y de la intensidad del mismo (Ojeda et al. 2001).

Diferentes estudios han hallado que la incidencia de la disponibilidad hídrica del viñedo es diferente según la familia fenólica de que se trate (Stoll et al. 2000; Kennedy et al. 2001, 2002). Se ha sugerido que los cambios en la concentración de antocianos y taninos se deben principalmente a efectos relacionados con el tamaño de la baya (Roby et al. 2004), pues el riego puede modificar la relación hollejo-pulpa de la baya, alterando la concentración de los compuestos fenólicos, responsables en gran medida de la calidad de los vinos tintos (Ojeda et al. 2002; Roby et al. 2004). Un tamaño de baya menor favorece la relación hollejo/pulpa, aspecto fundamental, ya que la mayor parte de las sustancias que determinan el color, el sabor y el aroma de los vinos se encuentra en la piel de la baya (Singleton 1972; Esteban et al. 2001; Kennedy et al. 2001; Peterlunger et al. 2002). Por otro lado, también se ha comprobado que el déficit hídrico incide en la biosíntesis de estos compuestos en la baya (Roby et al. 2004).

De lo anterior se deduce que el efecto del riego en la composición fenólica de la baya depende en gran medida, además de la dosis, del momento en que se aplique (Jordao et al. 1998; Ojeda et al. 2002; Roby et al. 2004; Santos et al. 2005), pues el estado hídrico de la planta en los distintos estados fenológicos puede llegar a ser más importante para la calidad del mosto que la cantidad total de agua aportada (Matthews et al. 1988, 1990). Así, antes del envero, el estrés hídrico reduce el tamaño potencial de la baya, con lo que aumenta la proporción de hollejo y, por tanto, la de compuestos presentes en el mismo, mientras que el déficit hídrico después del envero puede actuar como estímulo en la síntesis de compuestos fenólicos y, finalmente, durante la maduración influye en la cantidad de azúcares y muy especialmente en la de compuestos fenólicos acumulados durante dicho periodo.

Dado que son pocos los estudios que existen sobre la incidencia del riego en viñedos conducidos en vaso y que gran parte del viñedo español está conducido con este sistema, el cual está comenzando a ser regado en muchos casos, se considera necesario un trabajo de investigación de este tipo. Así, el trabajo, englobado dentro de un amplio estudio sobre la incidencia de dosis y época de riego en el *cv.* Tempranillo, analiza el efecto de la época de riego en la calidad de la uva, así como en la respuesta productiva, en cepas conducidas en vaso en el valle del río Duero.

MATERIAL Y MÉTODOS

El ensayo se ha llevado a cabo en el periodo 2005-2008 en Valladolid, en el valle del río Duero (España). Sobre la base de una dosis moderada de riego, se han aplicado los siguientes tratamientos experimentales de época de riego: P20 (20% ETo, a partir de la parada de crecimiento) y E20 (20% ETo, a partir del envero), en ambos casos hasta la semana anterior a la vendimia. La cantidad total de agua aplicada en el verano, a través de

una aportación semanal de riego por goteo, así como la pluviometría de cada año de estudio, está reflejada en la tabla 1. El diseño experimental fue en bloques al azar con 4 repeticiones y una parcela elemental de 9 cepas de control. En el suelo del viñedo experimental, plantado en 1993, se distinguen tres horizontes cuyas principales características se detallan en la tabla 2. La mayor parte del sistema radicular del viñedo se sitúa en los 60 cm más superficiales. Las cepas de Tempranillo/110R, con un marco de plantación de 2,7 m x 1,4 m, se han conducido en espaldera, con una poda corta en cordón Royat bilateral de 12 yemas por cepa. La temperatura media de los meses de junio, julio, agosto y septiembre del periodo 2005-2008 fue respectivamente de 19,2 ºC, 21,9 ºC, 20,6 ºC y 17,3 ºC.

Tabla 1. Riego total (mm) aplicado en los tratamientos E20 y P20 y pluviometría anual (mm) en 2005, 2006, 2007 y 2008.

	2005	**2006**	**2007**	**2008**
E20	50,3	37,5	25,8	33,5
P20	77,8	66,2	63,5	78,3
P	301	454	550	490

Las determinaciones experimentales se han orientado hacia la medida del desarrollo vegetativo, la producción y la calidad de la uva. El desarrollo vegetativo se determinó a través del peso de la madera de poda y del conteo de los sarmientos de cada cepa. El rendimiento en uva se determinó en el momento de la vendimia mediante conteo y peso de los racimos de cada cepa. En la fecha de vendimia se realizó un muestreo de bayas para el análisis de los componentes del mosto (concentración de azúcares, acidez total, pH y compuestos fenólicos). La concentración de sólidos solubles (ºBrix) se midió mediante refractometría. La acidez total (g ac. Tartárico/L) y el pH se determinaron según la metodología oficial CEE.

Los hollejos de las bayas se pesaron, antes y después de ser liofilizados, a fin de determinar su contenido en agua. La extracción de compuestos del hollejo se realizó añadiendo 4 mL de Metanol/Fórmico (95:5) a 0,5 g de una muestra homogeneizada en tubos de ensayo color topacio. Tras agitar en vortex y dejar 5 minutos en la oscuridad, se centrifugó a 2.500 rpm durante 5 minutos. La operación se repitió hasta conseguir un sobrenadante casi incoloro. Todos los sobrenadantes se reunieron en un matraz y se completó el volumen hasta 50 mL con Metanol/Fórmico (95:5). En el extracto se determinaron compuestos polifenólicos totales (expresados en mg ácido gálico/L), antocianos totales (en mg de cloruro de malvidina), catequinas (en mg de catequina) y proantocianidinas (en mg de cloruro de cianidina) mediante los correspondientes métodos espectrofotométricos (Riberéau-Gayon et al. 1999). Se llevaron a cabo 3 extracciones de cada repetición y se efectuó un análisis duplicado de cada una de ellas. La biosíntesis de compuestos fenólicos se ha estimado a través de la sustancia contenida en cada g de hollejo liofilizado, así como indirectamente a través de la concentración referida a cada kg de uva.

Los resultados se han sometido a análisis estadístico ANOVA-2 mediante el programa de análisis estadístico MSTAT-C (versión 2.0).

Tabla 2. Características físicas de los horizontes presentes en el suelo del viñedo experimental.

Profundidad (cm)	Elementos gruesos (%)	Textura U.S.D.A.			Clase textural
		Arena	Limo	Arcilla	
0-20	70,7	45,3	19,4	35,3	AcAr
20-45	68,8	47,4	19,5	33,1	FrAcAr
45-100	74,8	61,4	9,5	29,1	FrAcAr

RESULTADOS Y DISCUSIÓN

a. Desarrollo vegetativo

El peso de madera de poda se ha visto ligeramente beneficiado por el adelanto del inicio del riego, pues el tratamiento P20 ha mostrado valores ligeramente superiores al E20 en 3 de los 4 años de estudio (excepto en 2008), alrededor de un 5,5% de incremento medio, aunque sin diferencias estadísticamente significativas (tabla 3). El número de sarmientos totales por cepa sólo ha mostrado diferencias estadísticamente significativas, favorables al tratamiento P20, en 2006, debidas a la mayor proliferación de chupones, mientras que el resto de años el número de sarmientos fue similar en ambos tratamientos. El peso del sarmiento no ha mostrado diferencias constantes entre tratamientos, en ningún caso significativas, aunque P20 superó en un 15% a E20 en 2006.

En definitiva, el desarrollo vegetativo apenas ha sido levemente sensible a la época de aplicación del riego y, consecuentemente, al déficit hídrico inducido en E20 hasta el envero, con una tendencia ligeramente favorable al tratamiento cuyo riego se inicia antes, el P20.

Tabla 3. Madera de poda (kg/cepa); número de sarmientos totales; peso del sarmiento (g), de los tratamientos: P20 (riego de 20% ETo desde Parada de crecimiento) y E20 (riego de 20% ETo desde Envero), en 2005, 2006, 2007 y 2008. Nivel de significación estadística (Sig.): * = p < 5%; ** = p < 1%.

	2005		2006		2007		2008	
	E20	**P20**	**E20**	**P20**	**E20**	**P20**	**E20**	**P20**
Madera poda	0,89	0,94	0,86	0,91	1,88	1,98	1,77	1,74
Sarmientos	15,8	15,6	14,2	16,1**	17,6	17,7	14,7	14,0
Peso sarmiento	56	61	61	59	106	122	122	119

b. Rendimiento

Los tratamientos aplicados han provocado diferencias en el rendimiento aunque no hayan resultado estadísticamente significativas ningún año de estudio (tabla 4). El rendimiento alcanzado por P20 fue mayor que el de E20, con un aumento del 28%, 15%, 48% y 70% en 2005, 2006, 2007 y 2008 respectivamente. El rendimiento claramente inferior en 2007 y 2008 con respecto a 2005 y 2006 se debió a algunos problemas sanitarios ocurridos en las cepas en dichos años, que afectaron bastante a su rendimiento. El rendimiento de P20 se debió tanto al número de racimos como, sobre todo, al peso del racimo, aunque las diferencias en el número de racimos no han sido estadísticamente significativas. Las diferencias en el peso del racimo han sido estadísticamente significativas en 2007 y 2008. El mayor tamaño del racimo del tratamiento P20 se ha debido, en ambos años, al mayor número de bayas por racimo, ya que el incremento del peso de la baya en P20 ha sido menos notable. El peso de la baya ha mostrado la misma tendencia que el peso del racimo, mayor en el tratamiento P20, aunque las diferencias no hayan sido estadísticamente significativas ninguno de los 4 años, como consecuencia directa del estrés hídrico sufrido por el tratamiento E20 en julio y parte de agosto.

En definitiva, la modificación de la fecha de comienzo del riego ha supuesto una variación apreciable del rendimiento y de sus componentes.

Tabla 4. Rendimiento (t/ha), número de racimos por cepa, peso del racimo (g) y peso de la baya (g), de los tratamientos: P20 (riego de 20% ETo desde Parada de crecimiento) y E20 (riego de 20% ETo desde Envero), en 2005, 2006, 2007 y 2008. Nivel de significación estadística (Sig.): * = p < 5%; ** = p < 1%.

	2005		2006		2007		2008	
	E20	**P20**	**E20**	**P20**	**E20**	**P20**	**E20**	**P20**
Rendimiento	11,0	14,2	15,4	17,7	4,1	6,0	4,4	7,5
Racimos/cepa	18,4	19,5	19,2	23,1	16,7	16,5	14,5	17,1
Peso Racimo	216	268	204	206	92	137*	113	163*
Peso Baya	1,4	1,5	1,8	1,7	2,1	2,3	1,9	2,2

c. Calidad de la uva

c.1. Composición básica del mosto

La época de aplicación del riego ha afectado de forma variable a diversos aspectos de la composición básica de la uva (tabla 5). La concentración de sólidos solubles fue ligeramente superior en E20 que en P20, la mayoría de los años, excepto en 2005, sin que las diferencias fueran estadísticamente significativas. Este resultado parece estar relacionado con el menor rendimiento del tratamiento regado desde el envero (E20) en comparación con el regado desde la parada de crecimiento (P20), que también se corresponde con una reducción de tamaño de la baya, en general. La acidez total alcanzó un valor ligeramente mayor en el tratamiento P20, a la inversa que el pH, sin diferencias estadísticamente significativas en ninguno de los 4 años.

Tabla 5. Concentración de sólidos solubles (ºBrix), acidez total (g ac. tartárico/l) y pH, de los tratamientos: E20 (riego de 20% ETo desde Envero) y P20 (riego de 20% ETo desde Parada de crecimiento), en 2005, 2006, 2007 y 2008. Nivel de significación estadística (Sig.): * = p < 5%; ** = p< 1%.

	2005		2006		2007		2008	
	E20	**P20**	**E20**	**P20**	**E20**	**P20**	**E20**	**P20**
Sólidos solubles	21,4	22,4	23,7	22,3	22,7	22,4	23,7	23,2
Acidez total	4,53	4,63	4,96	5,03	5,98	6,05	5,99	6,16
pH	3,45	3,46	3,80	3,71	3,38	3,33	3,45	3,37

c.2. Constitución de la baya

El estrés hídrico puede alterar en diferente medida tanto la proporción de los diferentes constituyentes de la baya como la composición de éstos según han observado diversos autores. En este sentido, el diferente grado de estrés hídrico al que se vieron sometidas las cepas de los tratamientos aplicados apenas incidieron en la relación Peso de hollejo fresco/Peso de baya, como también se había visto en estudios realizados por nuestro grupo de investigación cuando se aplicaron los mismos tratamientos a cepas conducidas en espaldera (Valdés et al. 2008). Sin embargo, se ha observado un grado diferente de hidratación de los hollejos, de manera que en general los hollejos de las bayas de P20 (más riego) contienen mayor proporción de agua, derivada de que, en todas las campañas estudiadas, el peso de hollejo liofilizado fue mayor en E20 que en P20 (tabla 6). Este resultado es la causa de las diferencias de tendencia encontradas en la composición fenólica de los hollejos según se analicen los resultados en gramos de sustancia/gramo de hollejo liofilizado o en gramos de sustancia/kg de uva, como se puede ver a continuación.

c.3. Composición fenólica

Los resultados referidos a gramo de hollejo liofilizado muestran que el retraso en la aplicación del riego ha supuesto en todas las campañas una reducción de la cantidad de sustancias fenólicas totales (IPT en mg de gálico) contenidas en el hollejo. La cantidad de antocianos sintetizados en 2006, sobre todo, y 2007 fue mayor en el tratamiento más regado, P20, mientras que en 2008 fue similar en ambos tratamientos. La cantidad de catequinas sintetizadas por el tratamiento más regado fue mayor en todos los años de estudio. Contrariamente a las sustancias fenólicas citadas, y en concordancia con lo hallado por Jordao et al. (1998), la cantidad de proantocianidinas sintetizadas por el tratamiento más regado fue menor. Con excepción de las proantocianidinas y considerando las particularidades de cada campaña, los resultados parecen confirmar que la biosíntesis de sustancias fenólicas puede verse limitada por un fuerte estrés en el período de preenvero (Ojeda et al. 2002), aunque también aparece en 2007 y 2008 la relación positiva entre la biosíntesis de sustancias fenólicas y el tamaño de baya (Roby et al. 2004; Tarter y Keuter 2005), lo que, sin embargo, ha contribuido a una mayor dilución de dichas sustancias del hollejo en el conjunto de la baya.

Así, los resultados expresados en mg de sustancia/kg de uva fresca, debido a la dilución en el conjunto de la baya, muestran que el tratamiento más regado (P20) presenta un contenido de polifenoles totales claramente inferior, incluso en 2006, año en que el peso de baya fue menor que el de E20, dado que el hollejo de P20 está mucho más hidratado. Por la misma razón, la cantidad de compuestos antociánicos referidos a kg de uva ha sido claramente menor en el tratamiento más regado. Asimismo, el contenido de catequinas ha sido mayor en E20 que en P20, debido a la diferente proporción de agua de los hollejos, aunque en el último año la diferencia en dicha proporción fue menor y consecuentemente el contenido de catequinas/kg de uva resultó muy similar en ambos tratamientos. Finalmente, el contenido de proantocianidinas, que ya resultó mayor al referirlo a gramo de hollejo liofilizado, aumenta claramente la diferencia entre tratamientos a favor del menos regado, E20, al referirlo a kg de uva.

Tabla 6. Peso fresco de la baya (g), Peso seco del hollejo (g), Índice de polifenoles totales (IPT) (mg ácido gálico/g de hollejo seco), Índice de polifenoles totales (IPT) (mg ácido gálico/kg de uva), Antocianos (mg/g de hollejo seco), Antocianos (mg/kg uva), Catequinas (mg/g hollejo seco), Catequinas (mg/kg uva), Proantocianidinas (mg/g hollejo seco), Proantocianidinas (mg/kg uva), de los tratamientos: E20 (riego de 20% ETo desde Envero) y P20 (riego de 20% ETo desde Parada de crecimiento), en 2006, 2007 y 2008. Nivel de significación estadística (Sig.): * = p < 5%; ** = p < 1%.

	2006		2007		2008	
	E20	**P20**	**E20**	**P20**	**E20**	**P20**
Peso Fresco Baya (g)	1,95	1,88	2,10	2,22	2,25	2,37
Peso Seco Hollejo (g)	0,121*	0,072	0,174**	0,060	0,179*	0,155
IPT (mg ác. gálico/g hollejo seco)	102,7	122,3	82,4	93,9	64,0	70,4
IPT (mg ác. gálico/kg uva)	6231*	4759	6834**	2554	5077	4614
Antocianos (mg/g hollejo seco)	14,46	19,04*	14,95	15,94	12,57	11,48
Antocianos (mg/kg uva)	876*	739	1240**	434	1001*	745
Catequinas (mg/g hollejo seco)	1,72	2,30	1,52	1,54	1,06	1,32
Catequinas (mg/kg uva)	1053	876	1231	419	844	857
Proantocianidinas (mg/g hollejo seco)	8,78	5,08	5,75	5,34	7,09	6,42
Proantocianidinas (mg/kg uva)	5473*	1929	4962	1452	5643	4161

CONCLUSIONES

El retraso de la aplicación del riego hasta el envero apenas ha reducido el desarrollo vegetativo del viñedo de Tempranillo, mientras que ha provocado una reducción apreciable del rendimiento (27%) a través de sus diversos componentes. El adelanto del riego ha favorecido la acidez total del mosto, pero ha tendido a reducir el contenido en sólidos solubles (ºBrix) en la mayoría de los años. El diferente grado de estrés hídrico al que se vieron sometidas las cepas de los tratamientos aplicados modificó ligeramente el tamaño de la baya, pero apenas incidió en la relación peso de hollejo fresco/peso de baya, sin embargo, el hollejo del tratamiento regado desde más temprano estuvo más hidratado, es decir, mostró una mayor proporción de agua.

El retraso en la aplicación del riego ha supuesto en general una reducción de las sustancias fenólicas contenidas en el hollejo, excepto de las proantocianidinas, pero sus efectos, estimados en relación a cada kg de uva fresca, han favorecido la cantidad de las sustancias fenólicas analizadas, debido a la dilución de las mismas en el conjunto de la baya que sufre el tratamiento más regado.

REFERENCIAS BIBLIOGRÁFICAS

DOWNEY M.O., DOKOOZLIAN N.K., KRSTIC M.P., 2006. Cultural practice and environmental impacts on the flavonoid composition of grapes and wine: a review of recent research. *Am. J. Enol. Vitic*., 57 (3): 257-268.

DRY P.R., LOVEYS B.R., DURING H., 2000. Partial drying of the rootzone of grape I. Transient changes in shoot growth and gas exchange. *Vitis*, 39: 3-7.

ESTEBAN M.A., VILLANUEVA M.J., LISSARRAGUE J.R., 2001. Effect of irrigation on changes in the anthocyanin composition of the skin of *cv*. Tempranillo (*Vitis vinifera* L.) grape berries during ripening. *J. Sci. Food Agriculture*, 81 (4): 409-420.

GARCÍA ESCUDERO E., 1991. Influencia de la dosis y del momento de aplicación del riego sobre la producción, desarrollo vegetativo, calidad del mosto y nutrición mineral en la vid (*Vitis vinifera* L.). Departamento de producción Vegetal: Fitotecnica. *Tesis Doctoral*. Universidad Politécnica de Madrid.

JORDAO A., DA SILVA R., LAUREANO O., 1998. Influence of irrigation on phenolic composition of Touriga Francesa (*Vitis Vinifera* L.). *Cienc. Tecnol. Aliment.*, 2: 60-73.

KENNEDY J.A.. HAYASAKA Y., VIDAL S., WATERS E.J., JONES G.P., 2001. Composition of grape skin proanthocyanidins at different stages of berry development. *J. Agric. Food Chemistry*, 49 (11): 5348-5355.

KENNEDY J.A., MATTHEWS M.A., WATERHOUSE A., 2002. Effect of maturity and vine water status on grape skin and wine flavonoides. *Am. J. Enol. Vitic.*, 53 (4): 268-274.

MATTHEWS M.A., ANDERSON M.M., 1988. Fruit ripening in *Vitis vinifera* L.: Responses to seasonal water deficits. *Am. J. Enol. Vitic.*, 39 (4): 313-320.

MATTHEWS M.A., ISHII R., ANDERSON M.M., O'MAHONY M., 1990. Dependence of wine sensory attributes on vine water status. *J. Sci. Food Agriculture*, 51: 321-335.

MENDEZ M., SANCHEZ L., DOKOOZLIAN N., 2009. Interaction between crop load and irrigation management during late season, effects on vine water status and fruit composition of Merlot grapevines. *16th International GiESCO Symposium*, 12-15 Julio, 2009. Universidad de California, Davis (CA, USA).

NADAL M., AROLA L., 1995. Effects of limited irrigation on the composition of must and wine of Cabernet Sauvignon under semi-arid conditions. *Vitis*, 34 (3): 151-154.

OJEDA H., DELOIRE A., CARBONNEAU A., 2001. Influence of water deficits on grape berry growth. *Vitis*, 40 (3): 141-145.

OJEDA H., ANDARY C., KRAEVA E., CARBONNEAU A., DELOIRE A., 2002. Influence of pre- and postveraison water deficit on synthesis and concentration of skin phenolic compounds during berry growth of *Vitis vinifera* cv. Shiraz. *Am. J. Enol. Vitic.*, 53 (4): 261-267.

PETERLUNGER E.M, SIVILOTTI P., BONETTO C., PALADIN M., 2002. Water stress induces changes in polyphenol concentration in Merlot grapes and wines. *Rivista Vitic. Enol.*, 55 (1): 51-66.

RIBEREAU-GAYON P., GLORIES Y., MAUJEAN A., DUBORDIEU D., 1999. Handbook of Enology. Ed. Wiley and sons.

ROBY G., HARBERTSON J.F., ADAMS D.A., MATTHEWS M.A., 2004. Berry size and vine water deficits as factors in winegrape composition: Anthocyanins and tannins. *Aus. J. Grape Wine Research*, 10 (2): 100-107.

SANTOS T.P., LOPES C.M., RODRIGUES M.L., 2005. Effects of partial rootzone drying irrigation on cluster microclimate and fruit composition of field-grown Castelao grapevines. *Vitis***,** 44 (3): 117-125.

SINGLETON V.L., 1972. Effects on red wine quality of removing juice before fermentation to simulate variation in berry size. *Am. J. Enol. Vitic.*, 23: 106-113.

STOLL M., LOVEYS B., DRY P., 2000. Hormonal changes induced by partial rootzone drying of irrigated grapevine. *J. Experimental Botany*, 51: 1627-1634.

TARTER M.E., KEUTER S.E., 2005. Effect of rachis position on size and maturity of Cabernet Sauvignon berries. *Am. J. Enol. Vitic.*, 56 (1): 86-89.

VALDÉS E., ALBURQUERQUE M.V., GAMERO E., RUBIO J.A., YUSTE, J., 2008. Influencia del régimen hídrico en la composición fenólica de la variedad Tempranillo en el valle del Duero. XXXI Congreso Mundial de la Viña y el Vino, junio, Verona (Italia).

YUSTE J., GIL M., YUSTE R., 2002. Vaso y cordón vertical: análisis preliminar del desarrollo vegetativo y productivo, y del comportamiento en maduración de Tempranillo en la D.O. Ribera del Duero. *La Semana Vitivinícola*, 2937: 3990-4000.

EFFECTS OF COVER CROP IN PHYSIOLOGICAL, AGRONOMIC AND QUALITATIVE BEHAVIOUR OF TEMPRANILLO cv IN THE SOIL AND CLIMATE CONDITIONS OF THE A.O. RUEDA

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ABSTRACT

The study is focused on understanding the behavior of Tempranillo cv grown with different soil management alternatives. The trial was conducted under non irrigation conditions, during the period 2006-2009, with the following treatments: TIL: traditional tillage; BAR: barley (*Hordeum vulgare*); LEG: locust bean (*Vicia monanthos*) (2006) and vetch (*Vicia sativa*) (2007 to 2009); PER: natural grassing (2006) and fescue (*Festuca orundinacea*) with Ray-gras (*Lolium perenne*) 50% (2007 to 2009). The cultivation of cover crop species in the space between rows has variably affected vine water status and physiological activity, reducing leaf development and grape yield with respect to traditional tillage cultivation. The effects have been more pronounced with the cover crop of legume species and permanent grassing than with barley. The influence of cover crop on grape quality has depended on the cover crop species, as well as the annual conditions and level of yield reached.

RESUMEN

El objetivo del trabajo es conocer la respuesta del cv Tempranillo sometido a diversas alternativas de manejo del suelo. El ensayo se ha llevado a cabo en secano, en 2006-2009, con los siguientes tratamientos experimentales: TIL: laboreo tradicional; BAR: cebada (*Hordeum vulgare);* LEG: algarroba parda (*Vicia monanthos)* y veza (*Vicia sativa)*; PER: enyerbado natural y Festuca (*Festuca arundinacea)* con Ray-gras (*Lolium perenne)* al 50 %. El cultivo de cubierta vegetal en la calle ha afectado de forma variable al estado hídrico y a la actividad fisiológica del viñedo, reduciendo su desarrollo foliar y el rendimiento en uva respecto al cultivo mediante laboreo. Los efectos han sido más pronunciados con la cubierta de especies leguminosas y la de enyerbado permanente que con la de cebada. La influencia de la cubierta vegetal en la calidad de la uva ha dependido de la especie cultivada, así como de las condiciones anuales y del nivel de rendimiento alcanzado.

INTRODUCTION

Soils in wood crops, as the vineyard, suffer severe erosion problems, especially in drought conditions, so that the use of grass is employed as a method to combat erosion (Jimenez et al. 2007). In addition, problems of change and resistant emergence of flora, as well as the growing ecological pressure, bring into question the use of chemical herbicides (Morlat et al. 1993). That is why the vineyard soil maintenance is changing in recent years and the use of plant species covering the soil between the rows of vines is increasing. On the other hand, excessive vigor and yield are enhanced by both tillage and soil maintenance by herbicides. The beneficial effects of the use of cover crop (Ludvigsen 2002) include improvement of soil characteristics, limiting of water runoff and erosion (Coulon and Prud'homme 2003, Murisier 1986), vigor and yield reduction, reduction of Botrytis attack and microclimate improvement of vineyard (Lopes et al. 2004), which can help to improve quality and increase biodiversity. Also, the cover crop growing in the space between rows promotes biological activity and important effects on

the terroir (Doledec et al. 2003). The interaction between vines and cover crop in relation with the water demand as well as with nitrogen and nutrients demand, and the physiological activity that vines can develop influenced by competition from other growing species, are still not well known and there are a lack of quantitative data of the effects in many areas and environmental conditions.

Thus, the purpose of the trial carried out has been to know the physiological and agronomic performance of Tempranillo variety subjected to different soil management alternatives. In particular, it aims to analyze photosynthetic potential, water stress, leaf development and the productive response of vines, as affected by different cover crops, and their impact on grape quality in the Appelation of Origin Rueda, in the Duero river valley region, in Spain.

MATERIAL AND METHODS

The trial, located in the town of Nieva (Segovia province), belonging to the Appelation of Origin Rueda, was carried out with Tempranillo/110R. The planting was done in 2000, at a distance of 3.0 m between rows and 1.25 m between vines. The vineyard has been growing without irrigation, with rainfall of 443, 469, 421 and 252 mm in 2006, 2007, 2008 and 2009 respectively. The soil where the trial is established has sandy structure fairly homogeneous from the surface to 110 cm deep. From this depth the emergence of the water table can be found. The training has been bilateral Royat cordon with vertical positioning of the vegetation. The bud load was the same in all treatments, maintaining after green pruning about 1 shoot every 10 cm of cordon.

The treatments tested were as follows: TIL: traditional tillage; BAR: barley (*Hordeum vulgare*); LEG: carob bean (*Vicia monanthos*) (2006) and vetch (*Vicia sativa*) (2007-2009); ENY: perennial vegetation or natural grassy (2006) and fescue (*Festuca orundinacea*) with Ray-gras (*Lolium perenne*) 50% (2007-2009). The data were collected during the years 2006, 2007, 2008 and 2009. The ground cover crops were planted in prior October of each year. The line of vines remained without vegetative competition, by means of mechanical labour between vines. In the second half of May green operations of pruning were applied on vines, removing shoots that come from old wood buds and secondary shoots of regular pruning buds. The experimental design consisted of randomized blocks with 4 replication per treatment, being the elemental plot of 150 vines, with 50 control vines per replication. Experimental determinations have been made to estimate photosynthetic activity of vines at 9 and 12 hours of sunlight, vine water status (through leaf water potential measured at 9, 12 and 15 h), leaf development (by leaf area index, LAI), grape production, global productivity (through dry matter of the renewable parts of the vine) and grape composition.

RESULTS AND DISCUSSION

Leaf water potential at 9:00

Leaf water potential measured at 9:00 (solar time) in 2006, 2007 and 2008 has shown a steady decline throughout the cycle ending with values close to -1.6 MPa, depending on the year (table 1). Legume treatment (LEG) has shown unfavorable differences with other treatments that have been statistically significant in June 2007 and July 2008, although the treatment PER has shown values close to LEG, or even higher, with some frequency. Definitely, water status of individual leaf at 9:00 has not be very different between treatments, although treatments TIL and BAR have shown, in general, less negative values than LEG and PER, so, lower water stress than the vines of these latter treatments.

Table 1. Leaf water potential (-MPa) measured at 9:00, for treatments TIL, BAR, LEG and PER, in 2006, 2007 and 2008. Statistical significance level (Sig): ** p < 1%, * p < 5%, - not significant.

	2006	2007			2008			
	24 Jul	19 Jun	24 Jul	4 Sep	4 Jul	21 Jul	20 Aug	15 Sep
TIL	1.29	0.54[ab]	0.99	1.50	0.60	0.71[b]	1.28	1.61
BAR	1.26	0.51[b]	1.03	1.44	0.66	0.86[b]	1.32	1.59
LEG	1.32	0.58[a]	1.06	1.47	0.69	1.08[a]	1.30	1.62
PER	1.34	0.49[b]	1.13	1.48	0.53	0.86[b]	1.24	1.56
Sig.	-	*	-	-	-	**	-	-

Leaf water potential at 12:00

The leaf water potential was measured at 12 hours (solar time) in 2006, 2007 and 2008. In 2006, potential values were very similar for all treatments, being the grassing treatment (PER) which showed more negative values in July and August. In September, water stress was intensified, becoming similar in all treatments (table 2). The observed differences were not statistically significant. Water potential in 2007 was down from -0.55 in June, to about -2.0 MPa in September similarly in all treatments. Treatments LEG and PER quite often showed more negative values than the other treatments, although the differences were not statistically significant at any time of the cycle. In 2008, the differences between treatments were higher in early summer, showing treatment LEG more negative values than the rest, with statistically significant differences in the two measurements made in July. Definitely, the potential measured at 12 hours has shown, in general, a tendency of higher water stress for treatments LEG and PER, and better leaf water status of treatment TIL, mostly, and BAR.

Table 2. Leaf water potential (-MPa) measured at 12:00, for treatments TIL, BAR, LEG and PER, in 2006, 2007 and 2008. Statistical significance level (Sig): ** p <1%, * p <5% - not significant.

	2006					2007						2008			
	22 Jun	24 Jul	14 Aug	31 Aug	19 Sep	19 Jun	4 Jul	24 Jul	13 Aug	4 Sep	24 Sep	4 Jul	21 Jul	20 Aug	15 Sep
TIL	1.02	1.27	1.40	1.53	1.74	0.59	0.75	1.08	1.28	1.57	1.94	0.65[b]	0.86[b]	1.31	1.82
BAR	1.12	1.31	1.39	1.53	1.71	0.57	0.73	1.13	1.33	1.52	1.98	0.69[b]	0.99[b]	1.39	1.88
LEG	1.10	1.31	1.43	1.49	1.74	0.64	0.76	1.18	1.32	1.57	2.05	0.79[a]	1.25[a]	1.37	1.82
PER	1.11	1.36	1.55	1.59	1.64	0.55	0.76	1.19	1.35	1.60	1.96	0.64[b]	0.97[b]	1.39	1.78
Sig.	-	-	-	-	-	-	-	-	-	-	-	*	**	-	-

Leaf water potential at 15:00

The treatments applied did not affect leaf water potential measured at 15:00 hours (solar time) in 2007 and 2008 (table 3). Potential values were very similar in all four treatments, not having noticed, of course, statistically significant differences between them none of the two years.

Table 3. Leaf water potential (-MPa) measured at 15:00, for treatments TIL, BAR, LEG and PER, in 2007 (4 September) and 2008 (15 September). Statistical significance level (Sig): ** p < 1%, * p < 5%, - not significant.

2007	TIL	BAR	LEG	PER	Sig.	2008	TIL	BAR	LEG	PER	Sig.
	1.50	1.49	1.48	1.49	-		1.70	1.76	1.75	1.73	-

Photosynthesis at 9:00

Net photosynthesis measured at 9:00 (solar time) in 2006, 2007 and 2008, showed differences between treatments, with higher values, in general, in treatment TIL than in the

other treatments (table 4). In both years, 2006 and 2007, treatments BAR and TIL showed higher photosynthesis rates in July than PER and LEG, with statistically significant differences in 2006. In 2008, the evolution of photosynthesis throughout the cycle showed that treatment TIL reached higher rates in July than the other treatments, with statistically significant differences on July 21st with respect to LEG and PER (table 4). In August and September, when the level of photosynthesis is lower in general, treatments TIL and BAR approach their photosynthesis rates to those of LEG and PER.

Photosynthesis at 12:00

Photosynthesis was measured at 12 hours (solar time) in July 2007 and throughout the summer of 2008 (table 4). In general, treatment TIL showed the highest rate of photosynthesis at this time, with greater differences in the month of July compared to other treatments, being statistically significant the differences between treatments on July 21st, 2008. The evolution of photosynthesis has shown, in 2008, a clear decrease in the months of the 2nd half of summer, resulting all treatments with a similar photosynthesis rate in August and September.

Table 4. Net photosynthesis measured at 9:00 and 12:00 (solar time) of treatments TIL, BAR, LEG and PER, in 2006 (July 24), 2007 (July 24) and 2008 (July 4 and 21, August 20 and September 15). Statistical significance level (Sig): ** p < 1%, * p < 5%, - not significant.

	2006	2007		2008							
	9 hs	9 hs	12 hs	9 hs				12 hs			
	24 Jul	24 Jul		4 Jul	21 Jul	20 Aug	15 Sep	4 Jul	21 Jul	20 Aug	15 Sep
TIL	9,0[a]	14,1	11,0	21,5	18,2[a]	7,2	6,1	16,5	17,1[a]	6,1	4,2
BAR	9,2[a]	12,2	9,3	20,0	17,2[a]	8,9	5,9	16,2	14,5[ab]	5,2	4,2
LEG	6,5[ab]	10,8	9,1	20,0	10,6[b]	8,1	5,2	16,2	10,8[b]	6,2	4,1
PER	4,4[b]	9,6	8,0	20,4	14,7[ab]	8,4	5,6	15,4	15,0[ab]	6,4	3,9
Sig.	**	-	-	-	*	-	-	-	*	-	-

Leaf development

The total leaf area (LAI) was measured in 2007 and 2008. In general, leaf development was higher in 2008 than in 2007, reaching 2.5 m^2/m^2 in treatment TIL (table 5). The competition carried out by the soil maintenance treatment to the vines was lower in treatments TIL and BAR than in LEG and PER, in 2007 as well as in 2008. Otherwise, treatment PER got a leaf development at the end of the cycle of only 1.7 m^2 and 2.0 m^2 per each m^2 of soil, in 2007 and 2008, below, increasingly, of treatments LEG, BAR and TIL, which has shown greater development correspondent to a lower leaf water stress of the vines. The differences were not statistically significant neither in 2007 nor in 2008.

Table 5. Leaf area index (LAI) (m^2/m^2) of treatments TIL, BAR, LEG and PER, in 2007 and 2008. Level of statistical significance (Sig): ** p < 1%, * p < 5%, - not significant.

	2007		2008
	Jul	Sep	Aug
TIL	1,69	2,11	2,53
BAR	1,85	2,00	2,42
LEG	1,88	1,93	2,22
PER	1,74	1,72	2,01
Sig.	-	-	-

Yield

The use of cover crops between rows of vines has provoked significant differences in yield among treatments, so that vines with Tillage had higher production, with some proximity of treatment BAR, except in 2008 when Tillage was slightly exceeded by this treatment (BAR). Treatment TIL caused an average increase of yield, in overall years, of 7%, 31% and 34%,

compared to treatments BAR, PER and LEG respectively, with significant differences in 2006 and 2007 (figure 1). This yield increase was mainly due to cluster weight, which was significantly higher in TIL and BAR than in PER and LEG. Berry weight was also higher in TIL and BAR than in LEG and PER, although the differences were statistically significant only in 2006 and 2009. With regard to fertility, expressed as number of clusters per vine, treatment LEG has resulted less fertile while BAR and TIL have been more fertile, with statistically significant differences in 2007 and 2008. to sum up, the cultivation of a cover crop of any of the species tested, as opposed to the tilled soil, has caused a decrease in yield more evident with a legume species or with grassy cover than with a barley one.

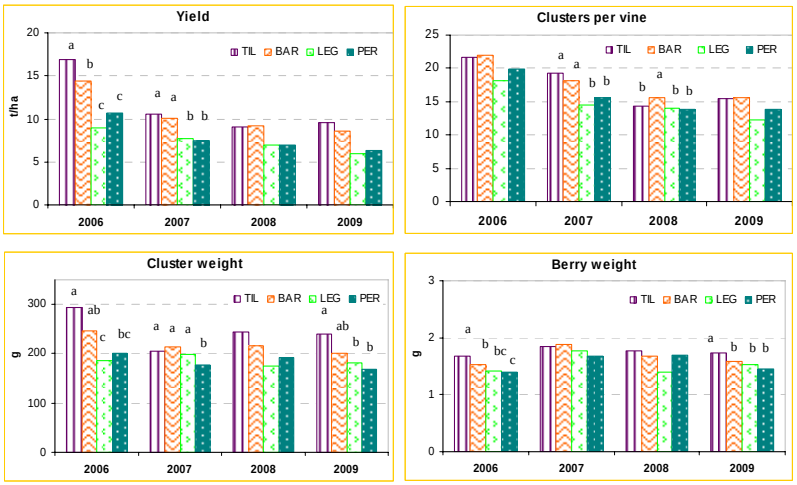

Figure 1. Yield (t/ha), number of clusters per vine, cluster weight (g) and berry weight (g) of treatments TIL, BAR, LEG and PER, in 2006, 2007, 2008 and 2009. Statistical significance level ($p < 5\%$): different letters indicate significant differences between means.

Dry matter of vines

Dry matter of vines was evaluated in 2007 and 2008. The use of cover crops between rows of vines has resulted in significant differences of productivity between treatments, so that the vines of treatment TIL had higher productivity, with a certain proximity of treatment BAR, than the vines with treatments LEG and PER. Treatment TIL caused an average increase in productivity, as average value of 2007 and 2008, of 3%, 25% and 30% with respect to treatments BAR, LEG and PER respectively, whereas treatment BAR provoked an average increase of 21% and 26% with respect to the previous treatments, with statistically significant differences in both years (table 6).

Table 6. Dry matter (g/m^2) of treatments TIL, BAR, LEG and PER, in 2007 and 2008. Statistical significance level (Sig): ** $p < 1\%$, * $p < 5\%$, - not significant.

	2007	2008
TIL	434[a]	432[a]
BAR	422[a]	422[a]
LEG	352[b]	343[b]
PER	327[b]	340[b]
Sig.	**	*

Grape quality

Sugar concentration has been slightly affected by the type of soil maintenance applied. The tendency has not been the same every year, possibly due to the different environmental conditions, and the annual yield level achieved. In 2006, treatment LEG showed a concentration greater than the other three treatments (1.5 °Brix more than BAR and PER and

2.7 °Brix more than TIL), being these differences statistically significant. In 2007 and 2008 the sugar concentration was very similar in all four treatments applied, as well as in 2009, even with the sugar concentration higher in LEG and lower in TIL that year. On the other hand, the titratable acidity has maintained a similar tendency each year, showing the treatment TIL significantly higher values than the other treatments (except in 2008), while treatment PER presented the lowest values.

Regarding the pH, the tendency has been similar all four years. Treatment LEG showed slightly higher values than the other three treatments, which reached similar values of pH. This difference between treatments was statistically significant in 2006 and 2007. The total phenolic content (IPT) has not shown a constant tendency all four years, possibly due to the annual environmental conditions and yield. Treatments PER and LEG have shown, in general, a higher level of polyphenols that TIL and BAR, being the differences statistically significant in 2008 (figure 2). In 2006 and 2008 treatment PER exceeded treatment LEG in phenolic content, while in 2007 and 2009 PER outperformed the treatment LEG in phenolic content, although these differences were not statistically significant. The total phenolic content was much higher in 2007 than in the other three years in all treatments tested, although the yield was not significantly lower in that year than in other years**.**

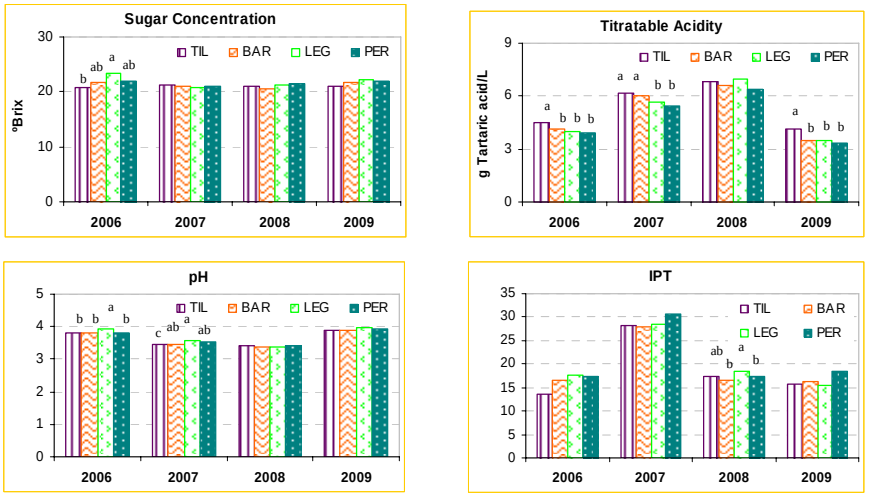

Figure 2. Sugar concentration (ºBrix), Titratable acidity (g tartaric acid/L), pH and total polyphenols index (TPI) of treatments TIL, BAR, LEG and PER, in 2006, 2007, 2008 and 2009. Statistical significance ($p < 5\%$): different letters indicate significant differences between means.

CONCLUSIONS

Water status and physiological activity of vines have been clearly affected by the use of soil cover crop, so that the vines of treatments TIL and BAR had higher rates of photosynthesis, at the same time that showed, in general, lower water stress at different times of the day, than treatments PER and LEG. This allowed the plants, mainly of treatment TIL, achieve greater leaf development and a higher final yield. Legume cover and Permanent cover have led to greater competition with vines than Barley cover, clearly reducing the productivity and leaf development with respect of this treatment. The influence of the type of soil maintenance in grape quality has not been decisive, with a tendency of Legume cover to show greater concentration of sugars and Tillage to have higher level of acidity, as well as a tendency of LEG and PER to show higher phenolic content, so that the effect of vegetation cover and the competition against vines has depended substantially on annual conditions and level of yield, as well as on the crop species grown.

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BIBLIOGRAFY REFERENCES

COULON T., PRUD'HOMME P.Y., 2003. Effet d´un enherbement permanent sur la physiologie de la vigne dans les vignobles du Bordelais. *Le Progrès Agricole et Viticole* 7: 154-161.

DOLEDEC A., PANIGAI L., DESCOTES A., MONCOMBLE D., CLUZEAU D., PERES G., CHAUSSOD R., 2003. Enherbement permanent et préservation des terroirs en Champagne. *Le Progrès Agricole et Viticole* 7: 151-154.

JIMÉNEZ L., JUNQUERA P., LINARES R., LISSARRAGUE J.R., 2007. Mantenimiento del suelo mediante *Festuca longifolia nana*, "Aurora Gold", como alternativa al manejo del suelo mediante laboreo convencional. *Viticultura/ Enología Profesional* 111: 5-18.

LOPES C., MONTEIRO A., RÜCKERT F., GRUBER B., STEINBERG B., SCHULTZ H., 2004. Transpiration of grapevines and co-habitating cover crop and weed species in a vineyard. A "snapshot" at diurnal trends. *Vitis* 43 (2): 111-117.

LUDVIGSEN K., 2002. Cover crops – are they a free ride?. *The Australian and New Zealand grapegrower and winemaker* 457: 37-38.

MORLAT R., JACQUET A., ASSELIN C., 1993. Principaux effets de l'enherbement contrôlé du sol, dans un essai de longue durée en Anjou. *Le Progrès Agricole et Viticole* 19: 406-410.

MURISIER F., 1986. Le point sur les techniques d'entretien des sols viticoles en Suisse. *2ème Symp. Intern. Sur la Non Culture de la Vigne,* Montpellier (Francia): 15-26.

VOLATILE PROFILE OF PRIMITIVO GRAPE AND WINE AS AFFECTED BY TRAINING SYSTEM

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KEYWORDS: volatile compounds, *Primitivo*, training system

ABSTRACT

The varietal character of wine deriving from non-aromatic grapes originate mainly from the transformation of odourless precursors, such as glycosidically bound volatile compounds, into odour-active compounds during winemaking. The use of several training systems involves changes in different conditions including exposure to light, intervine distance and vine density. All these factors affect the content of vine metabolites, including the ones that constitute the volatile profile of grape. The objective of this work was to evaluate the influence of different training systems such as little tree, trellis, and four rays training, on the volatile composition of *Primitivo* grape and the corresponding wine in Apulia region. Free and bound volatile fractions have been evaluated by gas-chromatography coupled to mass spectrometry. Results have shown that little tree and trellis training sistems improve the accumulation of aroma precursors and volatile compounds in grape and wine.

RIASSUNTO

Il carattere varietale dei vini derivanti da uve non aromatiche proviene principalmente dalla trasformazione dei precursori inodori, quali i composti volatili legati, in composti odorosi attivi durante la vinificazione. L'utilizzo di sistemi di allevamento diversi comporta cambiamenti quali l'esposizione alla luce, la distanza e la densità tra i filari. Tutti questi fattori influenzano il contenuto di metaboliti della vite, inclusi quelli che costituiscono il profilo volatile dell'uva. L'obiettivo di questo lavoro è stato valutare l'influenza di differenti forme di allevamento, come alberello, controspalliera e tendone, sulla composizione volatile di uve *Primitivo* nella regione Puglia. Le frazioni volatili libera e legata sono state analizzate mediante gas-cromatografia accoppiata alla spettrometria di massa. I risultati hanno dimostrato che il sistema ad alberello e a controspalliera migliorano l'accumulo di precursori di aroma e di composti volatili nelle uve.

INTRODUCTION

Quality of red wine is strongly affected by volatile fraction responsible for its aroma; nevertheless, volatile wine composition depends on the aromatic potential of the grape used, and this is in turn greatly affected by ground, agronomic techniques, climate and training system. The use of several training systems involves changes in different conditions including exposure to light, intervine distance and vine density (Jackson, Lombard, 1993). Vine training also influences distribution and orientation of foliage within a canopy, and vine size (Howell

et al., 1991). All these factors affect the content of vine metabolites, including the ones that constitute the volatile profile of grape (Reynolds *et al*., 1996) and consequently the aroma wine (Zoecklein *et al*., 1998). Grape aroma compounds are present as free volatiles, which may contribute directly to odour, or as bound sugar conjugates, which are non volatile aroma precursors.

Investigations on the improvement of grape aromatic potential and on the relationships between grape and wine composition are of great concern due to the growing interest in wines with distinctive varietal character.

The objective of this work was to evaluate the influence of different training systems such as little tree, trellis, and four rays training, on the volatile composition of *Primitivo* grape and wine in Apulia region. Free and bound volatile fractions of grape and wine have been evaluated by gas-chromatography coupled to mass spectrometry, with previous sample preparation.

MATERIAL AND METHODS

Field trials and winemaking

The experiments were carried out in 2008, on *Primitivo* grapes from vine planted in 1998 in the area around Fragagnano in Apulia Region, Southern Italy. Three different training systems (little tree, four rays training, and trellis) were compared for their effect on grape and wine volatile composition. The vines trained on a little tree and four rays training, were grafted on 1103 Paulsen, planted 2.5 m apart in rows and spaced at 2.5 m (1,600 plants/hectare), while the vineyard with trellis training system (TW) was spaced at 1.8 m x 1.2 m. All the vines were not irrigated. Grapes from little tree (LTG), four rays (FRG), and trellis (TG) training system were manually harvested, placed in 20 kg plastic boxes and transported to an experimental wine-production centre. Approximately, 700 kg of grapes were processed in each assay. The grapes were destemmed and crushed and then transferred into a 100 L stainless steel tank for maceration. The juice obtained was treated with 50 mg L^{-1} SO_2 in the form of potassium metabisulfite ($K_2S_2O_5$), and inoculated with *Saccharomyces cerevisiae (Zymasil, AEB),* previously rehydrated according to manufacturer instructions. Fermentation was carried out at a controlled temperature (25°C). Caps were punched down manually twice daily. All the tanks were emptied at same density after the end of alcoholic fermentation. Finally, the wines deriving from little tree (LTW), four rays (FRW), and trellis (TW) trained grape were bottled and stored at about 14°C until analysis. Each experiment was carried out in triplicate.

Determination of volatile compounds

Free and bounded fractions of grape volatile compounds were recovered by solid phase extraction (SPE), according to Di Stefano, 1991. The determination was performed using a gas chromatograph (Agilent Technologies 6890N) equipped with a mass spectrometer detector (Agilent Technologies 5975). The chromatographic column was a capillary column DB-WAX (60 m*0.25mm*0.25µm, J&W Scientific) and the oven temperature program was 40°C (15 min), 3°C/min up to 200°C (20 min). The flow of carrier gas (He) was 1 mL/min. The volume of injection was 1µL in splitless mode. Free volatiles of wines were recovered by solid phase extraction (SPE), according to the method proposed by Pineiro *et al*., 2004 and analyzed as grape deriving ones.

The identification of the volatile compounds was achieved by comparing mass spectra with those of the data system library (NIST 02, P>90%) and retention indexes with published data. Concentration of each volatile compound is expressed as mg internal standard equivalents L^{-1} wine, obtained by normalizing the compound peak area to that of the internal standard and multiplying by concentration of the internal standard (1.2 mg L^{-1}). Oneway analysis of

variance (ANOVA) using the Duncan test at level of significance $p<0.05$ was used for statistical analysis (Statsoft 6.0). Analysis Principal Component (PCA) was carried out by means of Statistica 6.0 software package.

RESULTS AND DISCUSSION

Bounded fraction of grape volatile compounds detected by gas-chromatography coupled to mass spectrometry have been reported in Tab.1, grouped according to similarity in structure.

Tab. 1 Volatile compounds detected in bound fraction of *Primitivo* grapes. *Data are expressed as means of 3 replicates ± standard deviation; nd, not detected.

Compound	Retention time	LTG* (µg/L)	TG* (µg/L)	FRG* (µg/L)
Aldehydes				
hexanal	14.73	2.1a ± 0.5	10.6b ± 1.5	10.18b ± 1.06
2-hexenal	20.60	1.9a ± 0.3	35b ± 5	79c ± 13
benzaldehyde	34.31	74a ± 3	85a ± 10	88a ± 7
Alcohols				
2-pentanol	16.31	6.5 ± 0.7	16 ± 3	10 ± 1.0
1-butanol	17.36	44a ± 4	138 ± 25	75 ± 13
1-Butanol-3 methyl	20.24	125a ± 2	251c ± 6	108b ± 12
3-buten-1-ol,3-methyl	22.12	43a ± 5	69b ± 13	86c ± 17
1-pentanol	22.19	51a ± 2	91b ± 11	58a ± 5
2-heptanol	25.38	8.8a ± 0.2	11.3a ± 1.3	9.5a ±0.8
2 Buten-1-ol,3 methyl	25.43	60a ± 4	254b ± 40	232b ± 40
1-hexanol	26.87	322b ± 2	402c ± 17	212a ± 2
3-Hexen-1-ol(E)	27.34	9.71b ± 0.16	11.4c ± 0.2	6.0a ± 0.2
3-Hexen-1-ol(Z)	28.27	33b ± 2	37b ± 5	25a ± 3
3-penten-1-ol,4-methyl	28.46	7.97a ± 0.19	10.2a ± 0.8	11a ± 3
2-hexen-1-ol(E)	29.21	33.5a ± 1.4	70c ± 7	49b ± 4
1-octen-3-ol	31.06	13.74b ± 0.16	15.9c ± 1.2	8.7a ± 1.3
1-heptanol	31.35	21.3b ± 1.7	23b ± 2	14a ± 2
1-hexanol,2-ethyl	32.80	7.48a ± 0.8	6.8a ± 1.1	7.20a ± 0.97
benzyl alcohol	47.93	1180a ± 110	1370a ± 100	1320a ± 16
phenyl ethyl alcohol	49.22	827b ± 40	1143c ± 120	656a ± 30
2-methoxybenzyl alcohol	57.83	31c ± 5	20b ± 3	13a ± 2
Terpenoids				
cis-linaloloxide	30.92	4.0 ± 0.3		
2,6-octadien-1-ol 3,7-dimethyl(Z)	45.10	44b ± 8	49b ± 3	30a ± 2
2,6-octadien-1-ol 3,7-dimethyl(E)	46.81	122a ± 11	135a ± 2	107a ± 3
2H-pyran-3-ol	42.84	25a ± 5	18a ± 2	26a ± 2
geranic acid	66.25	80c ± 9	36a ± 3	52b ± 2
Acids				
butanoic acid, 3-methyl	40.33	31 ± 13	nd	nd
3-hexenoic acid	51.55	40 ± 16	nd	nd
Others				
2 methoxy-4-vinylphenol	59.05	45a ± 20	83b ± 8	118c ± 17
methoxyphenyl ethanol	64.99	49b ± 20	nd	13a ± 2
3-Hydroxy-beta-damascone	80.02	57a ± 30	107b ± 6	107b ± 13
methyl salicylate	44.30	34b ± 14	20b ± 3	7.3a ± 1.2

Little tree and trellis training sistems seem to improve the accumulation of some aroma precursors, such as 2,6-octadien-1-ol 3,7-dimethyl(Z) and especially geranic acid, known to have a positive impact odor, and cause the decrease of C6-aldehydes, known to confer wine herbaceous odors. On the other hand, LTG showed the lowest content of 3-Hydroxy-beta-damascon. To determine groupings of *Primitivo* grapes and to evaluate relationships among the attributes and samples, Analysis Principal Component (PCA) was carried out. The PCA graph is shown in Fig. 1.

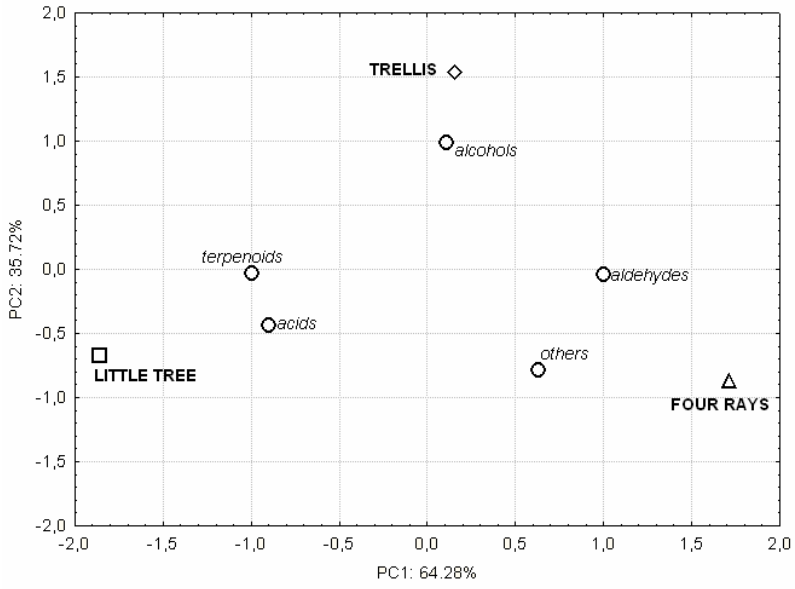

Fig. 1. PCA of bound aroma compounds in *Primitivo* varieties.

The PCA analysis of *Primitivo* grapes data accounted for a total of 100% of the variance in the data using two factors: factor I (64.28%) and factor II (35.72%). A good separation among TG, FRG, and LTG was observed. TG samples were characterized by alcohols; FRG by C6-aldehydes, and other components including phenols and 3-hydroxy-β-damascon; LTG samples were characterized by terpenoids and acids.

The analysis of volatile compounds of the corresponding wines revealed less marked differences between samples. A major presence of some esters, such as esanoic, octanoic, decanoic and lactic acid ethyl esters in LTW was detected. Terpenoids were found in no samples investigated. Further work should be addressed to investigate how grape volatile composition affects wine volatile composition.

CONCLUSIONS

Results have shown that little tree and trellis trained grapes seem to improve the accumulation of some terpenoids and cause the decrease of C6-aldehydes. No easy correlation was found between grape volatile composition and corresponding wine volatile composition. Further investigations should be done in this direction.

ACKNOWLEDGEMENTS

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BIBLIOGRAPHY

Di Stefano R. 1991. Proposal for a method of sample preparation for the determination of free and glycoside terpenes of grapes and wines. *Bulletin de l'ÓIV*, 721: 219-223.

Howell G.S., Miller D.P., Edson C.E., Striegler R.K. 1991. Influence of training system and pruning severity on yield, vine size, and fruit composition of Vignoles grapevines. *American Journal of Enology and Viticulture*, 42: 191-198.

Jackson D.I., Lombard P.B. 1993. Eviromental and management practices affecting grape composition and wine quality – A review. *American Journal of Enology and Viticulture*, 44: 409-430.

Pineiro Z., Palma M., Barroso C.G. 2004. Determination of terpenoids in wines by solid phase extraction and gas chromatography. *Analytica Chimica Acta*, 513: 209-214.

Reynolds A.G., Wardle D.A., Naylor A.P. 1996. Impact of training system, vine spacing, and basal leaf removal on *Riesling*. Vine performance, berry composition, canopy microclimate, and vineyard labor requirements *American Journal of Enology and Viticulture*, 47: 63-76.

Zoecklein B.W., Wolf T.K., Marcy J.K., Jasinski Y. 1998. Effect of fruit zone leaf thinning on total glycosides and selected aglycone concentration of Riesling (*Vitis vinifera* L.) grapes. *American Journal of Enology and Viticulture*, 49: 35-43.

Ecological Potential of Landscape and Uniqueness of Specific Viticulture Zones (Racha-Lechkhumi)

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ClAIM

Georgia is an ancient country of viticulture and oenology distinguished by its diversity; its geographical sides are characterized by rare mosaic structure, mountainous landscapes and very contrast climate. The relief is given preference in landscape division of the country; its geo-morphological condition enables to outline special disposition of scenery types. Harmonized microclimate, precipitation, sum of active temperatures, exposition, mixed soils together with technogenic factors selected for aboriginal varieties condition special characteristics of naturally semi-sweet wine.

Georgien ist ein ältestes Land des Wein- und Gartenbaus, das sich durch die reiche Natur unterscheidet. Seine einzelnen Regionen sind durch das seltene Mosaik der Natur, durch Berglandschaften und ziemlich kontrastreiches Klima charakterisiert. Bei der Teilung der Landschaften wird das Relief bevorzugt, dessen geomorfologischer Zustand ermöglicht es, die räumliche Lage der Landschaftstypen und die Verbreitungsarealien dieser Typen abzuteilen. Die Einung der ethnographischen Regionen Ratscha und Letskhumi, die von einander durch Relief unterscheiden, ist zusammen mit der territorialen Nähe durch die orographischen Bedingungen, durch die Aehnlichkeit der Natur- ind Wirtschaftstätigket der Bevoelkrung und durch die eigene Teilung der Klimafaktoren dieser Regionen bedingt.

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INTRODUCTION

Georgia is an ancient country of viticulture and wine making (area of 69,500 km^2, coordinates: North latitude $41^0 07$ and East Longitude $43^0 35$). Since past times Georgians have been familiar with grape growing and wine making art what is clearly demonstrated by the wine cellar and diversity of incredible wine tableware of ancient times found in archeological excavations (II-III millennium B.C.) as well as high quality, distinct wine (table dry, semi-dry, semi-sweet, sparkling, Kakhetian, Imeterian) produced using original technology.

Georgia is distinguished by diversity of relief and climatic conditions, in particular there are humid subtropical (Black Sea Coast) as well as hot continental (East Georgia plain) and cold (mountainous, permanently snow covered, glacial locations) climates in the country.

Potential of landscape ecology along with aboriginal varieties have a great impact on unique identity of original wine production. Due to the specific character of natural conditions the agro-ethnographical (combined with natural-traditional and farming economic systems) principle of zoning has been worked out since past times.

Almost none of the viticulture countries of the world has so rich aboriginal assortment of vine as Georgia having up to 550 varieties. Over the century those varieties had been developed and spread as endemic groups of Kakheti, Kartli, Imereti, Racha-Lechkhumi, Guria, Samegrelo, Adjara and Abkhazia in favourable ecological conditions.

In landscape zoning of the country the preference is given to the relief as geo-morphological conditions enable to line out special dislocation of landscape types and areas of their extrapolation. Uniting Racha and Lechkhumi - these different (in terms of relief) ethnographic provinces was conditioned by similarity of orographic conditions and natural-farming activities of local communities along with vicinity of territories. Peculiar distribution of climatic factors is conditioned by the fact that the territory is surrounded by high ridges from all sides and open to South-west side by narrow gorges of the rivers (Rioni and Tskhenistskali).

Racha

Location. Racha region is situated in West Georgia on Southern slopes of Caucasus upstream of the Rioni River. Industrial vineyards are mostly located on 450-750 m above the sea level.

The micro-zone of naturally semi-sweet wine “Khvanchkara” subject to the place of origin title control is located in Kvemo Racha on the right bank of the Rioni River along with latitudinal direction gorge over the distance of 35-40 km in the cave protected with high ridges; it covers the villages of Tsesi, Kvatskhuri, Ghviara, Sadmeli, Khvanchkara, Tola, Bugeuli and Chrebalo.

Soils. Humus carbonate soils established on limestone, marl and carbonate sand stone exhaustion crust (M. Alpenidze 2009) dominate in the lower viticulture zone; the unique vine varieties (cv) Alexandrouli and (cv) Mujuretuli produce their best yields on these soils. Most of locations (60%) where high quality wines are produced are situated on strong skeletal soils. The positive impact of skeletal soils is reflected by the product quality linked to its thermal features. In the daytime the skeletal soil heated up by sun radiation energy absorption moderates surface air temperature by night emission and by this positively affects vine physiological processes (T. Turmanidze 2003). Moreover, rare and dissipated elements of skeletal soil have great importance for vine nutrition. It has been confirmed that vineyards located on thin skeletal soils of slopes produce special high quality wines as in this case the merits of wine are impacted by not so much the physical features of soil but nutrition of vine by minerals from rock.

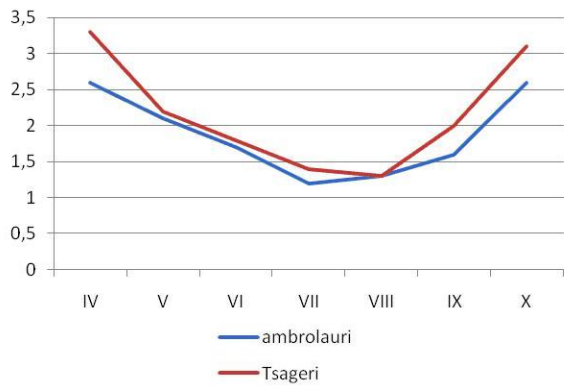
Vine has major requirements to the quality of soil air; Soil oxygen is an irreplaceable ecological factor which undergo relevant modifications in accordance with the variety. This fact is related to life cycle of vegetation of this particular landscape region as well as to strong emission of carbonic acid and huge consumption of oxygen (T. Turmanidze 2003).

Climatic Peculiarities

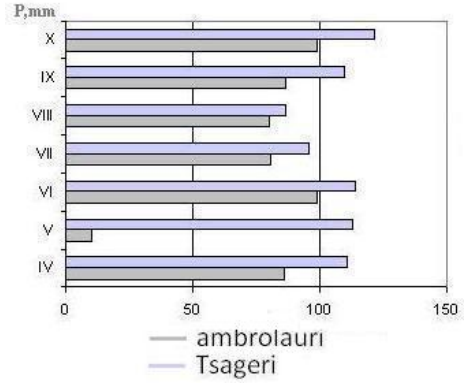
The weather formation is conditioned by subtropical and atmospheric processes developed in moderate latitudes and incoming from West and East. The region is located in sea

subtropical humid district and is characterized by moderately cold winter and hot, comparatively dry summer. The sum of active temperatures on the said elevation ranges between 3,750-3,350^0 and precipitation in vegetation period is 640-660 mm.

Production of original wine in the region is conditioned by main agro-climatic indicators established on Southward inclined foothills of latitudinal direction, in particular: sun radiation, thermal quantity, moderate tension of summer temperatures and moderate moistening of location. Here skeletal soils with their dark color create special condition of these soils and positively impact thermal conditions of vine aboriginal varieties what in its turn helps accumulation of more sugar in vine varieties of (cv) Mujuretuli and (cv) Alexandrouli and gives the fruit a specific taste.



Drawing 1: Distribution of summarized precipitation in Racha-Lechkhumi (Ambrolauri, Tsageri)



Drawing2: Distribution of hydrothermal coefficient by vegetation periods in Racha-Lechkhumi (Ambrolauri, Tsageri)

Varieties. (cv) Alexandrouli and (cv) Mujuretuli are main production varieties of Racha Region. They are characterized by normal growing and development; they accumulate sugar in big quantities (up to 28-30%) and give naturally semi-sweet red wine Khvanchkara (Tola-Khvanchkara micro-zone) subject to place of origin; in the remained locations table high quality red wine is produced. Both varieties are distinguished by low yield but due to merits of the product are more spread in Racha Region compared to the other varieties of the zone. Notwithstanding the fact that (cv) Alexandrouli variety can freely fructify in almost any viticulture region of Georgia it seldom goes beyond Racha borders; it is well adopted to Racha's droughts and slightly inclined immature, skeletal, limy, clay stone soils. Harvest and quality of vine variety (cv) Alexandrouli apart from vineyard location and soil conditions depends on meteorological conditions i.e. how warm the vegetation period is and how evenly precipitation is distributed during the said period. On the elevation of 450-800 m (cv) Alexandrouli vine variety starts maturing in the second part of August up to early September (12.VIII-22. VIII), (warmth accumulation 2,450-2,100^{0}C) and full fructification commences from 25.09 to 10.10 during accumulation of 3420-2950 ^{0}C total warmth. The total period from budbreak through full fructification consists of 169-172 days. As the elevation increases (for maturing start and full fructification) the sum of temperatures gradually reduces what can be explained by increase of direct sun radiation towards the said direction. Within the said elevation range on Southern slope of Lechkhumi Ridge the sugar content in (cv) Alexandrouli grape juice collected in late September early October is 20-26%, titratable acidity 6.5-9.5 g/dm^3. Sugar content in (cv) Alexandrouli vineyards located up to 600 m elevation is 24-26%,

titratable acidity – 6.5-7.5 g/dm3; on the elevation of 600-800 m sugar content is 24-20% and acidity ranges between 7.5-9.5 g/dm^3. Along with the above mentioned the following vine varieties are spread in Racha Region: (cv) Tsulukidzis Tetra, (cv) Tsolikouri, (cv) Rachuli Mtsvane (Kvishkhuri), (cv) Dzvelshavi, (cv) Saperavi, (cv) Rkatsiteli, (cv) Rko, (cv) Rachuli Kapistoni, (cv) Ojaleshi, (cv) Mgaloblishvili, (cv) Kundza, (cv) Usakhelouri, (cv) Aligote.

In the villages Tsakhi, Baji, Ghadishi, Bugeuli, Khimshi etc at the elevation of 450-800 m the sum of active temperatures ranges between 3,800 -2,900^{0}C; at the elevation of 800-900 m it reduces down to 2,900-2,700^{0}C. On up to 650 m elevation the sum of temperatures is 80-120^{0}C less and on 700-900 m it is 140-180 ^{0}C less compared to the other river side similar elevations. Here the vine varieties of (cv) Tachuli Tetra, (cv) Mtsvivani Rachuli and (cv) Tsolikouri having white grapes produce wine with good taste features.

Table 1

Temperature Indicators of Main Phenological Stage of Vine Varieties in Racha-Lechkhumi

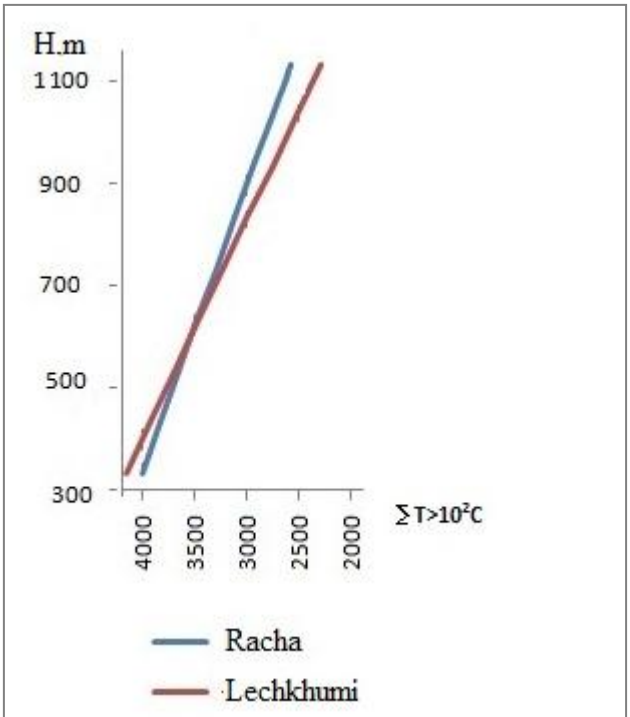
Variety	Bud Blossoming		Start of Budberak	Start of Ripening	Sum of Active Temp. (ΣT>10^{0}C)	Full Maturing	Sum of Active Temp. (ΣT>10^{0}C)	Defoliation	Period Duration (days)		Grape Quality		Elevation Limit of Spread
	Start	Average Air Temp. (^{0}C)							From Budberak to Full Maturing	From Budberak to Defoliation	Sugar Content (%)	Total Acidity (g/dm^3)	
Alexandrouli	10.IV	12,4	25.V	13.VIII	2450	28.IX	3400	15.XI	171	218	25,5	7,0	630
Mujuretuli	10.IV	12,4	25.V	15.VIII	2500	28.IX	3400	15.XI	171	218	26,8	5,8	630
Usakhelouri	14.IV	11,2	1.VI	15.VIII	2400	10.X	3450	15.XI	179	214	23-25	7-8	650
Rachuli Tetra	13.IV	11,0	25.V	25.VIII	2600	30.IX	3200	15.XI	170	215	20-22	7-8	650
Orbeluri	13.IV	11,0	25.V	25.VIII	2600	30.IX	3300	15.XI	170	215	22-23	7,5-8,0	650
Tsolikouri	10.IV	11,7	26.V	16.VIII	2700	8.X	3800	21.XI	182	225	22-24	7,5-9,5	600

Lechkhumi

Location. Lechkhumi is located on the right bank of the Rioni River in the coastline. The coordinates are 42^{0}31' North latitude and 42^{0}54' of East longitude at the elevation of 435 m above the sea level. The viticulture production zone of the semi-sweet wine "Tvishi" subject to the place of origin title control spreads on the far West plains of Racha Ridge and East slopes of Khvamli Massif on the right bank of the Rioni River and covers the villages of Tvishi and Alpana. In terms of relief these locations represent slightly and averagely inclined slopes of various exposition with minor terrace-like flatting. The total inclination is directed towards South-east and East.

Soil. Humus carbonate, delluvial and black soils with their diversity.

Climatic Peculiarities. It is characterized by humid climate moderately cold winter and long warm summer. The duration of vegetation period is 209 days. The sum of active temperatures is 3,700-3,800^{0}C, the annual precipitation is 1,095 mm.



Drawing 3: Distribution of Sum of Temperatures (ΣT>10^{0}C) above the sea level in Racha-Lechkhumi

Variety. (cv) Tsolikouri is a standard vine variety of local origin which is widely spread in viticulture zone (dry and humid) of West Georgia; duration of its vegetation period noticeably fluctuates compared to ecological conditions of separate districts.

Vine variety of (cv) Tsolikouri produces best quality wine yielded in vineyards located on plains of Lechkumi, far West slopes of Racha Ridge and East plains of Khvamli Massif as well as on slopes slightly and averagely inclined towards the Rioni River bank. It is notable that apart from high sugar content (28%) this variety maintains acidity and gives full, cheerful semi-sweet wine with high alcohol contents.

Due to similar relief, climatic and soil conditions vineyard lines grown on viticulture areas of Racha-Lechkhumi region are directed from North to Southward and the flowing agri-technological rules are maintained in particular: planting layout (2.5X1.5), free pruning, scheduled yield. The necessary agrotechnical activity for these varieties must be considered removing tips before blossoming.

Conclusion. Geographical location, climatic and soil diversity, spread of aboriginal vine varieties and traditional technology of wine making of Georgia have a great impact on producing original, semi-sweet wines “Kvanchkara” and Tvishi” subject to place of origin title control.

More than third of high quality, naturally semi-sweet wines of 15 trade names subject to place of origin title control existing in Georgia are produced in Racha-Lechkhumi region and have leading position in the country.

Bibliograghy:

1. Alpenidze M., Seperteladze Z., Davitaia E., Kharadze K., 2009, Georgia, Tbilisi, Physical Geography of pg. 119-178-182
2. Turmanidze T., 2003, Tbilisi, Vine Ecology, “Sulakauri”.
3. Ketskhoveli N., Ramishvili M., Tabidze D., 1961, Ampelography of Georgia, state publishing house of Science academy of Georgia
4. Gogitidze V., Vakhtangadze and others, 2008, Agro-Climatic Factors of Grapevine in the Conditions of West Georgia.

LAS YEMAS Y SU TRASCENDENCIA COMO RESERVORIO DE LEVADURAS EN LA PLANTA DE VID

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RESUMEN

Se investigó el modelo de circulación y permanencia de levaduras en los órganos aéreos de la vid, con énfasis en las *yemas*, que asumen el rol de reservorio a lo largo del ciclo anual. Se realizó muestreo aleatorio y sistemático en plantas de vid (*Vitis vinifera* var. *Malbec)*, cultivadas en espaldero alto en la zona vitivinícola de Mendoza (Argentina). Se efectuó la recolección aséptica de muestras a campo, observación, conteo y descripción morfológica de las levaduras en los diferentes órganos aéreos de la vid (yema en actividad, yema en reposo, hoja joven, hoja adulta, ritidomis, zarcillo, capullo floral, flor, fruto). Los resultados indican que existen dos momentos de máxima población de levaduras: a fines de otoño en *yema cerrada* y a mediados de verano en *yema axilar* de hoja adulta. En invierno las levaduras encuentran refugio protegidas por la pubescencia y la cámara de aire debajo de las pérulas en las yemas. La evolución en el fruto en función de su superficie muestra poca relación entre ambas variables, infiriéndose que el valor a considerar sería la cantidad de levaduras por baya, ya que en el momento de madurez se concentran en la región periférica del rodete. Se procedió a la selección de levaduras autóctonas en diferentes regiones vitivinícolas de Mendoza, utilizando bayas y jugos de vides. Esta metodología es novedosa y permite hacer un aporte en la selección y multiplicación de levaduras enológicas, de las cuales ya se están comercializando tres cepas, una de ellas también en Francia.

LES BOURGEONS ET LEUR TRANSCENDANCE COMME REFUGE DE LEVURES DANS LES PLANTES DE VIGNE

Resume: On a fait des recherches sur modèle circulation et permanence de levures dans les organes aériens de, avec emphase dans les *bourgeons*, qui assument le rôle de reserve le long du cycle annuel. On a effectué échantillonnage aléatoire et systématique dans les plantes de vigne ((*Vitis vinifera* var. *Malbec)*, cultivées dans la première zone vinicole de Mendoza (Argentine). On a effectué la récolte aseptique d'échantillons à domaine, l'observation, le comptage et la description morphologique des levures dans les différents organes aériens de la vigne (bourgeon en activité, bourgeon en repos, jeune feuille, feuille adulte, ecorce, vrille, floral, fleur, fruit). Les résultats indiquent existent deux moments de maximale population de levures: à la fin d'automne en bourgeon fermé et vers le milieu d'été en bourgeon axilar de feuille adulte. En hiver les levures trouvent refuge protégé par la pubescence et la chambre d'air sous les écailles dans les bourgeons. L'évolution de levures dans le fruit en fonction de sa surface montre peu de relation entre les deux variables, en impliquant qui la valeur à considérer serait la quantité de levures par baie, puisqu'au moment de maturité ils se concentrent la région périphérique du bourrelet. On a procédé à l'élection de levures autochtones dans différentes régions vinicoles de Mendoza, en utilisant des baies et des jus de vignes. Cette méthodologie est nouvelle et permet de faire un apport dans la sélection et la multiplication de levures oenologiques, de desquelles déjà on commercialise trois souches, une de d'elles aussi en France.

THE IMPORTANCE OF BUDS AS SHELTER OF YEASTS IN GRAPEVINE PLANTS

Summary: The model of circulation and permanence of yeasts in aerial organs of grapevine was investigated, with emphasis in the *buds*, that assume the roll of reserve throughout the annual cycle. Random and systematic sampling was realized in grapevine plants (*Vitis vinifera* var. *Malbec*), cultivated in the first wine producing zone of Mendoza (Argentina). The aseptic harvesting from samples in field, observation, count and morphologic description of yeasts in the different aerial organs of grapevine was accomplished (bud in activity, bud in rest, young leaf, adult leaf, ritidomis, tendril, flower bud, flower, fruit). The results indicate that exist two moments of maximum yeasts population: at end of autumn in closed buds and in middle of summer in axillary buds of adult leaf. In winter the yeasts find refuge protected by the pubescence and the air chamber underneath bracts in the buds. The yeasts evolution in the fruit based on its surface shows little relation between both variables, inferring that the value to consider would be the amount of yeasts by berry, since at the time of maturity they are concentrated in the peripheral region of the peduncule. The election of native leavenings in different wine producing regions from Mendoza was made, using berries and juice of grapevines. This methodology is novel and allows to make a contribution in the selection and multiplication of oenological yeasts, of which already three stocks are being commercialized, one of them also in France.

INTRODUCCIÓN

La fermentación alcohólica de los mostos es efectuada por las levaduras provenientes normalmente de las uvas y se acepta comúnmente que éstas viven en asociación con la vid. Las levaduras se hallan usualmente sobre la corteza, las hojas, las flores y en la pruina de la baya de uva (Van Zyl and Du Plessis, 1961; Ribéreau-Gayon et Peynaud, 1964; Ribéreau-Gayon *et al*, 2003). Su crecimiento en la superficie de las bayas de la vid está determinado por los diversos factores ambientales (temperatura, humedad), así como por el grado de madurez y el estado sanitario. Poco se ha investigado sobre la ecología de las levaduras en las vides argentinas y, por otra parte, su presencia como exclusivas de cada cepaje incide en las características distintivas de los vinos varietales. La obtención de vinos de excelencia contempla el proceso de fermentación natural llevado a cabo por levaduras privativas de cada variedad y su aislamiento e identificación.

OBJETIVOS

Verificar el papel de las yemas de la vid como reservorio de levaduras. Correlacionar los estados de desarrollo de los primordios de inflorescencias con la presencia de levaduras en las yemas de vid. Exponer el modelo de circulación y de permanencia de las levaduras en la parte aérea de la planta de vid. Suministrar nueva evidencia acerca de la interacción entre la planta de vid y las levaduras a lo largo del ciclo vegetativo.

MATERIAL Y MÉTODO

Se trabajó en plantas de vid var. *Malbec*, cultivadas en espaldero alto en Mendoza (Argentina: 33° 00’ 38,23’’ S; 68° 52´37,58’’ O). En una *primera etapa* se realizó un muestreo aséptico y sistemático de diferentes órganos aéreos, a intervalos regulares durante 12 meses. Se extrajeron al azar todos los órganos de cada punto de muestreo: yema terminal, yema lateral, hojita joven (junto al meristema apical), hoja adulta, inflorescencias con capullos y con flores en antesis (sin caliptra), baya en distintos estados de madurez, zarcillo y

ritidomis. Se evitó el uso de tests con medios de cultivo discriminatorios, privilegiando cultivos en jugo de uva estéril (pH=3,5) y a 25°C, un medio de cultivo natural acorde con las condiciones normales de vinificación, que previene el desarrollo de bacterias, y con elevado contenido de azúcares fermentables por las especies de levaduras asociadas con la vinificación. Los resultados se refirieron a 1 cm2 de superficie de los órganos considerados. En una *segunda etapa* se extrajeron *yemas* de vid en el período previo a la brotación y se realizaron cortes longitudinales para su observación microscópica. Se efectuaron cortes con micrótomo rotativo tipo Minot, previa deshidratación e inclusión en parafina. Los cortes se coloraron con safranina-fastgreen y safranina-hematoxilina y se conservaron en preparados permanentes con bálsamo de Canadá. En preparados para observación directa al microscopio se apreció la presencia de levaduras protegidas por los tricomas de los primordios foliares.

RESULTADOS

La variación de la presencia de levaduras en los órganos aéreos de la planta de vid, a todo lo largo del ciclo vegetativo (fig. 1), muestra que se dan dos momentos de máxima cantidad de levaduras: en yema cerrada a fines de otoño (16/junio/04) con 38,75 10^6 lev/cm^2 en yema terminal abierta con 43,75 x 10^6 lev/cm^2, así como en yema axilar a la hoja adulta con 38,25 x 10^6 lev/cm^2, a mediados de verano. Sólo durante el período primaveral, cuando se produce la brotación de las yemas para proveer a la planta de pámpanos, hojas y flores, las yemas son superadas por la ritidomis en cuanto a cantidad de levaduras/cm^2. Estos datos, evidencian la importancia de las yemas como reservorios de levaduras a todo lo largo del ciclo vegetativo, excepto en el momento de transición de yema del año anterior a las nuevas del ciclo siguiente. A medida que avanza la brotación en primavera, las yemas abiertas (en brotación) que poseían valores muy bajos en cuanto a levaduras presentes, sufren un incremento uniforme alcanzando su punto máximo a mediados de verano. Esta fecha concuerda con la estabilización de la expresión vegetativa de la planta y la maduración de los frutos. Durante los meses invernales las levaduras encontrarían refugio en las yemas, protegidas por la pubescencia que forma una cámara de aire debajo de las pérulas. Si se analiza la curva correspondiente a yema cerrada de invierno, se observa una continuidad con las yemas apicales en brotación y las yemas axilares a las hojas que se forman en los nuevos brotes en primavera, uniéndose a ambas en una línea de tendencia que sigue una curva polinomial de 2do grado, con un mínimo en el momento de la brotación, coincidiendo con el aumento de la masa vegetativa de la planta durante la primavera.

La planta de vid presenta crecimiento acrópeto, con yemas axilares protegidas por pérulas. Las yemas presentan una estructura compleja (fig. 4), formada por un cono vegetativo principal, acompañado por dos conos vegetativos secundarios. Este sistema está protegido externamente por dos escamas o “perulas”, de color pardo y disposición imbricada. Una tercer pérula acompaña a la yema pronta. El meristema apical está rodeado por primordios foliares; cada uno de los cuales está acompañado y protegido por dos estípulas cubiertas de abundante lanosidad denominada “borra”. Es en esta borra, que consiste de tricomas, simples unicelulares (epidermis principalmente abaxial de las escamas estipulares) donde se observa la presencia de abundante cantidad de células de levaduras que conviven en asociación con la planta de vid (fig. 3).

Figura 1:

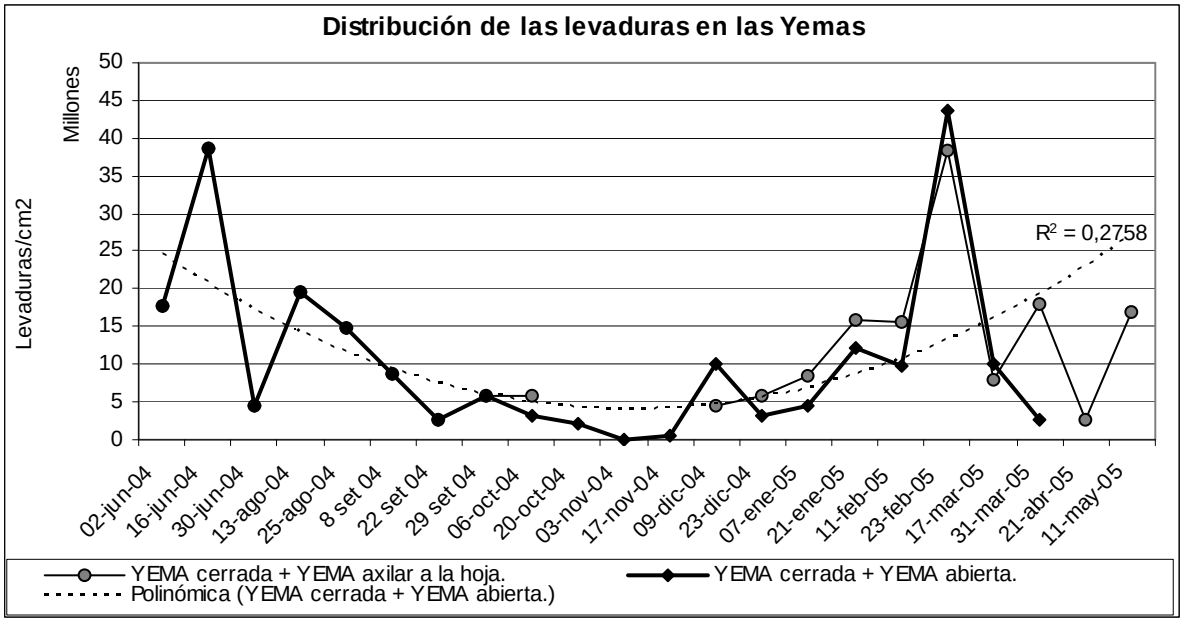


Figura 2: Yema de vid en el momento previo a su brotación ("desborre").

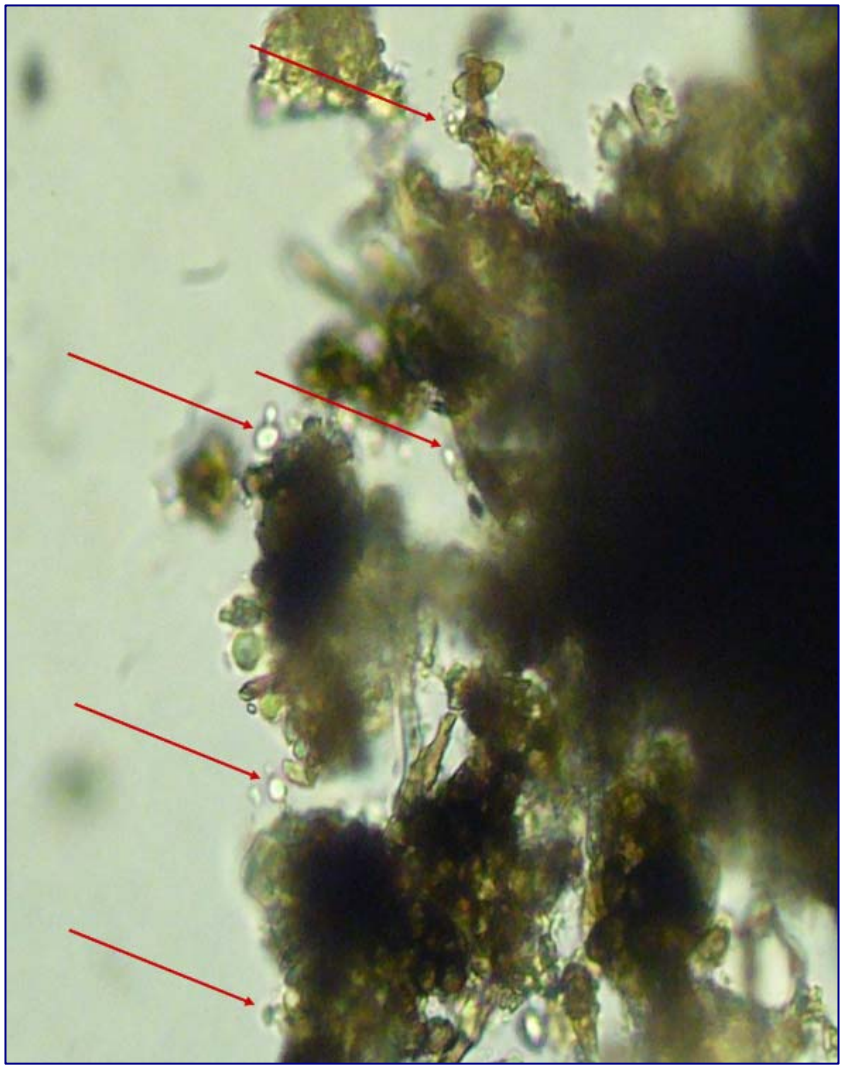
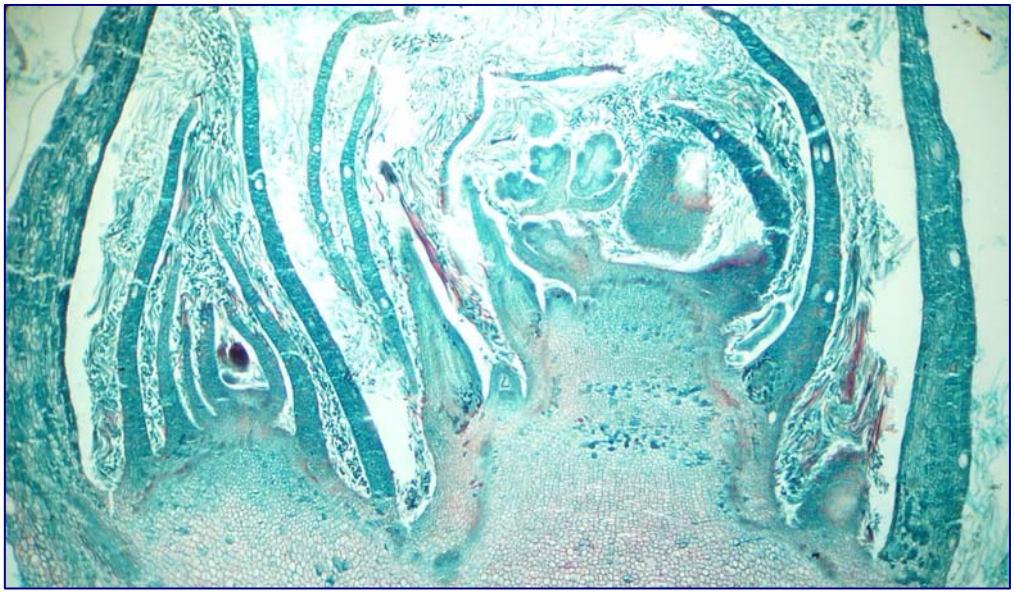


Figura 3: Células de levaduras conviviendo en los tricomas de la "borra" en yemas de vid (x400).

Figura 4: Corte longitudinal de una yema de vid (vista al microscopio óptico, x100)



CONCLUSIONES

Se ha verificado la importancia de las yemas de la vid como reservorio de levaduras. Este hallazgo permitirá seleccionar levaduras específicas de cada variedad de vid para la obtención de vinos de alta diferenciación. Se dan dos momentos de máxima cantidad de levaduras: en yema cerrada a fines de otoño y en yema terminal abierta así como en yema axilar a la hoja adulta a mediados de verano. Estos datos evidencian la importancia de las yemas como reservorios de levaduras, a las cuales brindan refugio debajo de las pérulas durante los meses invernales. La presencia de elevada cantidad de idioblastos de oxalato de calcio, en las células de las perúlas de las yemas, podría relacionarse con una función estructural de protección.

BIBLIOGRAFÍA

BELIN, J. M. 1972. Recherches sur la répartition des levures à la surface de la grappe de raisin. *Vitis* 11: 135-145.

CHAMBERS, T. C. and POSSINGHAM, J. V. 1963. Studies of the fine structure of the wax layer of Sultana grapes. *Austr. J. Biol. Sci.* 16: 818-825.

DOMERCQ, S. 1956. Etude et classification des levures de vin de la Gironde. Thèse Ingénierieur-Docteur. Bordeaux (según Belin, J. M. 1972).

FORMENTO, J. C. *et al.*. "Mosto concentrado tinto. Nueva variedad de vid para su elaboración". *La Semana vitivinícola*". Nº 2.763/64 del 24/31 de julio de 1999. España.

LÚQUEZ, C. V. Y FORMENTO, J. C. 2002. La flor y el fruto de la vid (*Vitis* v*inifera* L.).- Micrografía aplicada a la viticultura y la enología. *Rev. F. C. Agrarias* 34 (1): 109-121.

MARTINAND, V. and RIETSCH, M.. 1891. Des microorganismes que l'onrencontre sur les raisins murs et de leur développement pendant la fermentation. Comptes Rendus Acad. Sci. Paris 112: 736-749.

MARTINI, A.; CIANI, M. and SCORZETTI, G. 1996. Direct enumeration and isolation of wine yeasts from grape surfaces. *Am. J. Enol. Vitic.* 47 (4): 435-440.

RIBÉREAU-GAYON, *et al.* 2003. T*ratado de Enología.* Tomo I: *Microbiología del Vino. Vinificaciones.* (Dunod, París, 1998). H., Buenos Aires.

VAN ZYL, J.A. and DE W. DU PLESSIS, L. 1961. The microbiology of South African winemaking. Part I. The yeasts occurring in vineyards, musts and wines. *South African Journal of Agricultural Science* 4(3: September 1961): 393-403.

ORIGIN AND PECULIARITIES OF RARELY DISTRIBUTED GRAPEVINE VATIETY *ASURETULI SHAVI*

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ABSTRACT

It has been shown that Georgian variety of grapevine – *Asuretuli Shavi* – in conditions of the Kvemo Kartli region of Georgia manifests fairly good resistance against the environmental factors. It withstands extreme temperatures, well tolerates the spring-time frosts. Although the variety is functionally female, partial possibility of its fertilization with its own pollen has been determined. Therefore within the orchards it is feasible to search for such new clones, the flowers of which contain many bisexual flowers. The variety is high-yielding and, considering quality of its fruits, it could be implemented for production of naturally-pink wines.

ZUSAMMENFASSUNG

Von deutschen Siedler wurde in den Jahren 1825 -1845 in den Wäldern von Südgeorgien, eine Rotweinsorte „Assuretuli Schawi“ gefunden. Sie ist eine wenig verbreitete Sorte, aber ihre Herkunftsgeschichte und die Originalität der Produktion haben das Interesse für ihre Forschung erweckt.

Die Blüte ist funktionell weiblich. Die absolute Mehrheit der Staubkörner (96,6%) sind porenlos und steril, die anderen dreiporigen aber – fertil; Die Fertilität - 1,6%; Relative Frostwiderstandsfähigkeit „Assuretuli Schawi“ ist mehr als „Saperawi“ und „Tawkweri“. Sie ist auch relativ frostbeständig im Frühling.

In der Arbeit ist die Schlussfolgerung gezogen, dass „Assuretuli Schawi“ ausgehend von der Erntequalität und von der Widerstandsfähigkeit gegen die Umweltfaktoren zwecks der Herstellung von natürlichen Rosa – Weinen für die Verbereitung in der Weinbau – Zone von Kwemo Kartli gut geeignet ist.

INTRODUCTION

In 1825-1845, in Kvemo Kartli, in the woodlands of village Asureti, a German colonist Schall found wildly growing red-fruiting high-yielding variety of grapevine, which was named as *Schalltraube*. It was widely disseminated in the colonies inhabited by Germans and in 30s of XIX century it was described and entered into ampelography under the name of *Asuretuli Shavi*.

Following expulsion of the German colonists to the Central Asia in 1941, areas of cultivated *Asuretuli Shavi* decreased significantly, although a revived interest to this variety and its overall investigation according to current requirements did occur recently.

In order to successfully implement *Asuretuli Shavi*, as required by theoretical and practical views, and to polish existing information, under regulations of international institutes of plant genetic resources (OIV, Biodiversity International, UPOV), according to the grapevine descriptors, at the cellular and organism levels the following was investigated: cariology, processes for development of generation sphere, morpho-physiology of pollen; type of flower,

processes of pollination-fertilization and of fruit-bearing, resistance to abiotic factors; a new technology of wine making from this grapes has been elaborated, *etc*.

MATERIAL AND METHODS

The studies have been carried out according to the methods adopted in viticulture, cytology, biophysics, and biochemistry (Negrul, 1934; Pausheva, 1988; IPGRI, 1997; Kimura et al., 2006; Schreiber, 2005; Compendium , 2006), as well as according to the methods developed by us (Vashakidze, 2006; Ortoidze et al., 1990; Duering et al.,1990). The data obtained experimentally was processed by statistical methods (Lakin, 1990).

RESULTS AND DISCUSSION

Cytological investigation of the *Asuretuli Shavi* has shown that the meristem tissue of roots consists of small (length 16.2μm , width 10.0μm; nucleus diameter 4.9±0.1 μm) cells; chromosomal set of somatic cells is diploid (2n=38), number of the aberrant cells is within permitted range, Mitosis proceeds normally and is fairly active (7.0±0.3%), which, respective to general state of an organism, points at vigorous growth and high resistance against to the environmental factors (Vashakidze, 2007).

The flower of *Asuretuli Shavi* is female, although by other investigators (Cholokashvili, 1939; Ramishvili, 1970) on organism level in natural conditions, as well as by us (Vashakidze, 2006) in natural and on rooted canes in the laboratory conditions have shown developing of morphologicaly be-sexual flowers for many years; in conditions of flower isolation and self-pollination, some cases of normal berry set was noted along with small parthenocarpous ones; in castrated isolated inflorescences an apomixes did occur.

Pollino-morphological studies revealed that because of anemophily of the plant, pollen grains of *Asuretuli Shavi* are small. Their parameters (in air-dry condition) are: length 29.0±0.5μm , width 22.3±0.4μm , and diameter (colored in acetocarmine) 25.4±0.4μm. The pollen grain in air-dry condition has pointed shape and being moistened attains round shape. An absolute majority of pollen grain have no pores and keep sterile, unable thus to fertilize; however, they can produce a stimulating influence on formation of small, seedless grains. In the latter case also occasionally fertile, three-pore pollen (1.6±0.3%) have been found, germination of which on the glucose-agar medium nutrient attains 1.5%. The pollen retains viability for 7-10 days.

The above described experimental data excluded an absolute sterility of *Asuretuli Shavi*'s pollen; it was determined that partial fertilization with its own pollen was possible. Therefore it was determined a feasibility of searching for such clones within the vineyards, that contain high amount of bisexual flowers, which could be cultivated in mono cultivar vineyards and cumbersome and costly artificial pollination procedure could be hence avoided, as well as the problems linked with planting of multi cultivar vineyards (Vashakidze, 2008).

In order to assess relative frost-resistance of the grapevine varieties distributed in Kvemo Kartli – *Rkatsiteli*, *Saperavi*, *Tavkveri*, and *Asuretuli Shavi* – method of rapid and delayed fluorescence has been used. Experiments were carried out according to the following scheme: at the end of January (when the phase of quenching is already passed), the canes were cut off and placed into the deep-freeze chamber at -20ºC temperature, for different time spans. Afterwards changes of the chlorophyll fluorescence indices were evaluated in the apparatus of the cane pheloderm.

The results obtained are shown in the Tab. 1. It was found that stationary value of delayed fluorescence in the photosynthesis apparatus within the pheloderm of all varieties – S, decreases with time, although deceleration velocity in different varieties is different – it decreases relatively slowly in *Rkatsiteli* and *Asuretuli Shavi*, then in *Saperavi* and, finally, in *Tavkveri*. Nearly the same results have been obtained in a case of variable fluorescence Fv (Tab. 1). According to the experimental material obtained in our earlier studies (Ortoidze et al., 1990; Duering et al., 1990), at low temperature, as slower delayed fluorescence value decreases, as higher is the plant's frost-resistance. Therefore, the results obtained indicate that according to their frost resistance the varieties investigated should be arrange in the following sequence: *Rkatsiteli*, *Asuretuli Shavi*, *Saperavi* and *Tavkveri* – so, *Asuretuli Shavi* has equal to *Rkatsiteli* frost-resistance and higher than *Saperavi* and *Tavkveri*.

Table 1. Changes of the rapid and delayed fluorescence characteristics in yearly shoots of grapevine, incubated at -20ºC temperature

#	**Grape varieties**	**Fv variable fluorescence**					**S delayed fluorescence**				
		Cont.	1 h	2 h	4 h	6 h	Cont.	1 h	2 h	4 h	6 h
1	*Rkatsiteli*	0,662	0,610	0,580	0,540	0,528	3,6	3,0	2,8	2,8	2,8
2	*Saperavi*	0,713	0,658	0,612	0,540	0,510	3,2	2,2	2,3	2,0	2,5
3	*Tavkveri*	0,686	0,560	0,510	0,460	0,443	3,4	2,3	2,1	1,8	1,5
4	*Asuretuli Shavi*	0,719	0,680	0,580	0,510	0,480	3,0	2,5	2,5	2,1	1,8

Asuretuli Shavi manifested significantly higher spring frost resistance relativly to other varieties, including *Rkatsiteli*. In late April of 2006, in the village of Asureti ~~a~~ sharp decrease of temperature did occur (-2ºC at night). As a result, a yield of *Asuretuli Shavi* decreased only by 30%, while that of *Rkatsiteli* and other varieties – by 46%.

Considering the global warming trends during last years, a high importance attained a tolerance of grapevine against extremely high temperatures. Investigation of the grapevine resistance against high temperature was performed according to the following scheme: in a period of July-August (which is characterized with significantly high temperatures), sampled grapevine leaves were placed for 15 min at various temperatures. Then, the changes of delayed fluorescence stationary values – S, were evaluated in the leaves.

As shown in the Fig. 1, the delayed fluorescence value initially decreases rapidly, and then decrease velocity slows down. Fast decrease of the delayed fluorescence is concerned with inhibition of ATP synthesis and CO_2 binding process, while slow part of its decrease is concerned with decreased intensity of electrons, between the photosystems (Ortoidze et al., 1990). Meanwhile, as is evident from the figure, in *Tavkveri* stationary value of the delayed

fluorescence decreases more rapidly than in *Rkatsiteli*. This could be explained by the fact that *Rkatsiteli* is more resistant against high temperature than *Tavkver*i. *Asuretuli Shavi* holds a middle position in resistance against to high temperatures.

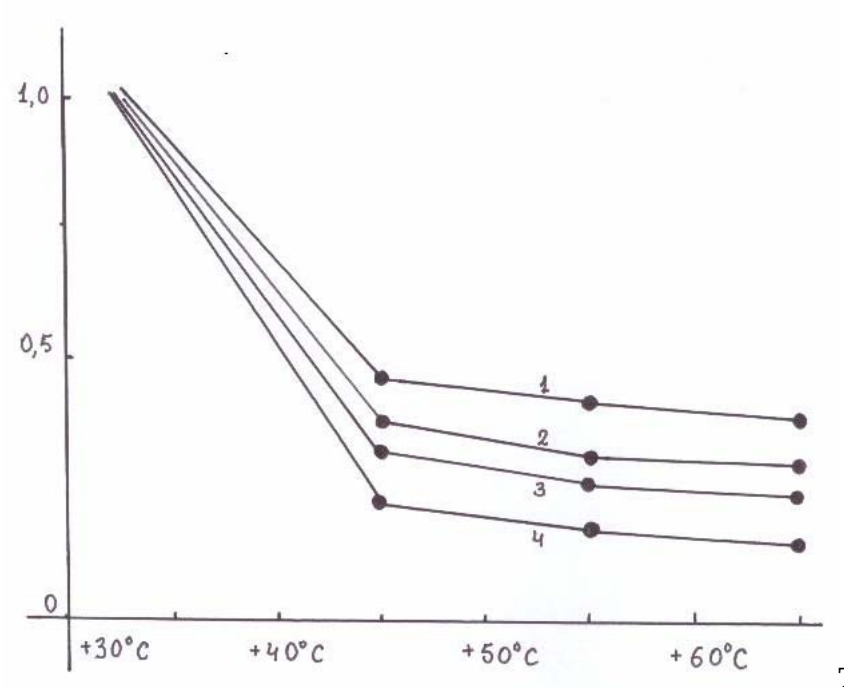

Fig. 1. Dependence of the stationary values of delayed fluorescence – S, on temperature.
1 – *Rkatsiteli*; 2 – *Asuretuli Shavi*; 3 – *Saperavi*; 4 – *Tavkveri*.

Coming from here the frost-resistance of tested cultivars is rank in the following order: *Rkatsiteli*, *Asuretuli Shavi*, *Saperavi* and *Tavkveri.* So *Asuretuli Shavi* has equaled to *Rkatsiteli* frost-resistance and higher than *Saperavi* and *Tavkveri*. As to its resistance against high temperatures, in our opinion it is at the same level of *Saperavi.* *Asuretuli Shavi* is sufficiently resistant against the spring frost bouts.

Above mentioned indices of *Asuretuli Shavi* reflect in the biochemical properties of a wine produced from this variety: a) the wines contain diglicoside of malvidine, although like representatives of *Vitis vinifera*, the malvidine monoglicoside is dominating among the anthocyanes (Bezhuashvili et al., 2007). Besides, unlike the Euro – American hybrids, in these wines no color alteration and misting has been reported; b) Similarly to representatives of *Vitis vinifera*, in the wines of *Asuretuli Shavi*, 76-87% of total phenol compounds are the polymer pro-anthocyanes; c) the juice of *Asuretuli Shavi* is of relatively low acid – 6.4 g/l, out of which 3.0 g/l is represented as potassium bitartrate and deposits in a sediment form in wine, which results decreasing of titratable acidity. Likewise, interrelation between the wine acid and apple acid does deteriorate.

Considering to mentioned peculiarities, *Asuretuli Shavi* should be proposed for production of naturally pink wine and addition of wine acid during technological process is advisable.

CONCLUSIONS

The results obtained allow concluding that Asuretuli Shavi, in conditions of Kvemo Kartli, demonstrates fairly high resistance against the environmental factors. It is resistant against extreme temperatures, well tolerates to the spring-time frosts.

Notwithstanding the fact that the variety is female, it was determined that its fertilization with own pollen is partially possible. As a result it becomes possible to search such clones in the vineyards, the flowers of which contain many bisexual flowers.

The variety is high-yielding and, considering its peculiarities, it is expedient to implement it for production of the pink wines.

BIBLIOGRAPHY

Bezhuashvili M., Jigauri G., Ortoidze T., 2007. Chemical peculiarities of the grape variety "Asuretuli Shavi" and their manifestation in wine products. Bulletin of the Academy of Agricultural Scienes of Georgia, B.21, pp. 66-69. (in Geogian)

Cholokashvili S., 1939. Handbook of viticulture. In 2 volumes. v.2 Amphelography. Tbilisi, Editor "Ganatleba". 278p. (in Georgian)

Compedium, 2006. Compendium of international methods of analysis OIV, HPLC – Determination of nine major anthocyanins in red and rose wine. Paris, v.1: 46-49

IPGRI, 1997. Descriptors for Grapevine (*Vitis ssp*.). IPGRI, Rome, 56p.

Duering H., Ortoidze T., Bushnell B. 1990. Effects subzero temperatures on chlorophyll fluorescence of grapevine Buds. Plant Physiology, 136: 758-760

Kimura P., Okomoto C., Hirano K., 1998. The mode of pollination and stigma receptivity in "Vitis coignetiae Pulliat". American Journal of Enology and Viticulture, 49(1): 1-5

Lakin G.F., 1990. Biometrics. Minsk, "Visshaia Shkola" publisher. 207p. (in Russian)

Negrul A.M., 1934. Contribution to the question as to parthenocarpy and apomixes in the grapevine. *Bulletin of Applied Botany, Genetics and Plant Breeding*. Saint-Petersburg. 2: 229-267p. (in Russian)

Ortoidze T., Matorin D.N., Venedictov P.S. 1990. Change in Luminescence of Phelloderm Chlorophyll during Dormancy in relation to Frost-hardiness of Grape. Plenum Publish. Corp. pp.661-667.

Pausheva Z.P., 1974. Method of plant cytology. Moscow, 284p. (in Russian)

Panarina A.M., 1974. Phonotypical variability of ampelographic characters. In "Grapevine breeding". Yerevan, Pp. 108-202 (in Russian)

Ramishvili M.A., 1970. Amphelography. Tbilisi, 304 p. (in Georgian)

Schreiber U., 2005. Chloropfylfluoreszenz und photosynthetische Energiumwandlung. Im: Experimente mit dem TEACHING-PAM Chlorophyll Fluorometer. H.Walz GmbH, Effeltrich/Germany, Pp. 79-102.

Vashakidze L.K., 2006. Scientific bases for identification of Georgian genotypes of grapevine and optimization of some phitotechnic activities. Tbilisi, Manuscript for dissertation. 284p. www.nplg.gov.ge (in Georgian)

Vashakidze L.K., 2007. Cell parameters of Georgian grapevine genotypes. Wine-making and Viticulture, Moscow, 6: 46-47 (in Russian)

Vashakidze L.K., 2008. Pollen peculiarities of Georgian grapevine genotypes. Wine-making and Viticulture, Moscow, 6: 46-47 (in Russian)

Identification of Grape Varieties via Digital Leaf Image Processing by Computer

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ABSTRACT

Grape variety identification is of great significance for resource statistics, new specie detection and protection of genetic resources. Based on the classical ampelographic grape identification method combined with machine learning and pattern recognition techniques in computer science, we proposed a new cheap and fast identification method via leaf image processing. We demonstrated its feasibility via the implementation of a prototype which could classify 354 leaf images belonging to 20 varieties with an accuracy rate of 87%. Our techniques can be applied to computer aided diagnosis of grape leaf diseases and new variety discovery, as well as to quantify the classical ampelographic identification method. We propose further work to transform this new method from a prototype into a practical software product.

Keywords: Grape variety identification; Ampelography; Pattern recognition; Image processing; Hu's moment invariants

RESUME

L'identification des cépages est très importante pour les statistiques de ressources, la détection des nouvelles espèces et la protection des ressources génétiques. Basés sur la méthode classique de l'identification ampélographique et grâce aux récents progrès en informatique, notamment la reconnaissance des formes et l'apprentissage automatique, nous proposons une nouvelle méthode efficace et automatique par ordinateur pour l'identification des cépages. Nous démontrons sa faisabilité via la réalisation d'un prototype qui a pu classifier 354 fichiers de feuilles, appartenant à 20 cépages avec une précision de 87%. Notre technique peut s'appliquer au diagnostic des maladies de vigne, à la découverte de nouvelles espèces et à la quantification de la méthode classique de l'identification ampélographique. Nous suggérons des directions de recherche future afin de transformer notre prototype en produit logiciel.

I. Introduction

There are over 10,000 grape varieties throughout the world. About 3000 of them are widely cultivated in production and many are wine varieties [Zhai, 2001]. Grape variety identification is of great significance for resource statistics, new specie detection and protection of genetic resources. OIV's Strategic Framework includes the task for recognising new viticultural varieties [OIV, 2005].

The classical identification method is based on ampelography [Galet，1990; Tassie and Blieschke, 2008]. Some new methods have been also developed recently, using different approaches such as DNA molecular genetic marker [Bower et al., 1993; Zhang et al., 1996; Testier et al., 1999], pollen morphology [Wang and Li, 2000], anthocyanin analysis [Wendelin and Barna, 1994], etc. All these methods need expert intervention and are hence quite expensive. Some of them need special devices and take a long time. Today, computer technologies have a wide range of applications in many fields including grape production. There are many successful examples where the computer has been used for image processing [Li et al., 2007; Barbu, 2009] and identification of plant species [Ye et al., 2004] based on pattern recognition. We look for a new method for identifying grape varieties combining the computer techniques and the classical ampelography. Based on the processing of digital grape leaf image, this new method would be rapid, efficient and nearly automatic with little or even no human intervention. Our research objective is to develop a software product, available on web, which will be able to tell a browser the variety of the grape leaf image that s/he uploads.

The ampelographic identification of grape varieties is based on the observation of features on some organs of a grape, such as flower, berry, shoot and leaf. OIV has produced 2 editions [OIV, 1983; OIV, 2009] of the document "OIV descriptor list for grape varieties and *Vitis* species" which defines as a standard the ampelographic characteristics for the identification of *Vitis* varieties and species. Using the 128 characteristics selected by [OIV, 1983] where each characteristics is signed a code and may take values from 1 to 9 for all grapes, [OIV, 2000] describes 250 wine grape varieties of its member states, by assigning a values to descriptor codes for each variety . For a given grape sample, if each its code has the same value as the variety V of the 250 in [OIV, 2000], this grape's variety is classified as V. All ampelographic experts agreed that the features of mature leaf are the most determinate for the varieties identification. For the 128 codes, 35 of them are for leaf and 29 for mature leaf. [OIV, 2009] adds another 18 codes from 601 to 618 on mature leaf. On the "Primary descriptor priority list" of 14 codes, there are 9 on leaf.

In our new approach based, the main idea is to let computer calculate all the code values instead of measuring them by a human being. Then the computer can compare these values against the known ones as in [OIV, 2000] to find the right variety. However, on one hand, it's not easy to calculate some code values and on the other hand, it is not necessary to know all these values for the identification purpose. Furthermore, some features not selected by [OIV, 2009] may also contribute to distinguish or identify varieties, for example, Hu's moment invariants for an image [Hu, 1962].

A digital image is composed of a pixel f(x, y) matrix where (x, y) is the index or coordinator of the matrix. Each pixel f(x, y) represents an image dot and is described by a series of numbers. For a binary image like a photo in an old news paper, a pixel f(x, y) is either 0 for white or 1 for black. For a colour image taken by a digital camera, a pixel may be a combination of three basic colours with different densities. Hu defined 7 moment invariants for any digital image. Each invariant can be easily calculated as the function of its pixels f(x, y). The 7 invariants' values are nearly independent of the rotation, position or size of the image of the matrix. They have been successfully used in computer pattern recognition applications such as car registration number [Liu and Lu, 2008], static hand gesture [Liu et al., 2008], tiger variety [Xu and Qi, 2009], human face [Gan and Zhang, 2002] and corn leaf disease recognition [Shen et al., 2008]. Yanhua YE and Chun CHEN of Hong Kong Polytechnic University have developed a Computer Plant Species Recognition System, CPSRS [Ye et al., 2004] which could provide a convenient and efficient way to search and identify plant species from a digital image file.

Departing from the works mentioned above, which consist of the cornerstone of our method, we present our method in detail and experiment it by the implementing a software prototype on an ordinary personal computer. We then analyze our experiment results and discuss on some choices that have been made, the remaining problems and possible improvements as well as applications. We conclude on the feasibility of our new method and point out the future work.

II. Materials and Methods

Our identification method is constructed on 4 steps: 1) collect typical mature grape sample leaves for the varieties we want to identify, 2) scan the leaves into digital image files, 3) select a set of characteristics or features useful for identification and computable by computer from the images, 4) build a software classifier based on the features calculated from the sample files.

1) Collect mature sample leaves

Following the requirements of OIV [OIV, 2009], for each variety, we collected about 10 mature leaves from different shoots at the third middle level, between berry set and veraison time. These leaves were collected from the grape variety culture field of College of Oenology, Northwest A&F University in Yangling, Shaanxi, China. There are a total of 500 leaves belonging to 3 wild local varieties and 47 cultured ones including Sauvignon, Riesling, Traminer, Sémillon, Chenin Blanc, Ugni Blanc, Müller-Thurgau, Cabernet Sauvignon, Carignan, Gamay, Syrah, Muscat, etc.

2) Obtain digital leaf files

For each leaf, we scanned both leaf sides with the default parameters of 3 A4 size ordinary scanners. We got a total of 1073 colour leaf image files at the resolution of 300 DPI (Dot Per Inch). In our software prototype, we used 354 leaf files of 20 varieties.

3) Select features for identification

Naturally, the 47 features on mature leaves, coded by OIV [OIV, 2009] have been considered. More researches have to be done for calculating some features, e.g. “density of prostrate hairs between the main veins on lower side of blade”, OIV code 84. We have found a way to calculate some of them, including size and circumference of blade, length of petiole, length of veins, etc. We select also some features, neither considered by OIV nor ampelography, which are easy to calculate and useful for identification, e.g. Hu’s 7 moment invariants. In order to quickly build our prototype, with the criteria of both computable and useful, we finally selected the size and circumference of blade and Hu’s 7 moment invariants to form the feature set or vector of 9 dimensions.

4) Build a software classifier

Let’s explain the mathematical basis of our method. Each leaf image is represented by a feature vector $L_j = (f_{j1}, \dots, f_{j9})$ where f_{ji} is a real number. Such vector L_j is a point in the 9 dimension feature space in mathematics. We imagine the Euclidean distance $D(L_1, L_2) = \sqrt{(f_{11} - f_{12})^2 + \cdots + (f_{19} - f_{29})^2}$ between 2 grape leaves L_1 and L_2 of the same variety should be in average smaller than that of 2 different varieties. For the variety i, its mass centre $C_i = (c_{i1}, \dots, c_{i9})$ Where $c_{im} = \sum_{j=1}^{n} \frac{f_{jm}}{n}$, f_{jm} is the m-th feature of the j-th leaf sample Lji of the variety Vi, and its radius R_i=maximum of $D(C_i, Lji)$ for j=1 to n. For a given leaf j’s vector L_j, if we only find one variety S which can satisfy $D(L_j, C_S) \leq R_S$, we can conclude that the leaf L_j belongs to the variety S.

Unfortunately, for the 354 vectors of 20 varieties, their mass centres are so close and their radiuses are so big that the 9 dimension sphere of a variety S_i at centre C_i with radius R_i has intersection with the spheres of other varieties. To reduce the space occupied by each variety, we improve the above method by detecting the 9 dimension cube which inscribed the sphere. This can be done by finding the value range of the vector’s each dimension for every variety. Some of leaves still cannot be distinguished. For this case, based on the fact that the values of each dimension for each variety should satisfy the normal distribution, we introduce the probability of a leaf L belonging to a variety S by the following formula:

$$P(L, S) = 1 - \sum_{j=1}^{9} \frac{2 * dL(j)}{r(S,j)} * \frac{1}{9}\ ,\ dL(j) = \left| L(j) - \frac{1}{2} * r(S, j) \right|,\quad r(S, j) = \max(f_{sj}) - \min(f_{sj})$$

where $\max/\min(f_{sj})$ means the maximum/minimum value of j-th dimension for all samples of the variety S.

Finally, we build our classifier with the following algorithm:

1) Find the vector L_i of a leaf image i and compare L_i (f_{ij}) with the value range max/min(f_{sj}) of all varieties S (S=1 to 20 and j= 1 to 9).
2) If max(f_{sj})>=f_{ij}>=min(f_{sj}) holds for only one variety S with j=1 to 9, then the leaf L_i belongs to the variety S.
3) Else, leaf L_i satisfies the relation in step 2) for m varieties $S_1, \cdots, S_m$,. We find out all the probabilities $P(L_i, S_j)$. L_i belongs to the variety S_j with the probability $P(L_i, S_j)$ which is the maximum of $P(L_i, S_j)$ for j=1 to m.

III. Results and Discussion

We developed a software prototype in Matlab implementing the above algorithm to verify our method. The following figure 1 shows an execution of our prototype under Matlab environment. The user selects a leaf image and then asks for the classification. The prototype displays the image in 3 modes and prints out the variety name:

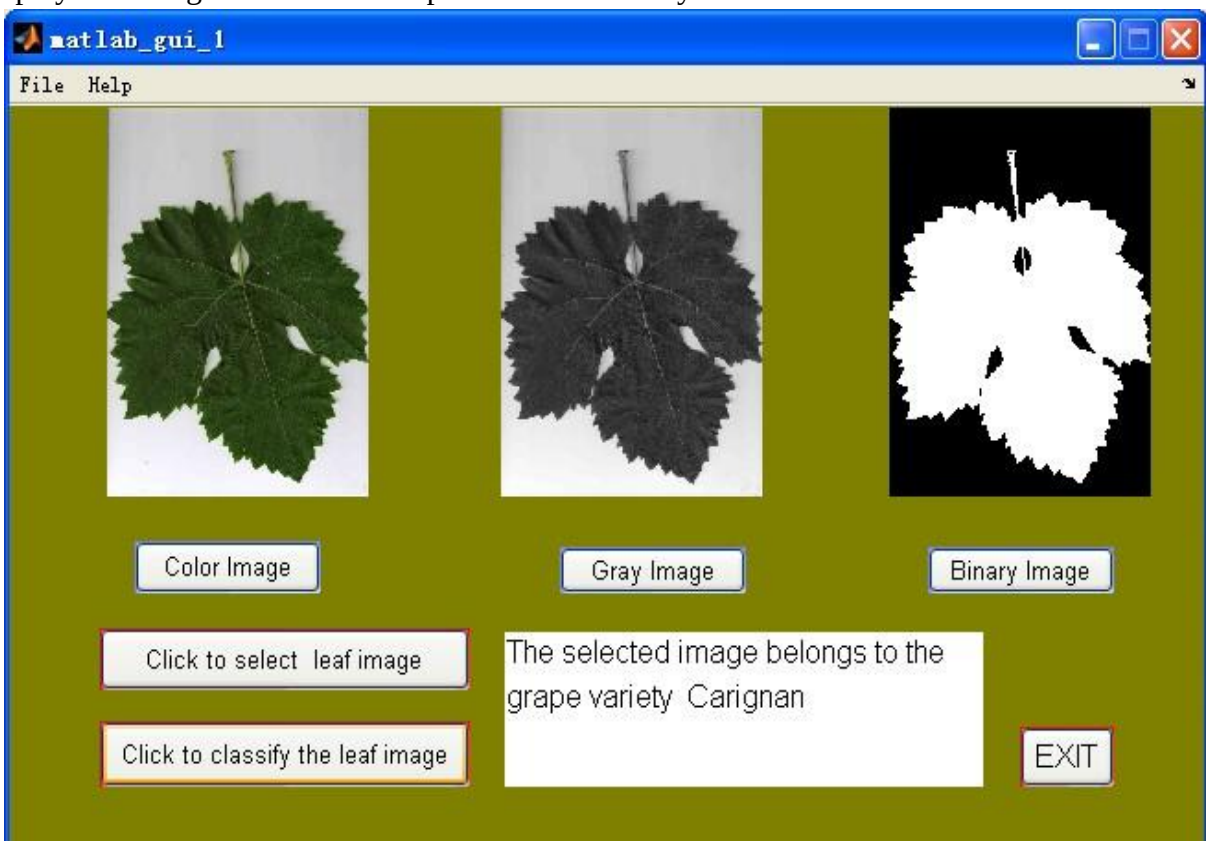

Figure 1. Execution of classification prototype

We have tested our algorithm to classify the 354 images files belonging to 20 varieties. The correct classification rate is of 87%. This rate is obtained by calling the classifier on all the 354 files and count the present of corrected classified ones.

The accuracy decreases when the number of varieties increases. We may resolve this problem by increasing the feature vector's dimensions, i.e. to find more features, and use better classification algorithm such as SVM [Wu et al., 2008]. The latter is a well known machine learning method for classification. It is in fact the capacity of computer to learn from examples. After having trained it by giving many leaf samples belonging to each grape variety, the software can decide to which variety a new leaf belongs to.

Our prototype can only classify scanned images. In order to classify digital camera images, we have to consider factors such as photography distance and focus. On the other hand, a camera may take photos from different angles. This may allow us to distinguish the prostrate hairs of a leaf from the erect hairs. However, this problem can be better resolved by a 3 dimensions camera or scanner. Based on the 3D image technique, we can more easily calculate other leaf features such as the profile of leaf (OIV code 74 [OIV, 2009]). Light wave lengths other than visible ones, such as infrared, microwave and terahertz [Lu, 2002; Xing and Baerdemaeker, 2005] etc. can also be used to obtain digital leaf images. These images should supply complementary features, useful for the variety identification. By combining these mentioned techniques, we are expected to be able to calculate all the 45 codes selected by OIV and hence to identify all grape varieties based on digital image processing by computer.

This new identification method may not only simplify the identification procedure, but its techniques can also improve the classical ampelographic identification method. The current OIV Descriptor List [OIV, 2009] uses the code values in a qualitative way. For example, the code 65 for the size of blade takes values 1, 3, 5, 7 and 9 which means respectively, very small, small, medium, large and very large. Our method can calculate the size of blade in $inch^2$ or cm^2 effectively and automatically. We may do this for all quantifiable codes of all known varieties. With these quantitive values, we may give a value range for each current qualitative value on one hand, and check if the code values for the 250 varieties in [OIV, 2000] are coherent. These techniques can be used to detect new variety and guess the parent varieties of a new hybrid variety. In fact, the feature vector of a new variety will not belong to any known varieties, but a hybrid one should be close to its parents' ones. Computers software can easily find out all the similar varieties and sort them according to the similitude.

IV. Conclusions

Based on the classical ampelographic grape identification method combined with machine learning and pattern recognition techniques in computer science, we proposed a new cheap and fast identification method via leaf image processing. We demonstrated its feasibility by implementing a software prototype which could classify 354 leaf images belonging to 20 varieties with an accuracy of 87%.

We are continuing our research to increase both the number of grape varieties and the accuracy by calculating more features from digital images and improving the classification algorithms.

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Bibliography

Barbu T., 2009. Content-based image retrieval using Gabor filtering. In: Proceedings - 20th International Workshop on Database and Expert Systems Applications. New York: Institute of Electrical and Electronics Engineers Inc. 2009: 236-240.

Bower J.E., Bandman E.B., Meredith C.P., 1993. DNA fingerprinting characterization of some wine grape cultivars. American Society for Enology and Viticulture, 44: 266-271.

Galet P., 1990. French grapevine varieties and vineyards. Volume 2. The French ampelography. 2nd edition. Montpellier.

Gan J.Y., Zhang Y.W., 2002. Face Recognition Based on Moment Invariants and Neural Networks. Computer Engineering and Applications, 38(7): 53-57.

Hu M.K., 1962. Visual Pattern Recognition by Moment Invariants. IRE Transaction Information Theory, 8(2): 179-187.

Li Y., Chi Z.R., Feng D.D., 2007. Leaf vein extraction using independent component analysis. In: 2006 IEEE International Conference on Systems, Man and Cybernetics. New York: Institute of Electrical and Electronics Engineers Inc. 5: 3890-3894.

Liu J.M., Lu K., 2008. The identification of car logo based on Hu's invariant moments. scientific and technical information (Academic Edition), 36: 76-81.

Liu Y., Gan Z.J., Sun Y., 2008. Static Hand Gesture Recognition and its Application based on Support Vector Machines. In: Software Engineering, Artificial Intelligence, Networking, and Parallel/Distributed Computing. Ninth ACIS International Conference, Phuket: 2008(9): 517-521.

Lu R., 2002. Detection of bruises on apples using near-infrared hyperspectral imaging. Information & Electrical Technologies Division of ASAE, vol.46(2): 1-8.

OIV, 1983. 1st Edtion of the OIV Descriptor list for grape varieties and Vitis species. http://www.oiv.int.

OIV, 2000. Description of world vine varieties. http://www.oiv.int.

OIV, 2005. RESOLUTION AG 1/2005, OIV Strategic Framework 2005-2008. . http://www.oiv.int.

OIV, 2009. 2nd Edtion of the OIV Descriptor list for grape varieties and Vitis species. http://www.oiv.int.

Shen W.Z., Wu Y., Chen Z.L., Wei H.D., 2008. Grading method of leaf spot disease based on image processing. In: Proceedings - International Conference on Computer Science and Software Engineering. NJ: IEEE Computer Society. Vol. (6): 491-494.

Tassie L., Blieschke N., 2008. Ampelography: do you know what variety you are planting in your vineyard or nursery. Australian & New Zealand Grape grower & Winemaker: national journal of the grape and wine industry, No.537.

Testier C., Daivd J., This P., Boursiquot J. M., Charrier A., 1999. Optimization of the choice of molecular markers for varietal identification in Vitis Vinifera L. Theoretical and Applied Genetics, 98(1): 171-177.

Wang X.D., Li C.L., 2000. A study on pollen morphology of the genus Vitis L. Acta Phytotaxonomica Sinica, 38(1): 43-52.

Wu D.K., Xie C.Y., Ma C.W., 2008. The SVM classification leafminer-infected leaves based on fractal dimension. In: 2008 IEEE International Conference on Cybernetics and Intelligent Systems, CIS 2008. NJ: Inst. of Elec. and Elec. Eng. Computer Society. 2008: 147-151.

Xing J., Baerdemaeker J.D., 2005. Bruise detection on ‘Jonagold’ apples using hyperspectral imaging. Postharvest Biology and Technology, 37(2005): 152-162.

Xu Q.J., Qi D.W., 2009. Parameters for Texture Feature of Panthera tigers altaica Based on Gray Level Co-occurrence Matrix. Journal of Northeast Forestry University, 37(7): 125-130.

Ye Y.H., Chen C., Li C.T., Fu H., Chi Z.R., 2004. A Computerized Plant Species Recognition System. In: Proceedings of 2004 International Symposium on Intelligent Multimedia, Video and Speech Processing. Hong Kong: Institute of Electrical and Electronics Engineers Inc. 2004: 723-726.

Zhai H., 2001. The main grape varieties used for processing. In: Wine Grape Growing and Processing Techniques. Yang T.Q., 1st edition. Beijing: China Agriculture Press. 1: 48.

Zhang L.P., Lin B.N., Shen D.X., 1996. The extraction, purification and identification of RFLP of the chromosomal DNA of Grape. Journal of Fruit Science, 13(2): 71-74.

Proteomic approach to study the influence of cluster thinning on protein expression on Bombino and Falanghina grapes.

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RIASSUNTO

I vitigni di varietà Bombino e Falanghina coltivati in vigneti situati nella regione Puglia, Italia meridionale, sono stati diradati a due livelli (0% e 30%) per due anni consecutivi (2007 e 2008). Il diradamento è stato effettuato prima dell'invaiatura (fine luglio) eliminando i grappoli distali. Le proteine solubili delle bacche mature sono state estratte con acetone, acido tricloroacetico ed frazionate in elettroforesi bidimensionale. Circa 300 spot sono stati rivelati per le due varietà al livello 0% (t_0) e circa 200 spot per quelle al livello del 30% (t_{30}). Il volume totale degli spot per le due varietà era molto simile a t_0, nel Bombino tuttavia si registrava un volume leggermente superiore, mentre a livello t_{30} il volume totale degli spot rimaneva più alto nella Falaghina. Questi risultati hanno dimostrato che, a livello t_0, il Bombino pur avendo un minor numero di spot rispetto alla Falanghina l'espressione proteica è stata maggiore mentre si riduceva il numero di proteine al livello t_{30}. In conclusione la gestione agronomica influenza fortemente l'espressione delle proteine nelle due varietà.

ABSTRACT

Vines of cultivars Bombino and Falanghina grown in vineyards located in the Apulian region, Southern Italy, were thinned at two levels (0%, and 30%) for two consecutive years (2007 and 2008). The cluster thinning was done just before veraison (end of July) eliminating the distal clusters. Major soluble proteins of grapevine ripe berries were extracted with trichloroacetic acid acetone and resolved in two dimensional electrophoresis. About 300 spots were detected for the two cultivars thinned at 0% level (t_0) and about 200 spots for those thinned at 30% level (t_{30}). Total spot volume was similar at t_0 level for the two cultivars, but slightly higher in Bombino, whereas at t_{30} level spot volume remained higher in Falaghina than in Bombino. These results demonstrated that, at t_0 level, this latter cultivar also having a lower number of spot with respect to Falanghina the protein expression was higher whereas reduced the number of protein expressed t_{30} level. In conclusion agronomic management strongly influence the protein expression in the two cultivars.

INTRODUCTION

Grapevine (*Vitis vinifera* L.) is a perennial woody vine that produces the most economically important fruit crop in the world. Grapes contain naturally a wide range of different proteins. They are not high in protein compared with some other fruits. However, they assume a considerable technological and economical importance because they greatly affect the clarity and stability of wines. Most notably, these proteins include chitinases, thaumatin-like proteins

and osmotins (Monteiro et al., 2001; Waters, et al., 1998), which are particularly stable under winemaking conditions (low pH, proteolysis), passing selectively using to the wine. Several authors (Koch and Ajak, 1959; Moretti and Berg, 1965; Baily and Berg, 1967) indicated that the proteins found in grapes or must prior to fermentation may be dictated by factors such as grape cultivar, viticultural practices, soil, climate or year, in a complex manner. Among viticultural practices cluster thinning aims to adjust crop load so grape maturation may be advanced and potential wine quality improved. This may be especially important in vines that are overcopped and thus out of balance. Naor et al. (2002) reported a negative correlation between crop load (varied by shoot and cluster thinning) and wine sensory score despite no consistent differences in fruit composition of field-grown Sauvignon blanc studied in Israel over three years. In a field study with Nebbiolo in the Italian Piedmont region, also conducted over three years, removing half of the clusters at the pea-size stage (one month after bloom) reduced yield by 43% and increased berry soluble solids by 7% and anthocyanin concentration by 18% (Guidoni et al., 2002). In contrast, another three-year field study with Cabernet Sauvignon in Napa Valley, California, where either one-third or two third of clusters were removed two weeks after bloom, found that although yield was reduced by about 20% and 33%, respectively, juice composition and wine quality were very little affected by cluster thinning (Oughg and Nagaoka, 1984).

Grapevine protein profiles have been characterized by proteomic studies (Tesnierres and Robin, 1992; Sarry et al., 2004; Carvlho et al., 2005; Vincent et al., 2006). Although powerful mass spectrometry-based techniques have emerged recently, such as isotope-coded affinity tagging (ICAT; Gygi et al., 1999), mass-coded abundance tagging strategies (MCAT; Cagney and Emili, 202), stable isotope labelling by amino acids in cell culture (SILAC; Ong et al., 2002), or isobaric tags for relative and absolute quantitation (iTRAQ; Ross et al., 2004), as well as direct isotopic labelling methods using ^{18}O (Rao et al., 2005) or D_2O (Che and Fricker, 2005), two dimensional electrophoresis (2-DE) remains one of the most efficient strategies to isolate proteins showing quantitative variation in response to a treatment (Rabilloud, 2002; Gorg et al., 2004). The reproducibility of 2-DE and the accuracy of sample comparison were recently improved with the introduction of difference gel electrophoresis (DIGE; Unlu et al ., 1997) technique.

Here, a proteomic approach to the effect of the cluster thinning on protein expression on Bombino and Falanghina grapes, is presented. Quantitative analysis of two-dimensional gels from grapes showed that this viticultural practice strongly influence the protein expression in the two cultivars.

MATERIALS AND METHODS

Preparation of total protein extract from grapevine berries

The berries of two *Vitis vinifera* L. (cv. Bombino and Falanghina) were harvested at ripe stage in the experimental vineyard (CRA, Apulia, Italy). The reproducibility of the whole protocol was evaluated upon conducting three independent extractions on ripe Bombino berries. Fresh berries were washed in tap water and weighed before extraction. All procedures described below were carried out at 4°C. Fresh grapevine berries berries were homogenized by manual crushing in cold 12.5% TCA-acetone solution containing 28 mM mercaptoethanol in a ratio of 1/10 w/v, which allows the skin and seeds to stay intact. After filtration on a 40 microM Miracloth mesh, the homogenate wasincubate at – 20 °C for 60 min and the proteins were precipitated by centrifugation at 10 000 x g for 15 min. After two washes with 85% ethanol, the pellet was resuspended in 1mL of IEF solubilisation buffer containing 2M

thiourea, 7 M urea, 4% CHAPS, 0.5% Triton X-100, 0.2 % Ampholine pH 3-10, 0.1 % Ampholine pH 4-7 and 65mM DTT. The resulting mix was frozen and thawed, and centrifuged at 100 000 x g for 45 min in order to remove insoluble polymers. The protein concentration was determined according to Bradford's method (Bradford, 1976) using bovine serum albumin as standard.

Isoelectric focusing with immobilized pH gradient and second-dimensional gel

Proteins (700 μg) dissolved in IEF solubilisation buffer were loaded by passive overnight rehydratation on Immobiline gradient pH 3-10 NL drystrip (GE Healthcare Bio-Sciences, Piscataway, NJ, USA). Isoelectric focusing was conducted at 120 kVh in a Multiphor II (LKB, Amersham Biosciencence) tank at 15 °C according to the following program of migration:active rehydratation at 50V during 9H, ramping at 1.5 kV for 9H, and focusing at 3.5 kV for 30 h. Strips were then equilibrated in 50 mM Tris-HCl (pH 8.8) containing 6M urea, 30% glycerol, 2% SDS, and 2% DTT in order to reduce disulfide bridges (20 min upon gentle stirring) followed by alkylation upon addition of 2.5% iodoacetamide for 20 min. Second-dimensional SDS-PAGE was performed in 10% acrylamide gels using an Isodalt apparatus. Running was first conducted at 40V for 12 h then at 150 V for 6 h. The spots were stained with colloidal Coomassie Brilliant Blue G-250 (GE Healthcare Bio-Sciences, Piscataway, NJ, USA) and the gels were scanned and analyzed with ImageMaster 2D Platinum (GE Healthcare Bio-Sciences, Piscataway, NJ, USA).

RESULTS AND DISCUSSION

2-DE and Image analysis

2-DE analysis was performed on ripe grape berries of Bombino and Falanghina cultivars. 2-DE gels of the cultivars are shown in Figure 1.The average number of detected spots was about 300 for both Bombino and Falanghina thinned at 0% level (t_0) (Fig 1 A) and about 200 spots for those thinned at 30 % level (t_{30}) (Fig 1B). In particular, at t_0 level Falanghina showed 363 spots, most of that concentrated in the zone 1; whereas Bombino showed 303 spots, most of that concentrated in the zone 3; at t_{30} level the two cultivar decreased the spot number: 233 for Falanghina, equally distributed in three zones (Fig. 1B), and 184 for Bombino, which tend to express more protein at low molecular weight (zone 3) (Fig. 2B). Zhang et al. (2008) demonstrated that the most berry plasma proteins (PM), which was resolved by two-dimensional gel electrophoresis, were involved in metabolic and cellular processes, and that during berry ripening the PM is a vigorous system in signal transduction and transportation of metabolites; dynamic for energy metabolism, protein trafficking, and proteolysis. Moreover they found that during grape berry ripening process, total PM protein content gradually decreased. Among all identified proteins, 12 showed significant differences in terms of their relative abundance. Increasing ubiquitin proteolysis and cytoskeleton proteins were observed from pre-véraison to post-véraison. Zeatin Oglucosyltransferase peaked at véraison, while ubiquitinconjugating enzyme E2-21 was down-regulated at this stage. Therefore we suppose that the increase of protein at low molecular weight and differential expression in the two cvs is due to proteome evolution during berry grape ripening process, gene expression of cv constitutive proteins and interaction between genotype and environmental condition as response to agronomic managements.

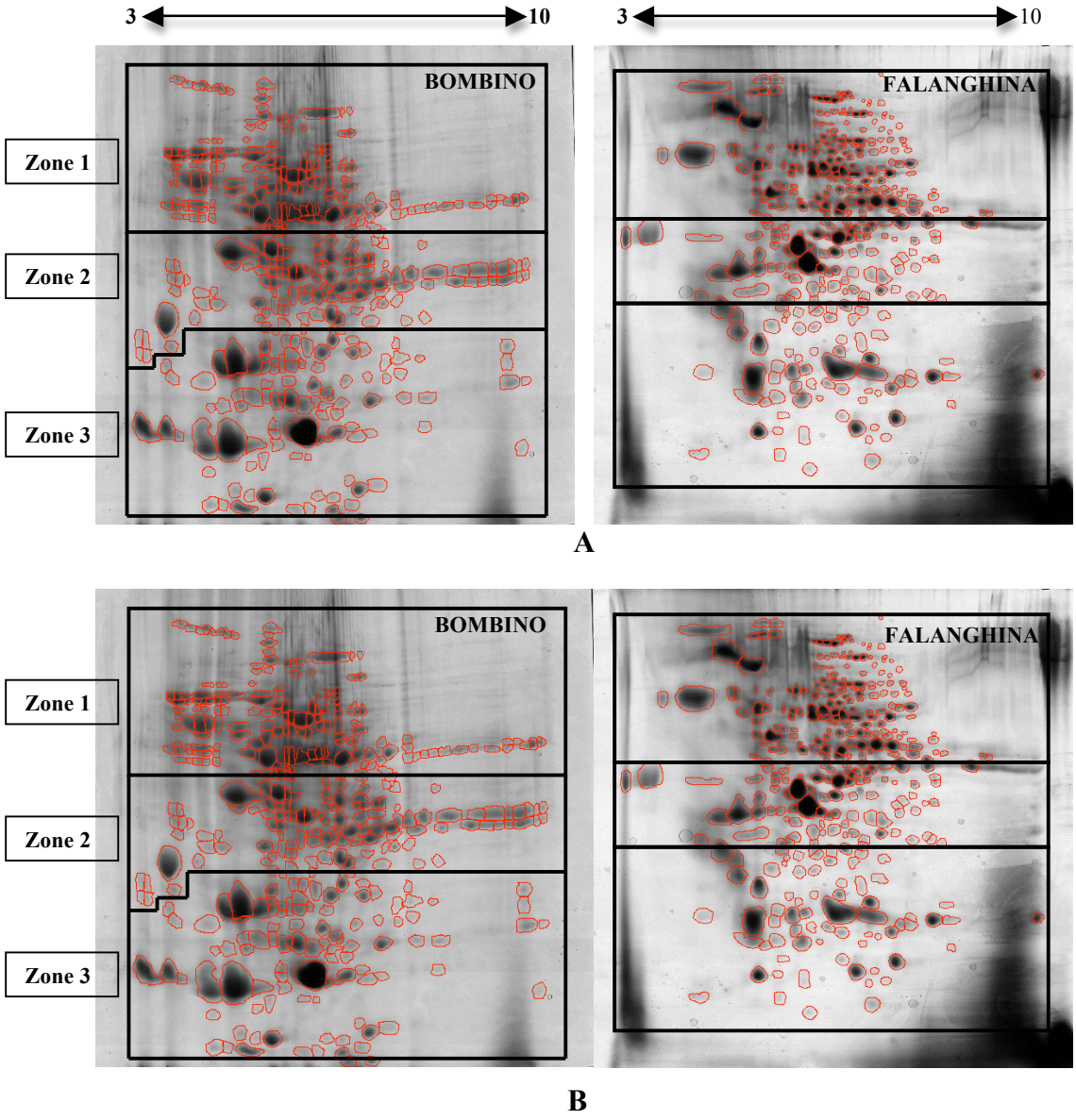
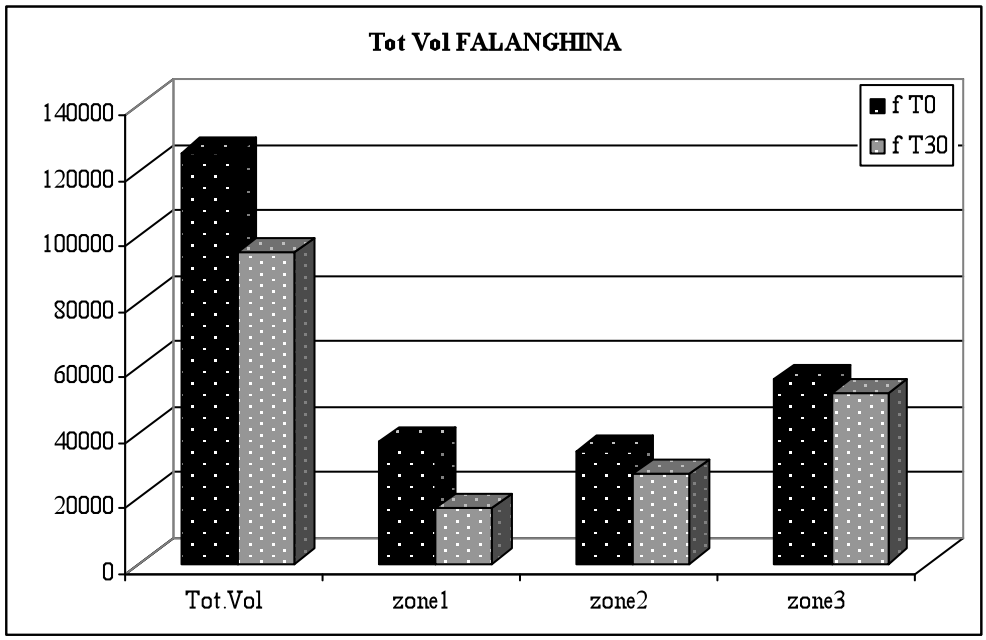
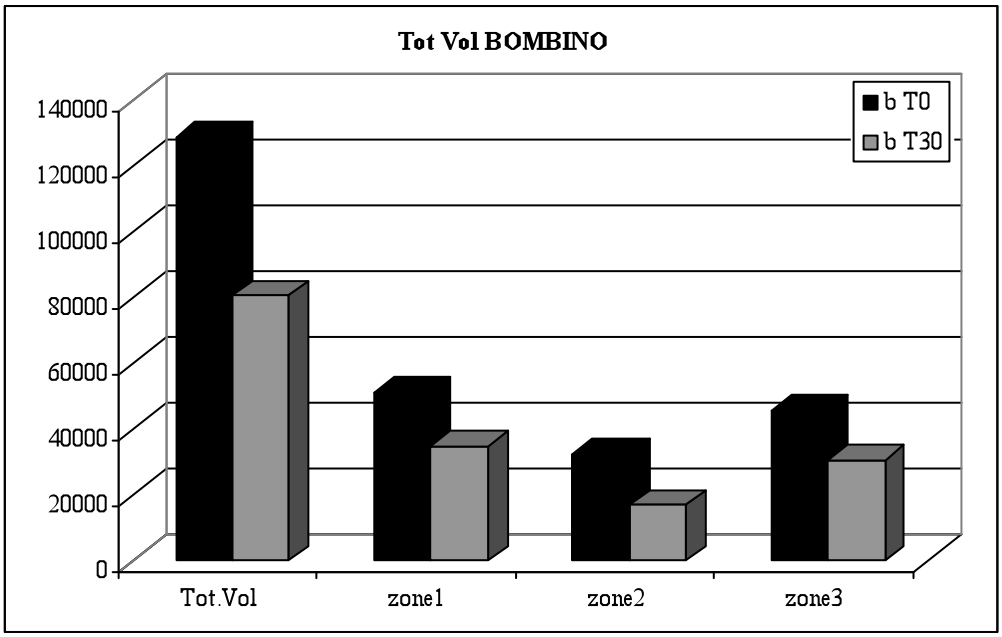


Figure 1. 2-DE maps of Bombino and Falanghina grape berries thinned at 0% level (t_0) **A** and thinned at 30 % level (t_{30}) **B**. **Zone 1**, **Zone 2** and **Zone 3** indicate respectively high, medium and low molecular weight area.

To ascertain the quantitative changes in the proteomics maps, their relative spot volumes (%Vol) were evaluated by software-assisted analysis. In Figure 2 is reported the Falanghina (Fig. 2A) and Bombino (Fig. 2B) spot total volume. As can be inferred from Figure 2 A, the Falanghina t_0 spot area total volume is larger than t_{30}, showing that the expression of the protein at higher molecular weight are those that differentiate more the two types of viticulture practice. Also in Bombino cultivar is possible to observe that the t_0 spot area total volume is larger than $t_{30,}$ while a larger volume in all three zone of the t_0 is shown. It is interesting to note that spot volume was similar at t_0 level for the two cultivars (Fig. 3 A), but slightly higher in Bombino, whereas at t_{30} level spot volume remained higher in Falanghina than in Bombino.

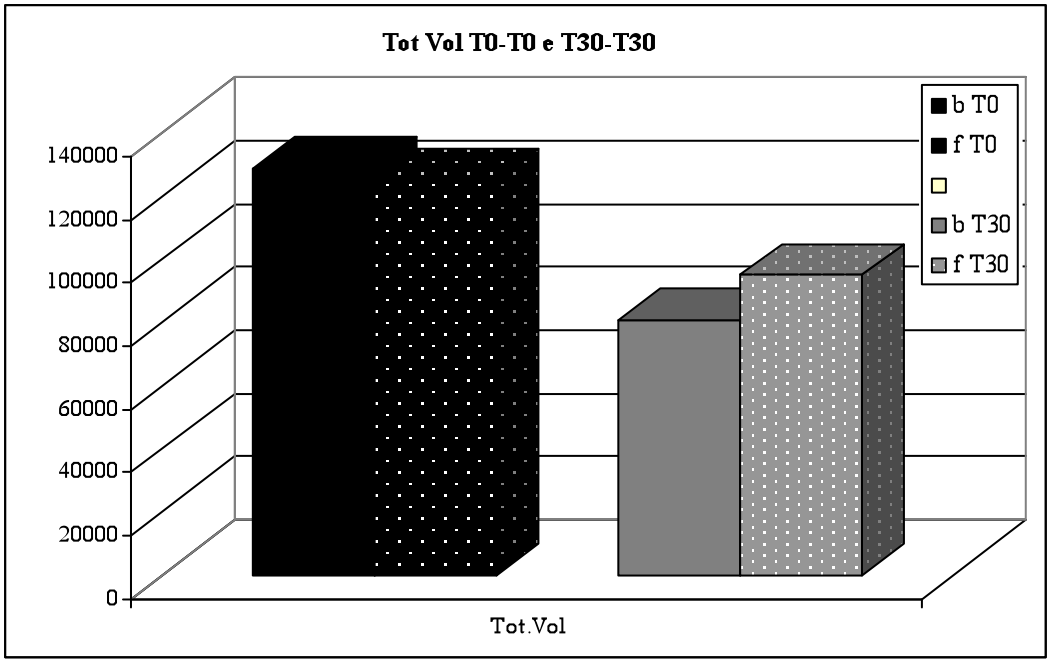


A

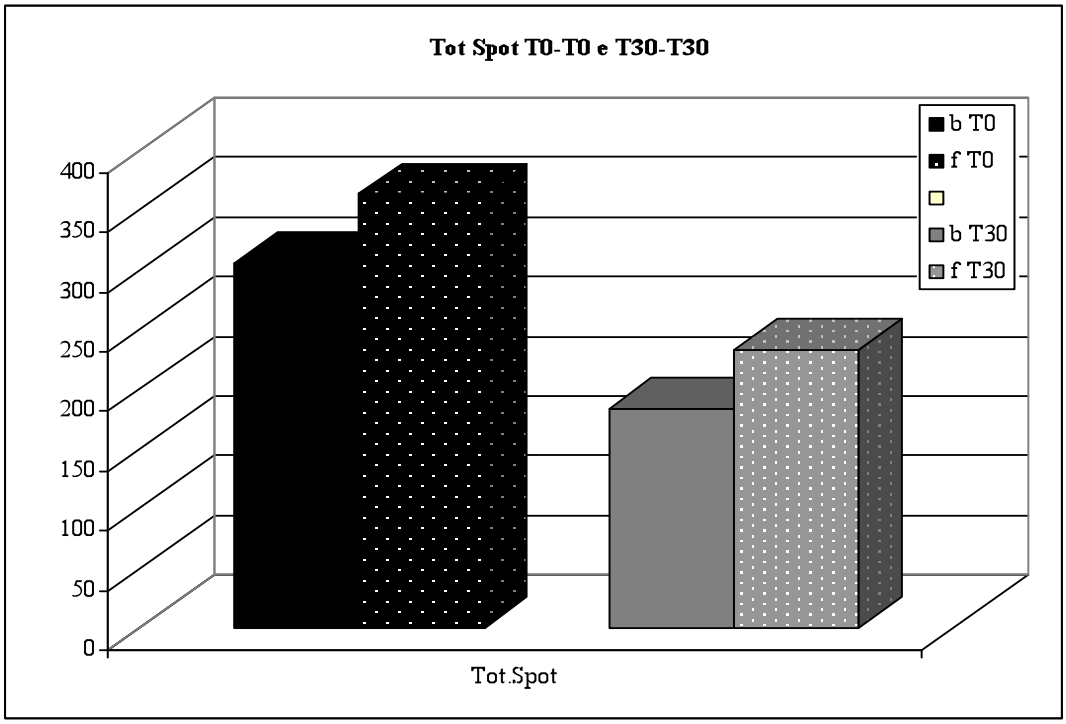


B

Figure 2. Spot area total volume of Falanghina (**A**) and Bombino (**B**) cultivars thinned at 0% level (t_0) and thinned at 30 % level (t_{30}).



A



B

Figure 3. Comparison of spot area total volume between Falanghina and Bombino cultivars thinned at at 0% level (t_0) and thinned at 30 % level (t_{30}) (**A**). Comparison of total spot between Falanghina and Bombino cultivars thinned at at 0% level (t_0) and thinned at 30 % level (t_{30}) (**B**)

Results demonstrated that, at t_0 and t_{30} level, this latter cultivar also having a lower number of spot with respect to Falanghina (Fig. 3B). So, it can be deduced that for the same expressed proteins the Falanghina cultivar produces more protein species than Bombino, highlighted in the higher molecular weight zone of the 2-D map.

CONCLUSION

The proteomic analysis proved a powerful tool in the revealing the influence of agronomic management on the protein expression in Bombino and Falanghina cv. On the other hand the increase of wine quality in thanned grape is well assessed and the diminution of protein expression can reduce the risk of protein precipitation concurring to increase wine quality.

ACKNOWLEDGEMENTS

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REFERENCE

Bayly F.C., Berg H.W., 1967. Grape and wine proteins of white wine varietals. *American Journal of Enology and Viticulture*, 24: 18-32.

Bradford M.M., 1976. A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry*, 72: 248-554.

Cagney G., Emili A., 2002. *De novo* peptide sequencing and quantitative profiling of complex protein mixtures using mass-coded abundance tagging. *Nature Biotechnology*, 20: 163-170.

Carvalho L.C., Esquivel M.G., Martins I., Ricardo C.P., Amancio S., 2005. Monitoring the stability of Rubisco in micropropagated grapevine (*Vitis Vinifera* L.) by two-dimensional electrophoresis. *Journal of Plant Physiology*, 162: 365-374.

Che F.Y., Fricker L.D., 2005. Quantitative peptidomics of mouse pituitary: comparison of different stable isotopic tags. *Journal of Mass Spectrometry*, 40: 238-249.

Gorg A., Weiss W., Dunn M.J., 2004. Current two dimensional electrophoresis technology for proteomics. *Proteomics*, 4: 3665-3685.

Guidoni S., Allara P., Schubert A., 2002. Effect of Cluster Thinning on Berry Skin Anthocyanin Composition of *Vitis Vinifera* cv. Nebbiolo. *American Journal of Enology and Viticulture*, 53:3: 224-226.

Gygi S.P., Rist B., Gerber S.A., Turecek F., Gelb M.H., Aebersold R., 1999. Quantitative analysis of complex protein mixtures using isotope-coded affinity tags. *Nature Biotechnology*, 17: 994-999.

Koch J., Sajak E., 1959. A review and some studies on grape protein. *American Journal of Enology and Viticulture*, 10: 114-123.

Monteiro S., Picarra-Pereira M.A., Mesquita P.R., Loureiro V.B., Teixeira A., Ferreira R.B., 2001. The wide diversity of the structurally similar wine proteins. *Journal of Agiculture and Food Chemistry*, 49: 3999-4010.

Moretti R.H., Berg H.W., 1965. Variability among wines to protein clouding. *American Journal of Enology and Viticulture*, 16: 69-78.

Naor A., Gal Y., Bravdo B., 2002. Shoot and cluster thinning influence vegetative growth, fruit yield, and wine quality of 'Sauvignon blanc' grapevines. *Journal of the American Society for Horticultural Scence*, 127: 628–634.

Ong S.E., Blagoev B., Kratchmarova I., Kristensen D.B., Steen H., Pandey A., Mann M., 2002. Stable isotope labeling by amino acids in cell culture, SILAC, as a simple and accurate approach to expression proteomics. *Molecular and Cellular Proteomics*, 1: 376-386.

Ough C.S., Nagaoka R., 1984. Effect of Cluster Thinning and Vineyard Yields on Grape and Wine Composition and Wine Quality of Cabernet Sauvignon. *American Journal of Enology and Viticulture*, 35: 1: 30-34.

Rabilloud T., 2002. Two-dimensional gel electrophoresis in proteomics: old, old fashioned, but it still climbs up the mountains. *Proteomics*, 2: 3-10.

Rao K.C., Carruth R.T., Miyagi M., 2005. Proteolytic ^{18} O labeling by peptidyl-Lys metalloendopeptidase for comparative proteomics. *Journal of Proteome Research*, 4: 507-514.

Ross P.L., Huang Y.N., Marchese J.N., *et al*., 2004. Multiplexed protein quantitation in *Saccharomyces cerevisiae* using amine reactive isobaric tagging reagents. *Molecular and Cellular Proteomics*, 3: 1154-1169.

Sarry J.E., Sommerer N., Sauvage F.X., Bergoin A., Rossignol M., Albagnac G., Romieu C., 2004. Grape berry biochemistry revisited upon proteomic analysis of the mesocarp. *Proteomics*, 4: 201-215.

Tesnières C., Robin J.-P., 1992. Two-dimensional electrophoresis of the total polypeptides in ripe red grape berries. *Electrophoresis*, 13: 93-96.

Unlu M., Morgan M.E., Minden J.S., 1997. Difference gel electrophoresis: a single gel method for detecting changes in protein extracts. *Electrophoresis*, 18: 2071-2077.

Vincent D., Wheatley M.D., Cramer G.R., 2006. Optimization of protein extraction and solubilization for mature grape berry clusters. *Electrophoresis*, 27: 1853-1865.

Waters E.J., Hayasaka Y., Tattersall D.B., Adams K.S., Williams P.J., 1998. Sequence analysis of grape (*Vitis Vinifera*) berry chitinases that cause haze formation in wines. *Journal of Agricultural and Food Chemistry*, 46: 4950-4957.

Zhang J, Ma H, Feng J, Zeng L, Wang Z, Chen S. 2008. Grape berry plasma membrane proteome analysis and its differential expression during ripening. *Journal of Experimental Botany*, 59: 2979-2990.

EVALUATION OF FATTY ACID CONTENT, TOTAL FAT AND TOTAL PHENOLIC COMPOUNDS IN THE SEED OF NATIVE GRAPE VARIETIES (*Vitis vinifera* L.) GROWN IN KONYA PROVINCE, TURKEY

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ABSTRACT

Grape seeds contain high amounts of unsaturated fatty acids, phenolic substances and antioxidants. Therefore, their use is becoming more and more widespread in making cooking oil, confectionery and, thanks to their antimicrobial properties, cookies and sesame rolls. The present study was conducted on two commercially grown local white grape varieties, namely Emir (Nevşehir), Gök üzüm (Konya) and a black variety Karadimrit (Nevşehir) in 2009. The Emir variety is green-yellow in colour and used for making wine; the Gök üzüm variety is also green-yellow and used for table, or for obtaining fermented grape juice or raisins; the Karadimrit grape variety, on the other hand, is red-purple in colour and used for making wine, fermented grape juice or raisins. The amounts of defined fatty acids vary by varieties. According to the varieties, linoleic acid (C18:2) was determined max. in the Emir (67,63%) min. Gök üzüm varieties (66,45%); oleic acid (C18:1) max. Gök üzüm (19,84%) min. in the Karadimrit varieties (17,40%); palmitic acid (C16:0) max. Karadimrit (8,60%) min. in the Gök üzüm varieties (6,96%). The highest amount of total phenolic compounds was found in the seeds of the Gök üzüm variety with 87031,32 mg GAE/kg. The seeds of the grape varieties used in the study were obtained from the pulps (waste) of fruit juice and wine factories. Grape seeds are used as a food source for humans and animals and thus contribute both to economy and protection of environment by providing less contamination.

Keywords: Grape; local varieties; grape seed oil; fatty acids; phenolic compounds

RESUME

La graine de raisin contient un pourcentage élevé d'acide gras insaturé,de matiere phenolique et d'antioxidant. C'est pour cela, que son emploi a augmenté pour l'obtention d'huile comestible,dans la fabrication de confiserie, grâce à sa propriété antimicrobiale est utilisé pour la fabrication de gâteau et de simit. Le travail;) a été réalisés en 2009 sur les especes de raisins régionaux, deux éspéces blanches cultivées dans les vignes commerciales; Emir (Nevşehir), Gök üzüm (Konya), une espece noire; Karadimrit (Nevşehir). L'espece de raisin Emir, étant vert jaune est utilisé pour le vin; l'espece Gök üzüm, étant vert-jaune et est utilisé à table, pour la cidre et pour être séchés. La quantité d'acide gras définie, change selon les especes. Selon les especes, l'acide linoléique (C18:2) dans les especes max. Emir (67,63%) min. raisin Gök üzüm (66,45%); acide oléique (C18:1) dans les especes raisin Gök üzüm max. (19,84%) Karadimrit min. (17,40%); acide palmitique (C16:0) dans les especes

karadimrit max. (8,60%) raisin Gök üzüm min. (6,96%). Le total de quantité le plus élevé de matiere phénolique est de 87031,32 mg GAE/kg dans la graine de l'espece raisin Gök üzüm. Les graines de raisins des especes étudiées ont été pris dans les dechets des usines de jus de fruits et de vins. La graine de raisin, dans l'alimentation des humains et des animaux utilisés comme complément d'alimentation apporte un plus à l'économie et aussi à l'environnement qui se sâlie moins.

Mots clés: raisins, variétés régionales, total de l'huile de graine de raisin; les acides gras; total des composants phenoliques.

INTRODUCTION

Located on a climatic zone on earth best suited for viticulture, Turkey is the genetic source for vine and at the same time possesses a very old and established viticulture tradition. According to recent statistical data, the total grapevine area in Turkey is 482789 ha; the total fresh grape production is 3918440 tons; and crop yield is 8116,2 kg/ha (FAO, 2008). While 71% of world grape production is used for wine, Turkey is also approximately 3%.

Grape seeds, a by-product of the winemaking industry or juice. The importance of grape seed oil is the high content of the unsaturated fatty acids such as linoleic acid and oleic acid. Fatty acids in grape seed varieties are examined that linoleic acid (C18:2) has the highest rate, followed by oleic acid (C18:1), palmitic acid (C16:0) and stearic acid (C18:0). Of the other fatty acids; although myristic acid (C14:0), palmitoleic acid (C16:1), margaric acid (C17:0), margaroleic acid (C17:1), linolenic acid (C18:3), arachidic acid (C20:0), ecosanic acid (C20:1), behenic acid (C22:0), and lignoseric acid (C24:0).

As well as grapes are accepted as fruit, they have high amounts of phenolic compounds and anthocyanins as a natural antioxidants source (Ames *et al.,* 1993). For the production of grape seed oil, only the seeds are in use, which consist to about 7–20% of oil (Matthäus, 2008). The phenolic substances are primarily located in the seeds and skins of the berry (Anonymous a, 2008). 60-70% of the Ployphenols, which can be exctracted from grape textures, are in the seed, 28-35 are % in the fruit skin and 10% are in the fruit flesh (Shi *et al*., 2003).

Grape seed oil is used for salad dressings, marinades, deep frying, flavoured oils, baking, massage oil, sunburn repair lotion, hair products, body hygiene creams, lip balm and hand creams. Additionally, this oil is reputed to contain plentiful antioxidants, as well as to lower cholesterol levels, vitamins (vitamin E, vitamin C and b-carotene), and phenolic compounds (Joshi *et al*., 2001). The grape seed extract is a natural antioxidant that is 50 times as strong as vitamin E and 20 times as strong as vitamin C. Antioxidants neutralise harmful substances, i.e. free radicals, that have formed in our body as a result of chemical reactions or that have been received from outside through stress, sun rays, cigarettes, alcohol and environmental pollution. It helps skin texture to keep its elasticity, prevents wrinkles and adds beauty. Thus, they play a vital role in slowing the ageing process. Grape seed oil, on the other hand, is also called vitamin F and is a precious skin care product with its high content of mono and polyunsaturated fatty acids. 50 kg of grape seed is needed to obtain a litre of cold pressed grape seed oil (Khanna *et al*., 2002). Grapeseed oil contains a high percentage of Omega 6 (Linoleic) essential omega fatty acids (EFAs), which can significantly raise HDL (good) cholesterol and reduce LDL (bad) cholesterol levels (Anonymous b, 2008).

Total phenolic compounds in grapes vary according to grape varieties, grown in climate and soil conditions, cultural practices and maturity levels (De La Hera Orts *et al*., 2005).

The present study aimed at investigating fatty contents and total phenol contents of grape seeds extracted from three grape varieties. It further aims to explore possibilities of using pulp

residues and especially seeds of pulp residues, which are by-products of wine, grape juice and molasses factories and which were not utilised economically until very recently, because they can be used as a new source of food for humans due to their high antioxidant, fatty acid and phenolic substance contents. It also aims to determine how factory product costs can be reduced and whether the properties that were examined have any effect on one another or not.

MATERIALS AND METHODS

The present study was conducted on two commercially grown local white grape varieties, namely Emir (Nevşehir), Gök üzüm (Konya) and a black variety Karadimrit (Nevşehir) in Turkey in 2009. The seeds were excised from berries and air-dried at the room temperature under shade conditions. They were stored at room temperature until their analysis.

Determination of fatty acids

After grape seeds were dried for three hours in the drying oven at 40 ºC, they were ground using an agate mortar. Soxhlet system (S&H Labware, United States) was used to extract oil from grape seeds. Oil was extracted from a 5 g sample thus obtained by the help of a hexane solvent. Efficiency percentages were calculated by using the amounts of oil obtained after the solvent was removed in the evaporator and the weighing of oil after extraction process was performed. Fatty acids were determined by using the Agilent-6890N gas chromatography device. The derivation process was performed according to Slover and Lanza, (1979). 200 mg oil is obtained, and a mixture of 3 ml Metanol+KOH (0.5 mol/L) is added. It is kept in the drying oven at 100 ºC for 10 minutes, and then warmed to room temperature. 2 ml of 12% BF3 (borontrifluoride) is added to this mixture, then kept in the drying oven at 100 ºC for 10 minutes, and then warmed to room temperature. 1 ml hexan is added, and then 1 ml of 0.6% (w/v) NaCI is added. Organic phase is removed using a Pasteur pipette, dried using Na2SO4, and read using GC. The experimental conditions in which the GC-FID spectrum of the oils was taken are as follows: Temperature 1: 60 ºC, Time 1: 2 minutes, Speed: 5 ºC/minute, Temperature 2: 250 ºC, Total duration of analysis: 60 minutes, The amount of sample injected: 1 microlitre, Injector temperature: 230 ºC, Capillary column: HP-5 column (30 m in length, 0.32 mm in diameter, film thickness 0.25 μm), Carrier gas: Hydrogen, 13,38 psi. Means and standard deviation values of fatty acid analysis results given in 3-parallel in Table 1 (X ± SD) were calculated using Microsoft Excel 2003 software and given as %.

Determination of total phenolic compounds

For the extraction procedure, 5 g of seed sample were mixed with 100 g of methanol in a nitric atmosphere environment for 2 hours. For the derivation procedure, solid liquid phase was separated by straining. The total phenol compound absorbances of the sample that was taken from the liquid phase were measured using the Folin-Ciocalteu reactive according to Slinkard and Singleton (1977) on a spectrophotometer (Shimadzu UV-1601 Japan) at 760 nm. In order to identify the amount of substances in the samples, appropriate standard solutions (gallic acid) were prepared, their absorbance was measured on the spectrophotometer and their calibration graphic was drawn. Total phenolic compound contents of the samples were determined using the linear equation obtained from the calibration graphic. Total phenolic substance averages and standard deviation values of the 3-parallel analysis given in Table 1 (X ± SD) were calculated using Microsoft Excel 2003 software and given as mg GAE/kg.

Statistical analysis

The research was planned in the completely randomized block design as simple factorial experiment and variance analyses and multiple comparison tests were done by JUMP statistical package program.

RESULTS AND DISCUSSIONS

Fatty acids, total fat and phenolic compounds determined as a result of the analysis conducted on the seeds of grape varieties are given in Table 1.

Table 1. Fatty acid (%), total fat (%) and phenolic compound (mg GAE/kg) contents of grape seeds

Yağ Asitleri	Üzüm Çeşitleri			LSD
	Emir	Gök Üzüm	Karadimrit	(%5)
C14:0	0,12 AB	0,16 A	0,09 B	0,04
C16:0	8,08 B	6,96 C	8,60 A	0,08
C17:0	0,10 B	0,15 A	0,09 B	0,04
C18:0	5,63 A	4,09 C	4,86 B	0,73
C20:0	0,13 B	0,15 AB	0,18 A	0,04
C22:0	0,13 A	0,17 A	0,09 B	0,04
C24:0	0,12 B	0,18 A	0,08 C	0,04
Σ SFA	**14,31**	**17,14**	**13,99**	**-**
C16:1	0,10 B	0,16 A	0,10 B	0,02
C17:1	0,10 B	0,15 A	0,08 B	0,02
C18:1	17,51 B	19,84 A	17,40 B	0,26
C18:1 trans	0	0	0	0
C20:1	0,15 A	0,17 A	0,13 A	0,05
C22:1	0	0	0	0
Σ MUFA	**17,86**	**20,32**	**17,71**	**-**
C18:2	67,63 A	66,45 A	66,74 A	2,92
C18:2 trans	0	0	0	0
C18:3	0,77 A	0,39 B	0,44 B	0,20
Σ PUFA	**68,40**	**66,84**	**67,18**	**-**
Yağ İçeriği	8,50 A	6,31 C	6,80 B	0,18
Fenolik Madde	71192,96 C	87031,32 A	83927,18 B	646,74

The amount of total saturated fatty acids in all varieties (**SFA)** varies between 13,99% and 17,14%, with a total amount of monounsaturated fatty acids (**MUFA)** and polyunsaturated fatty acids (**PUFA)** varies between 84,89% and 87,16%. The amount of saturated fatty acids is lower than the amounts of mono and polyunsaturated fatty acids. Among the defined fatty acids, linoleic acid (C18:2) was the highest in all varieties, which was followed by oleic acid (C18:1), palmitic acid (C16:0) and stearic acid (C18:0).

The highest amount of linoleic acid (C18:2) was determined in the Emir variety (67,63A%) and it was followed by Karadimrit (66,74A%) and Gök üzüm (66,45A%) respectively. Linoleic acid content between varieties are not statistically significant.

The highest amount of oleic acid (C18:1) was determined in the Gök üzüm variety (19,84A %), which was followed by Emir (17,51B%) and Karadimrit (17,40B%) respectively. Oleic acid content between varieties are statistically significant.

The highest amount of palmitic acid (C16:0) was found in the Karadimrit variety (8,60A%), which was followed by Emir (8,08B%) and Gök üzüm (6,96C%) respectively. Palmitic acid content between varieties are statistically significant.

The highest amount of stearic acid (C18:0) was found in the Emir variety (5,63A%), which was followed by Karadimrit (4,86B%) and Gök üzüm (4,09C%) respectively. Stearic acid content between varieties are statistically significant.

Of the other fatty acids, myristic acid (C14:0), margaric acid (C17:0), arachidic acid (C20:0), behenic acid (C22:0), lignoceric acid (C24:0), palmitoleic acid (C16:1), margaroleic acid (C17:1), eicosanic acid (C20:1) and linolenic acid (C18:3) were found in lower amounts though they demonstrated differences depending on varieties. Erucic acid (C22:1) could not been identified in our varieties. Of the trans oils, linoleic acid (C18:2 trans) and oleic acid (C18:1 trans) was not found.

The results are in parallel to the results obtained (fatty acids in Emir grape variety: linoleic acid 68,10%, oleic acid: 17,80%, palmitic acid: 8,60%, stearic acid: 4,70%) by Gokturk Baydar and Akkurt (2001); (linoleic acid: between 62,53% and 69,24%, oleic acid: between 18,14% and 22,88%, palmitik asit: between 7,96% and 10,01%, stearik asit: between 3,14% and 4,96%) by Gok Tangolar *et al*. (2007); (linoleic acid: between 61,16% and 71,37%, oleic acid: between 16,07% and 22,57%, palmitic acid: between 7,42% and 10,63%, stearic acid: between 2,95% and 4,68%) by Gokturk Baydar *et al*. (2007).

The amount of total fat in all varieties varies between 6,31% and 8,50%. The highest amount of total fat was found in the Emir variety (8,50A%), which was followed by Karadimrit variety (6,80B%) and Gök üzüm (6,31C%) respectively. Total fat content between varieties are statistically significant.

The results are slightly lower to the results obtained (the total fat concentration of seeds ranged from 11,6 to 19,6%. Emir variety is 15,3%) by Gokturk Baydar and Akkurt (2001); (the total fat concentration of seeds ranged from 10,45 to 16,73%) by Gok Tangolar *et al*. (2007); (the total fat concentration of seeds ranged from 7,6 to 16,0% by Maier *et al*., (2009).

The amount of total phenolic compounds varies between 87031,32 and 71192,96 mg GAE/kg. The highest amount of phenolic compound was determined in the seeds of the Gök üzüm variety (87031,32A mg GAE/kg), which was followed by the seeds of Karadimrit (83927,18B mg GAE/kg) and Emir (71192,96C mg GAE/kg) respectively. Total fat content between varieties are statistically significant.

The contents of total phenolic compounds of the grape seed extracts are similar to the results found; 171,32 mg GAE/g (Kalecik karası), 133,69 mg GAE/g (Emir) and 238,47 mg GAE/g (Narince) by Gokturk Baydar *et al*. (2007); (between 817 and 3062 µg/ml GAE by Orak (2007); (33945 mg GAE/100 g (Muskule), 52216 mg GAE/100 g (Razaki), 50150 mg GAE/100 g (Emir), 51532 mg GAE/100 g (Hasandede), 58 30 mg GAE/100 g (Narince), 39627 mg GAE/100 g (Karadimrit), 53947 mg GAE/100 g (Muscat of Hamburg), 54137 mg GAE/100 g (Alphonse Lavallee´), 45121 mg GAE/100 g (Okuzgozu), 51179 mg GAE/100 g (Kalecik karasi), 53434 mg GAE/100 g (Alicante Boushet), 55431 mg GAE/100 g (Papaz karasi) by Yemis *et al*. (2008); (3170 mg/kg (Chardonnay), 2376 mg/kg (Merlot), 1968 mg/kg (Shiraz), 1805 mg/kg (C. Sauvignon) by Ozden and Vardin (2009); (between 188.70 mg GAE/kg and 1116.5 mg GAE/kg) by Maier *et al*., (2009); (53370.52 mg GAE/kg (Kızıl uzum), 47427.36 mg GAE/kg (Ak uzum), 42813.45 mg GAE/kg (Dokulgen), 17769.66 mg GAE/kg (Hesap Ali), 15517.22 mg GAE/kg (Eksikara) by (Akin *et al*. (2009); (55,98 mg GAE g DW^{-1} using ethanol as solvents, 67,88 mg GAE g DW^{-1} using methanol as solvents) by Casazza *et al*. (2010).

Differences observed among different varieties of grapes in terms of total phenolic compounds are primarily attributed to the genetic structure of the variety, but they may have arisen due to soil and other cultivation conditions. As a result, the seeds of these grape varieties can be used as additional food source.

BIBLIOGRAPHY

Akin, A., Altindisli, A. and Buyukhelvacigil, M., 2009. Evaluation of Fatty Acid Content and Total Phenolic Compounds in The Seed of Native Grape Varieties (*Vitis vinifera* L.) Grown in Konya Province, Turkey. 32. World Congress of Vine and Wine and the 7 th General Assembly of O.I.V. June 28th –July 3rd 2009, Zagreb-Croatia:OIV, n:34-35.

Ames, B.N., Shigena, M.K. and Hagen, T.M.,1993. Oxidants, Antioxidants and The Degenerative Diseases of Aging. *The Proceedings of the National Academy of Sciences* (U.S.A). 90, n:7915-7922.

Anonymous a., 2008. Composition of Grapes. (www.extension.iastate.edu)

Anonymous b., 2008. Grapeseed Oil. (www. newleafbeauty.com)

Casazza, A.A., Aliakbarian, B., Mantegna, S., Cravotto, G. and Perego, P., 2010. Extraction of Phenolics from Vitis vinifera Wastes Using Non-Conventional Techniques. *Journal of Food Engineering.* (Article in Press).

De La Hera Orts, M.L., Martinez-Cutillas, A., Lopez-Roca, J.M. and Gomez-Plaza, E., 2005. Effect of Moderate İrrigation on Grape Composition During Ripening. *Spanish Journal of Agricultural Research*. 3(3), n:352-361.

FAO., 2008. Faostat Statistical Database. (http://www.fao.org)

Gok Tangolar, S., Ozoğul, Y., Tangolar, S. and Torun, A., 2007. Evaluation of Fatty Acid Profiles and Mineral Content of Grape Seed Oil of Some Grape Genotypes. *International Journal of Food Sciences and Nutrition*. ISSN 0963-7486 print/ISSN 1465-3478, n:1-8.

Gokturk Baydar, N. and Akkurt, M., 2001. Oil Content and Oil Quality Properties of Some Grape Seeds. *Turk J. Agric. For.* Tübitak. 25, n:163-168.

Gokturk Baydar, N., Ozkan, G. and Cetin, S., 2007. Characterization of Grape Seed and Pomace Oil Extracts. *Grasas Y Aceites*, 58 (1), Enero-Marzo, ISSN: 0017-3495, n:29-33.

Joshi, S.S., Kuszynski, C.A. and Bagchi, D. 2001. The Cellular and Molecular Basis of Health Benefits of Grape Seed Proanthocyanidin Extract. *Curr Pharm Biotechnol* 2, n:187-200.

Khanna, S., Venojarvi, M., Roy, S., Sharma, N., Trikha, P., Bagchi, D., Bagchi, M. and Sen, CK., 2002. Dermal Wound Healing Properties of Redox-Active Grape Seed Proanthocyanidins. *Free Radic Biol Med.* Oct 15; 33(8), n:1089-1096.

Maier, T., Schiber, A., Kammerer, D.R. and Carle, R., 2009. Residues of Grape (*Vitis Vinifera* L.) Seed Oil Production As A Valuable Source of Phenolic Antioxidants. *Food Chemistry* 112, n:551-559.

Matthäus, B., 2008. Virgin Grape Seed Oil: Is It really A Nutritional Highlight? *Eur. J. Lipid Sci. Technol.* 110, n:645–650.

Orak, H.H., 2007. Total Antioxidant Activities, Phenolics, Anthocyanins, Polyphenoloxidase Activities of Selected Red Grape Cultivars and Their Correlations. *Scientia Horticulturae*, Volume 111, Issue 3, 5, n:235-241.

Ozden, M. and Vardin, H., 2009. Şanlıurfa Koşullarında Yetiştirilen Bazı Şaraplık Üzüm Çeşitlerinin Kalite ve Fitokimyasal Özellikleri. *J.Agric.Fac.HR.U.*, 13(2), n:21-27.

Shi, J., Yu, J., Pohorly, J.E. and Kakuda, Y., 2003. Polyphenolic in Grape Seeds Biochemistry and Functionality. *J.Med. Food*, 6 (4), n:291-299.

Slinkard, K. and Singleton, V. L., 1977. Total Phenol Analyses: Automation and Comparison with Manual Methods. *Am. J. Enol. Vitic*. 28, n:49-55.
Slover, H.T. and Lanza, E., 1979. Quantitative Analysis of Food Fatty Acids by Capillary Gas Chromatography. *Journal of the American Oil Chemists' Society*, Volume 56, Number 12/December, 1979.
Yemis, O., Bakkalbasi, E. and Artik, N., 2008. Antioxidative Activities of Grape (Vitis vinifera) Seed Extracts Obtained from Different Varieties Grown in Turkey. *International Journal of Food Science and Technology*. 43, n:154–159.

CONSTAT ET INCIDENCES DU CHANGEMENT CLIMATIQUE DANS LA REGION DE COGNAC

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RÉSUMÉ

Une étude a été menée pour évaluer l'évolution du climat dans le vignoble de Cognac au cours des décennies passées et son impact sur le comportement de la vigne. Une analyse de l'évolution des températures et des précipitations depuis 1918 a été réalisée et plusieurs indicateurs viticoles ont été étudiés. Les moyennes mobiles sur 30 ans des températures annuelles moyennes ont augmenté d'environ 1°C depuis 1980. Le nombre de jours de très forte chaleur a également progressé. A l'inverse, la fréquence des gels de printemps a diminué. L'indice héliothermique de HUGLIN passe progressivement de la catégorie « climat frais » jusqu'à la décennie 1980, à celle d'un « climat tempéré ».

Depuis 1979, on observe une tendance nette à l'avancement de la date des vendanges alors que la date de débourrement de la vigne est peu modifiée. Une augmentation de 1°C de la température maximale diurne de la période de croissance de la vigne (avril-août) se traduit par une avancée d'environ 10 jours de la date des vendanges dans le vignoble de Cognac.

INTRODUCTION

La Région Délimitée de production du Cognac (figure 1) se situe dans l'ouest de la France, au nord du bassin aquitain, en bordure de l'océan Atlantique. Elle se termine à l'ouest par les bords de Gironde et les îles (Ré et Oléron), et à l'est, à Angoulême, aux premiers contreforts du Massif Central. Le vignoble s'étend sur la quasi-totalité du département de la Charente-Maritime, une grande partie de celui de la Charente, ainsi que sur quelques communes de la Dordogne et des Deux-Sèvres.

Le climat de la région viticole de Cognac est un climat océanique tempéré, assez homogène à l'exception des régions côtières, qui sont plus ensoleillées et présentent une moindre amplitude des températures. Du fait de la proximité de l'océan, même si elles sont plus abondantes l'hiver, les pluies peuvent intervenir à tout moment de l'année. Les sécheresses sont rares, permettant une alimentation hydrique régulière de la vigne. La température moyenne annuelle est de 13°C environ, avec des hivers assez doux. Les températures sont suffisantes pour assurer une bonne maturité du raisin destiné à la production des eaux-de-vie, mais sans être excessives. Le climat de la région de Cognac a notamment été décrit au début du vingtième siècle par RAVAZ (1900) puis par LAFON et al (1964).

En 2007, le IVe rapport du Groupe d'Experts Intergouvernemental sur l'Evolution du Climat (GIEC, 2007) conclue à un réchauffement climatique mondial sans équivoque. Selon ce rapport, au cours des 100 dernières années, le climat mondial s'est réchauffé de 0,74°C. Ce réchauffement s'est fortement accéléré depuis les 50 dernières années. A l'échelle de la France, la température moyenne a augmenté de 0.1°C par décennie depuis le début du XXe siècle. Le réchauffement s'est accéléré depuis 30 ans pour atteindre 0.6°C/10 ans (Dandin, 2006).

Différentes études évaluent les conséquences pour la vigne des évolutions du contexte climatique. Les conditions thermiques pendant la phase de maturation des raisins sont prépondérantes pour atteindre les caractéristiques technologiques recherchées. Ainsi pour Daux et al. (2007), il existe une forte corrélation dans de nombreux vignobles (Alsace, Champagne, Bourgogne, Touraine, Bordeaux) entre la date des vendanges et les moyennes d'avril à août des températures maximales diurnes (période de croissance et de maturation de la vigne). Quelque soit la précocité des cépages et quelque soit la situation géographique, une augmentation de 1°C de la température maximale diurne de la période de croissance (avril-août) entraîne une avancée 10 jours de la date des vendanges.

Une étude réalisée par Seguin en 2007, souligne une diminution sur le long terme de la durée du cycle végétatif dans différents vignobles de France. La modification des pratiques culturales ne peut expliquer à elle seule cette précocité. L'augmentation des températures a impacté pratiquement toutes les manifestations phénologiques des cultures pérennes, et en particulier les dates de floraison et de vendange.

Afin d'évaluer l'incidence de ce changement climatique dans le vignoble de Cognac, une étude a été mise en place en 2007 par la Station Viticole du BNIC. L'objectif est de préciser l'évolution du climat passé sur la région des Charentes et d'évaluer les impacts viticoles des changements observés. La première étape de cette étude a consisté en l'analyse de l'évolution des températures et des précipitations depuis 1918 sur 2 stations météorologiques représentatives du vignoble de Cognac. Différents indicateurs viticoles ont également été étudiés.

MATERIELS ET METHODES

Les données climatiques utilisées pour cette étude ont été acquises par le BNIC auprès de Météo France. Deux stations météorologiques ont été retenues pour analyser l'évolution du climat de la région de production du Cognac (figure 1) : Cognac (Charentes) et Saintes (Charente Maritime).

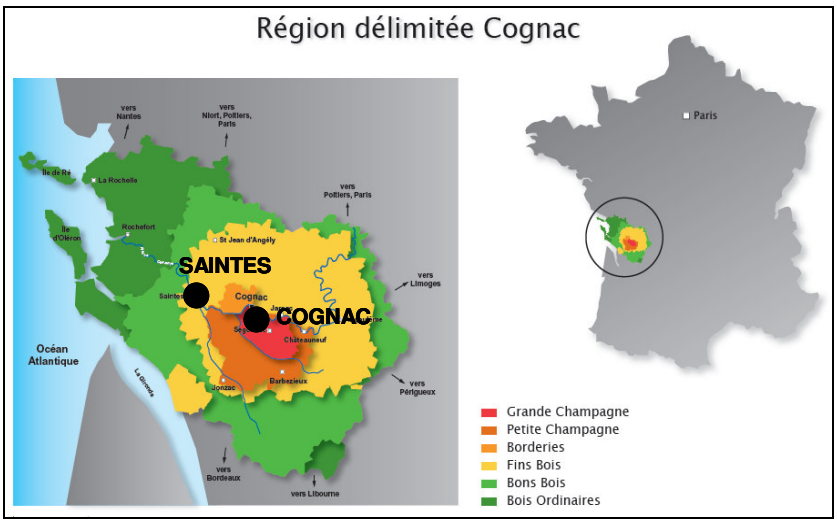

Figure 1 : Carte de la région de production du Cognac et localisation des 2 stations météorologiques retenues dans l'étude

Le Tab. 1 résume les caractéristiques des stations et des données utilisées dans le cadre de cette étude. L'évolution de chaque paramètre (température et pluviométrie) a été étudiée pour les deux stations météorologiques.

Tableau 1 : Caractéristiques des stations et variables climatiques utilisées

	COGNAC (CHATEAUBERNARD)	SAINTES
Département	Charente (16)	Charente –Maritime (17)
Propriétaire	Météo France	1918/89 : Météo France 1990/07 : Commission météorologique départementale
Altitude	30 mètres	38 mètres
Site	Non viticole, périurbain	Viticole, rural
Variables utilisées	températures (min, max) – précipitations données journalières	températures (min, max) – précipitations données journalières
Période	1948-2007	1918-1937 et 1948-2007

RESULTATS ET DISCUSSION

Evolution climatique

Evolution des températures moyennes

Les évolutions des variables climatiques des 2 stations météorologiques sont très semblables et conformes aux scénarios décrits par Météo France à l'échelon national (Dandin, 2006). Les températures moyennes des 2 stations météorologiques montrent une tendance nette à l'augmentation depuis la décennie 1980 (figure 2).

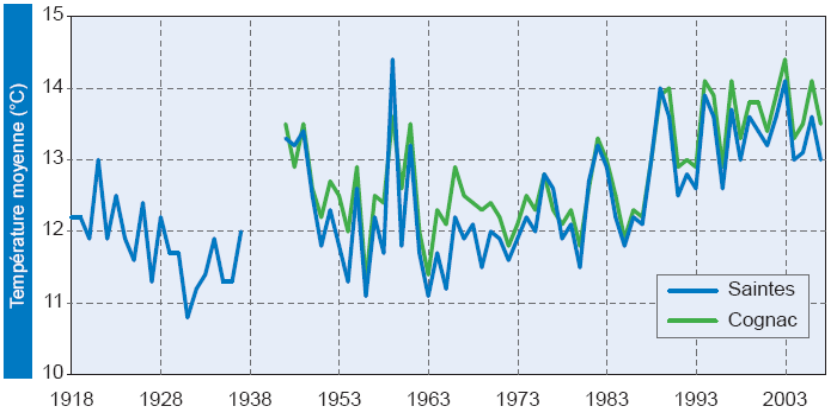

Figure 2 : Températures moyennes annuelles des stations de Saintes et Cognac

L'anomalie de la température moyenne annuelle par rapport à la normale 1970-2000 est représentée sur la figure 3 pour la station de Saintes qui permet de disposer de la série chronologique la plus complète. Pour obtenir cette anomalie, la température moyenne annuelle est soustraite à sa moyenne sur la période 1970-2000. Les anomalies négatives sont en bleu, les positives en vert. Les anomalies sont presque toutes positives à partir de l'année 1988. Cela signifie donc que les températures annuelles moyennes sont toutes supérieures à la normale depuis cette date.

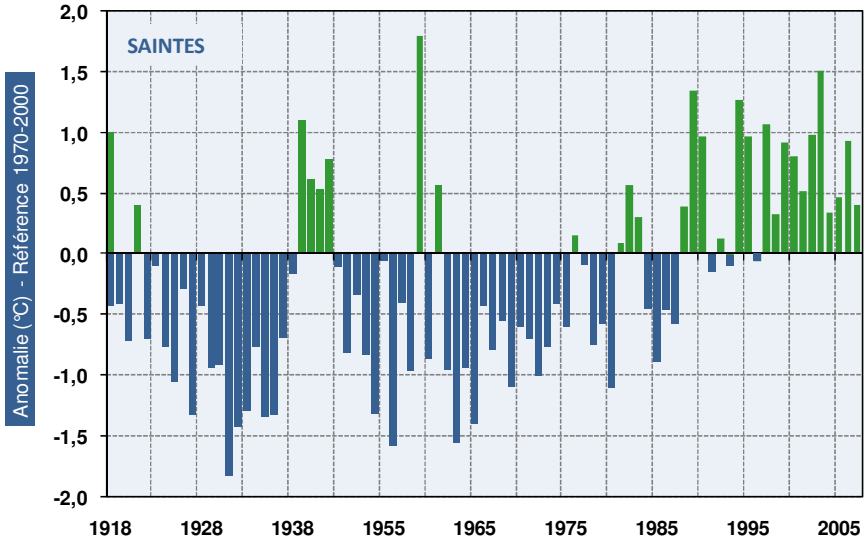

Figure 3 : Evolution de l'anomalie de la température moyenne annuelle par rapport à la normale 1970-2000 sur la station de Saintes

Afin de s'affranchir des variations annuelles, les moyennes mobiles sur 25 ans des températures moyennes annuelles ont également été calculées pour les deux stations étudiées (figure 4). Entre 1980 et 2004, elles ont augmenté de 0.8°C pour la station de Cognac et de 1°C pour celle de Saintes.

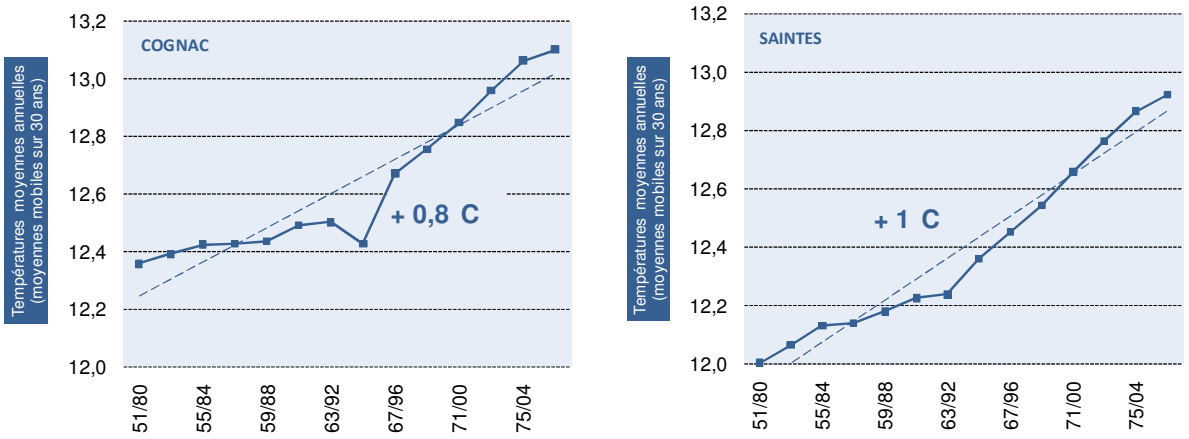

Figure 4 : Températures moyennes (moyennes mobiles sur 30 ans) sur les stations de Cognac et Saintes

Evolution des températures extrêmes

Le constat réalisé au paragraphe précédent peut être précisé par des observations concernant l'évolution des températures extrêmes.

Les hausses des températures minimales et maximales annuelles sont de même ordre que celle de la température moyenne annuelle. Par contre, le nombre de jours de très forte chaleur (>35°C) a augmenté, et l'effet de canicule observé pendant les mois d'été s'est accentué.

Ainsi, la moyenne mobile des températures maximales du mois d'août a progressé de 2°C sur la station de Cognac et de 1.8°C sur la station de Saintes au cours des 25 dernières années (figure 5).

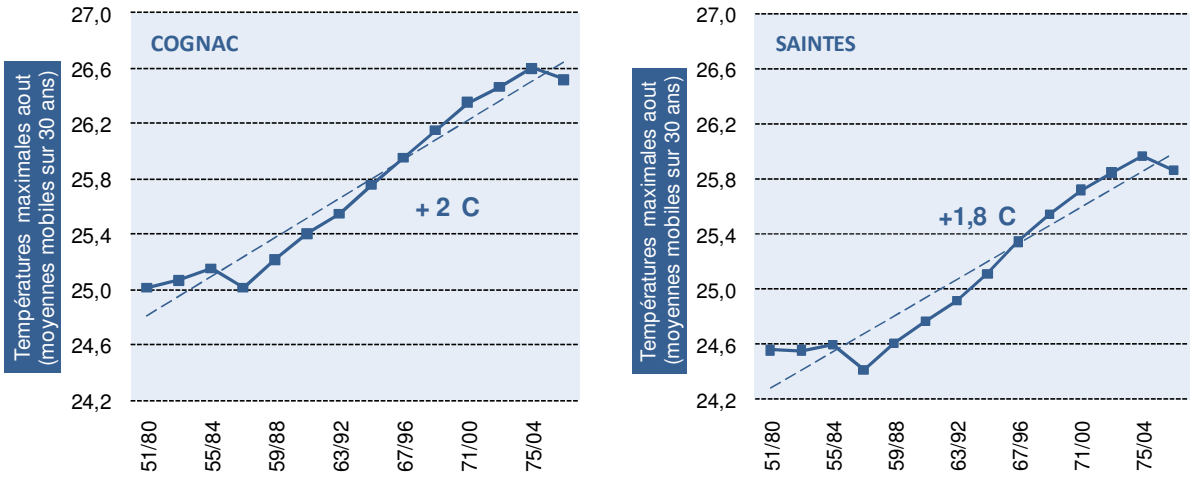

Figure 5 : Températures maximales du mois d'août (moyennes mobiles sur 30 ans) sur les stations de Cognac et Saintes

A l'inverse, le nombre de jours de températures très basses (<-5°C) et la fréquence du nombre de jours de gel de printemps ont diminué significativement. Ainsi, pour la station de Saintes, le pourcentage du nombre de jours avec gel pendant les mois de mars et d'avril est passé d'environ 12 % avant 1987 à 6 % sur la période 1988-2007 (figure 6).

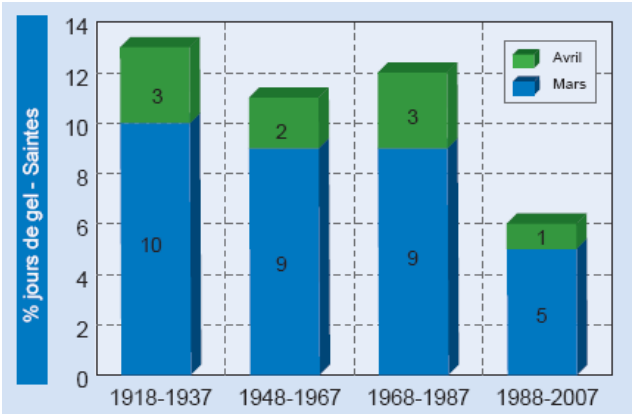

Figure 6 : Pourcentage du nombre total de jours de gel en mars et avril par groupe d'années sur la station de Saintes

Evolution des précipitations

Une légère tendance à l'augmentation des moyennes des précipitations annuelles est constatée sur les 2 stations étudiées. Les moyennes mobiles sur 30 ans sont en augmentation de 6 % (+ 54 mm) sur la station de Saintes et de 2 % (+ 14 mm) sur la station de Cognac depuis 1980. Ces évolutions sont en cohérence avec l'évolution moyenne décrite à l'échelle de la France : 7 % sur le cumul annuel des précipitations en un siècle (Dandin, 2006).

Incidence viticole du changement climatique

Evolution de l'indice héliothermique de Huglin

Différents indices peuvent être utilisés pour mesurer la quantité de chaleur reçue par la plante pendant sa période de végétation. L'indice héliothermique de Huglin (IH), s'exprime en degrés par an, et comptabilise les températures moyennes journalières supérieures à 10°C, sur la période du 1er avril au 30 septembre. Cet indice permet de caractériser de façon globale le climat d'une région viticole (Huglin, 1986). Il est calculé par la formule suivante :

$$IH = \sum_{01/04}^{30/09} \frac{(Tm-10) + (Tx-10) \times k}{2}$$

Avec : Tm : Température moyenne de l'air (°C)
Tx : Température maximale de l'air (°C)
k : Coef. longueur du jour (1,02 à 1,06 entre 40 et 50 ° de latitude)

L'évolution de l'indice de Huglin pour la région de Cognac est présentée sur la figure 7. Il connaît bien entendu des variations inter-annuelles importantes (effet millésime), mais passe progressivement d'un climat majoritairement considéré comme frais jusque dans les années 1980 à la catégorie « climat tempéré ». Certaines années récentes se situaient même dans la catégorie de climat « tempéré chaud » (2005), voire « chaud » pour l'année 2003.

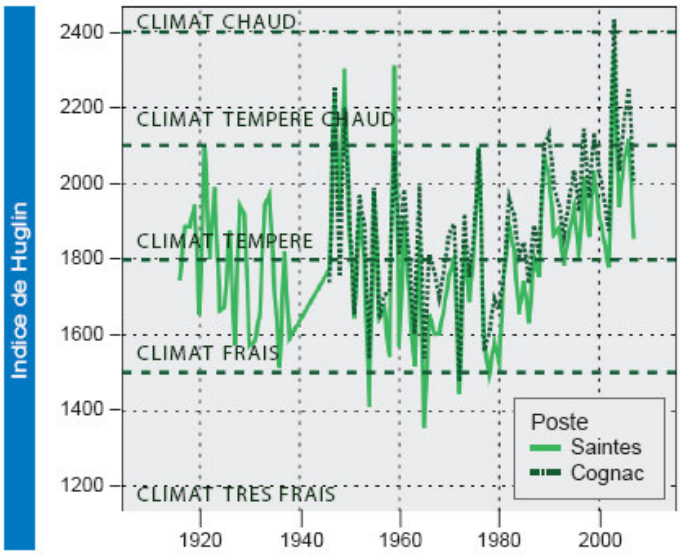

Figure 7 : Indice de HUGLIN calculé par les stations de Saintes et Cognac

Incidence sur le cycle végétatif

Depuis 1979, on observe dans le vignoble de Cognac, une tendance nette à l'avancement de la date des vendanges qui peut être rapprochée d'une diminution de la durée du cycle végétatif (figure 8). La figure 9 présente, de façon analogue à l'étude conduite par Daux et al. (2007), la relation entre la date des vendanges et les moyennes, d'avril à août, des températures maximales diurnes (période de croissance et de maturation de la vigne). Les résultats obtenus pour le vignoble de Cognac apparaissent en cohérence avec ceux publiés pour d'autres vignobles français : d'après la corrélation obtenue sur la période 1979-2008, une augmentation de 1°C de la température maximale diurne sur la période avril-août se traduit par un avancement de 10 jours de la date de récolte.

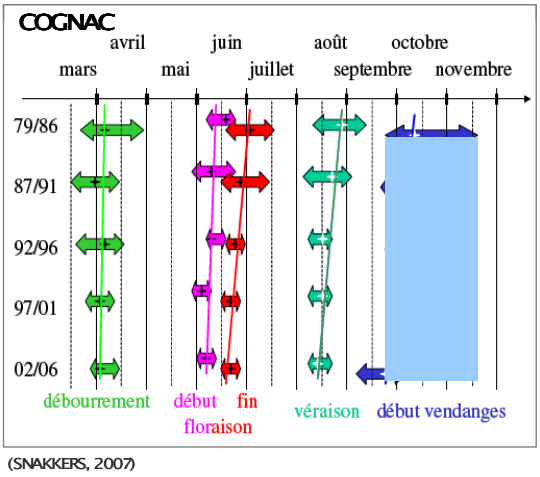

Figure 8 : Evolution des stades physiologiques de 1979 à 2006 sur le vignoble de Cognac (Snakkers, 2007)

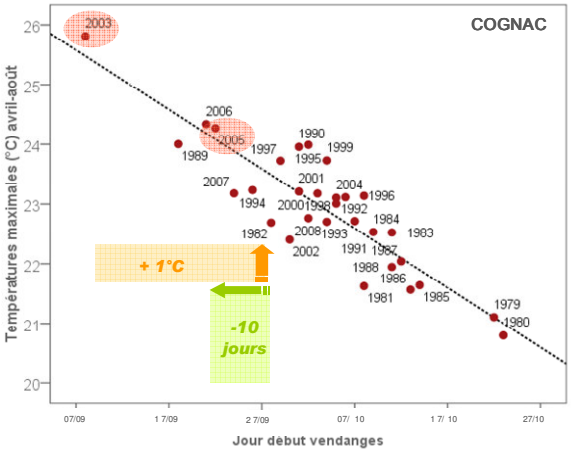

Figure 9 : Corrélation entre dates de vendanges et moyennes des températures maximales diurnes (avril/août)

Incidence sur la maturation

Une des conséquences du raccourcissement du cycle végétatif est la modification du régime thermique auquel est soumis la vigne pendant la phase de maturation des baies, qui débute dès la véraison. L'augmentation des températures accélère l'accumulation des sucres par stimulation de l'activité photosynthétique. De plus, les températures élevées favorisent la dégradation de l'acide malique, directement corrélée à l'acidité des moûts (Gaudillère, 2005). Le réchauffement climatique entraîne donc une accélération du processus de maturation par augmentation de la teneur en sucre, et donc du degré, et une baisse de l'acidité.

La figure 10 présente les corrélations entre le TAV déclaré aux vendanges, l'acidité du début de vendange et l'indice de Huglin depuis l'année 1979 dans le vignoble de Cognac. Plus la somme de températures reçue par la vigne est élevée et plus le TAV à la récolte est fort. A l'inverse, plus l'indice de Huglin est élevé et plus l'acidité de la vendange est faible.

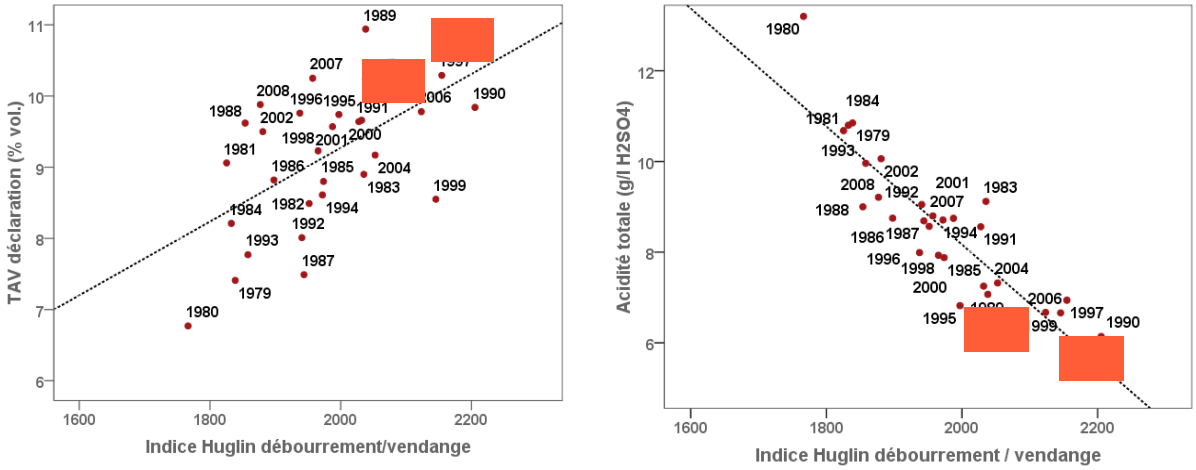

Figure 10 : Corrélation entre le TAV déclaré à la récolte, l'acidité totale au début des vendanges et l'indice de Huglin du débourrement aux vendanges depuis 1979

CONCLUSIONS

Cette étude permet de préciser le constat des évolutions climatiques de ces dernières décennies à l'échelle du vignoble de Cognac. Elle confirme les constatations déjà réalisées dans d'autres régions viticoles, tant sur l'amplitude des phénomènes observés que sur leur incidence sur le comportement de la vigne. Elle constitue ainsi une base utilisable par la filière pour anticiper les changements en cours et mettre en place les adaptations nécessaires à ces évolutions du contexte de production.

BIBLIOGRAPHIE

DANDIN P., 2006. Evolution du climat et sécheresses. Congrès de la FNCCR, Bordeaux, sept. 2006. Météo-France, Direction de la Climatologie.

DAUX V., LE ROY LADURIE E., MESTRE O., CHEVET JM., 2007. Températures et dates de vendanges en France. Actes du colloque «Réchauffement climatique, quels impacts probables sur les vignobles ? », Dijon 2007.

GAUDILLERE J.P., 2005. Site, climat, pratiques culturales : qu'est-ce qui détermine le rendement et la composition des moûts ? Données physiologiques. Journée technique Station Viticole BNIC 2005.

GROUPE D'EXPERTS INTERGOUVERNEMENTAL SUR L'EVOLUTION DU CLIMAT (GIEC), 2007. Bilan 2007 des changements climatiques : rapport de synthèse. Contribution des Groupes de travail I, II et III au 4ème rapport d'évaluation du Groupe d'experts intergouvernemental sur l'évolution du climat. GIEC, Genève, Suisse, 103 pages.

LAFON R., LAFON J., COUILLAUD P., 1964. Le Cognac, sa distillation. J.-B. BAILLIERE et fils, éditeurs, 19 rue Hautefeuille, Paris VI.

HUGLIN P., 1986. Biologie et écologie de la vigne. 372 pages. Editions Lausanne, Paris.

METEO-FRANCE, 2006. Compte rendu d'activité. Direction interrégionale Sud-Ouest.

RAVAZ L., 1900. Le Pays du Cognac. Louis COCQUEMARD, imprimeur éditeur à Angoulême.

SEGUIN B., 2007. Le changement climatique et son impact sur la viticulture. Journée Technique de la Station Viticole. p.5-13.

SNAKKERS G., 2007. Evolution des stades phénologiques et des caractéristiques de la production constatées pendant les dernières décennies. Journée technique Station Viticole BNIC 2007.

UNDERSTANDING THE LEVERAGE OF THE COLOMBARD WINE AROMATIC QUALITY PRODUCED IN GASCONY BY MODELING CLIMATIC, AGRONOMICAL, ENOLOGICAL AND ANALYTICAL DATA

COMPRÉHENSION DES LEVIERS DE LA QUALITÉ AROMATIQUE DES VINS DE COLOMBARD PRODUITS EN GASCOGNE A PARTIR DE DONNÉES CLIMATIQUES, AGRONOMIQUES, ŒNOLOGIQUES ET ANALYTIQUES.

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RÉSUMÉ

La perception aromatique des vins de Colombard produits dans le vignoble de Gascogne provient principalement d'une surexpression de deux thiols variétaux : le 3-mercaptohexan-1-ol (3MH) et le 3-mercaptohexylacetate (3MHA). Le but de cette étude est de comprendre les leviers qui influencent la qualité aromatique d'un vin blanc de Colombard. Au cours de 3 millésimes (2006 à 2008), environ 60 échantillons par an de 50kg de raisins ont été prélevés et vinifiés dans des conditions standards. Deux classes de vins ont été élaborées à partir du dosage des thiols variétaux dans les vins et de l'expertise sensorielle d'un jury entrainé. Une base de données a été générée à partir de variables climatiques, agronomiques, œnologiques et analytiques sur raisins associées à chaque lot vinifié. Différents traitements statistiques ont été testés pour évaluer les relations entre les variables et les classes aromatiques de vins. Le traitement par AFD pas à pas propose le meilleur résultat avec un optimal de 6 variables pour une erreur de validation de 16%. Ces variables sont en partie similaires aux résultats obtenus par régression PLS. Les deux principales variables sélectionnées sont la vitesse de fermentation des premiers jours (facteur à influence positive) et la concentration en cuivre des raisins (facteur négatif). Les autres variables sont les concentrations des moûts en potassium, polyphénols, acides aminés et une donnée climatique, la fréquence des précipitations estivales. Ces facteurs influents ont été sélectionnés par traitement statistique et sont à replacer dans le champ de variation de l'étude. Cependant, ces résultats permettent de hiérarchiser des variables déjà connues pour leur influence et peuvent ainsi inciter les producteurs à agir pour optimiser le potentiel aromatique de leurs vins.

The aromatic sensing of Colombard wine produced in the Gascony vineyard, mainly comes from the over-expression of two varietal thiols : 3-mercaptohexan-1-ol (3MH) and 3-mercaptohexylacetate (3MHA). The main objective of this project is to understand the leverage influencing the aromatic quality of a white wine from Colombard variety. During three vintages - 2006, 2007, and 2008 - around sixty samples of 50 kg of grapes per year have been processed in wines following a strictly controlled protocol. Two classes of wines have been determined -low or high aromatic intensity- based on the combination of the varietal thiols analysis and the wine sensory evaluation by an expert group. A data base has been generated from climatic, agronomical, oenological and grapes analytical variables associated

with each sample processed. Different statistical models have been derived to interpret the relationships between the data and the wine aromatic classes. The step-DFA -Discriminant Factor Analysis- model has shown the best result with six main variables and an error in cross validation of 16%. The influent variables selected are almost the same using PLS (Partial Least Square) regressions. The two main selected variables are the alcoholic fermentation rate during the first days -as positive influent factor- and the concentration of copper in grape juice -as negative factor-. The other ones are the concentrations of potassium, total phenolic compounds, amino-acids in must and the climatic "frequency of rainfall in summer period" variable. These influent factors, selected by statistical treatments, have to be set on the context of the study. But, these results also organize some well known influential factors into hierarchy and then allow wine producers to act at the vineyard or at the winery to optimize the aromatic potential of their product.
Key words: varietal thiols, aromatic quality, Colombard, white wine, model

INTRODUCTION

La perception aromatique des vins de Colombard produits dans le vignoble de Gascogne provient principalement d'une surexpression de deux thiols variétaux : le 3-mercaptohexan-1-ol (3MH) et le 3-mercaptohexylacetate (3MHA). Ces composés sont libérés au cours de la fermentation alcoolique par action d'enzymes produites par la levure. Le 3MH présente alors des caractéristiques aromatiques rappelant le pamplemousse et les fruits tropicaux. Pendant la fermentation, une partie du 3MH est transformé en acétate de 3-Mercapto-Hexile (3MHA). Cet autre composé est très odorant, sa perception rappelle le buis avec une nuance de fruit exotique. (Tominaga et al., 1996).

De très nombreux travaux internationaux sont menés sur ces composés aromatiques communément appelés thiols variétaux, depuis leur origine sous forme de précurseurs dans le raisin, leur transformation par la levure jusqu'à leur conservation dans les vins. (Du Plessis, 1981 ;Tominaga et al., 1995 ; Peyros des Gachons, 2000 ; Murat et al., 2001 ; Sweigers, 2007 ; Subileau, 2008). Il ressort ainsi qu'il existe une chaîne complexe et multivariée de facteurs qui agit sur la présence de ces arômes dans les vins. Ces facteurs peuvent avoir une action positive ou négative. Ainsi au vignoble, certaines techniques viticoles peuvent influencer le potentiel aromatique comme l'effeuillage (Dufourcq et al, 2007) ou la pulvérisation d'azote foliaire (Dufourcq et al., 2009). La présence de certains composés dans le moût perturbe le potentiel aromatique. Il s'agit du cuivre, de l'oxygène, des polyphénols, des oxydases du raisin. Ils sont pour la plupart connus et agissent à différentes périodes du processus de production du vin.

Il apparaît donc que la qualification, à priori, de la matière première sur son potentiel à révéler ce type d'arômes reste floue et difficile à mettre en œuvre de manière simple et rapide pour orienter les choix de vinification.

Le but de cette étude est de comprendre les leviers qui influencent la qualité aromatique d'un vin blanc de Colombard par une approche globale en essayant de mettre en évidence les principaux facteurs qui déterminent le potentiel aromatique recherché des vins de Colombard dans la zone de production Armagnac-Gascogne. Pour répondre à cette question, nous avons essayé d'évaluer la matière première et sa transformation par une approche multicritère à partir de données climatiques, agronomiques et analytiques sur raisins et sur vins.

MATERIELS ET METHODES

Echantillonnage et élaboration des vins.

Au cours de 3 millésimes (2006-2008), 184 échantillons de 50 kg de raisins de Colombard ont été prélevés à l'arrivée des bennes de vendanges au quai de réception de différents chais de Gascogne. De ces 50 kg, un sous-échantillon d'environ 2 kg a été prélevé pour analyse. Le reste est transporté vers notre chai expérimental pour être vinifié en conditions contrôlées à échelle pilote de 30 litres. Cette vinification est réalisée en mode « réducteur », avec une protection maximale contre l'oxygène (inertage au CO_2) à tous les stades opératoires (éraflage, macération pelliculaire, pressurage). Une macération pelliculaire, qui favorise l'extraction des précurseurs aromatiques soufrés, est effectuée à froid (4°C), pendant 16 h. Le pressurage est réalisé à l'aide d'un pressoir horizontal à vis. Le jus est enzymé à 2g/hl avec des pectinases, et sulfité à 2g/hl. Les jus sont mis à stabuler pendant 24 h en chambre froide (4°C) pour permettre le débourbage. La turbidité est réajustée aux environs de 150 NTU. L'ensemencement des moûts est réalisé avec la souche VL3 (Laffort) à la dose de 20g/hl. Du saccharose est ajouté si nécessaire de façon à obtenir un vin titrant au minimum à 11%vol d'alcool. Des nutriments azotés sont aussi incorporés de façon systématique à la dose de 50 mg/l d'ion ammonium. La température de fermentation est maitrisée à 17°C. En fin de fermentation les vins sont soutirés, sulfités (5g/hl) et stockés au froid jusqu'à la mise en bouteille. La dégustation a lieu dans les trois mois suivant la vinification lorsque le caractère primeur s'exprime à plein.

Classification des vins

Après la mise en bouteille, les thiols variétaux (3MH et 3MHA) sont analysés dans les vins par la société Nyséos (Montpellier, France) selon la méthode décrite par Schneider et al. En 2003. Tous les vins ont été dégustés par un jury régional d'experts et classés sur l'intensité aromatique du caractère « thiol » en quatre catégories (Très Faible, Faible, Bon, Très Bon).
Ensuite des classes de références sont établies pour **<l'ensemble des vins>** par la combinaison du consensus des classes sensorielles et de l'analyse des thiols donnant ainsi deux classes de qualité (Faible et Bon). Afin d'affiner les classes de vins, un sous ensemble d'échantillons a été sélectionné en se basant sur un pourcentage (supérieur à 70% des votes) de consensus du jury dans la caractérisation de l'intensité thiols perçue dans les vins associé à une quantité de thiols dosée minimale. Au final 128 échantillons ont répondu aux critères et ont constitué **<des échantillons fiabilisés>** répondant aux deux classes de qualité (Faible et Bon).

Les variables explicatives.

La base de données des mesures et le nom abrégé des variables correspondantes sont listés dans le tableau 1.

Traitement des données

Deux types de traitements ont été appliqués sur tous les échantillons, puis uniquement sur le sous-ensemble des échantillons fiabilisés. Tout d'abord une sélection pas à pas de variables informatives a été pratiquée, après élimination des variables corrélées entre-elles, en utilisant l'analyse factorielle discriminante (AFD). Ensuite une tentative de modélisation des deux classes de vins définies a été envisagée par régression linéaires aux moindres carrés partiels et analyse factorielle discriminante (PLS-AFD). Les traitements ont été réalisés avec MATLAB (The Mathwoks, NATIC, USA).

Tableau 1 : Données agronomiques, climatiques, oenologiques et analytiques déterminées pour 184 échantillons de Colombard

DONNEES VITICOLES	
coordonnées GPS longitude Est. (°)	Lg
coordonnées GPS latitude Nord. (°)	La
altitude (m)	Alt
orientation des rangs (°/nord)	Or
âge de la parcelle (an)	AGE
Ecartement entre rang [m]	E
Distance entre pieds [m]	L
DONNEES AGRONOMIQUES	
enherbement juin/septembre (%)	ENH
Hauteur feuillage [m]	H
épaisseur feuillage [m]	Ep
SECV (m^2/m^2)	SECV
SECV-PR (m^2/kg)	SECV/PR
Date de récolte (jour julien)	REC
poids de récolte (kg/m^2) rendement	PR
ANALYSES DES RAISINS (avant pressurage)	
Sucres V [g/l]	S v
Acidité Totale V [g/l H_2SO_4]	AT v
S/AT V	S/AT v
pH V	pH v
Acide Malique V [g/l]	M v
Acide Tartrique V [g/l]	T v
Rapport TH2/MH2 V	T/M v
IPT V Jus	PP v
Acide gluconique (mg/L) V	AG v
Cuivre (mg/L) V	CU v
Rapport isotop. $\delta^{13}C$ V	C
Azote ammoniacal (mg/L) V	NH4 v
Azote A. aminés (mg/L) V	AA v
Azote assimilable (mg/L) V	YAN v
%acides aminés/YAN V	AA/YAN v

DONNEES CLIMATIQUES	
Σ de température (Tmoy -10) du 1/4-30/9 (°C.jour)	SjT
IF (indice fraicheur nuit) du 1/9 au 30/9 (°C)	IF
Cumul précipitation (Cp) 1/4 au 30/9 (mm)	Cpc
Cumul de précipitations 1/6 au 30/9 (mm)	Cper
fréquence précipitations 1/6 au 30/9 (jour)	Fper
Cumul de précipitations 1/6 au 31/8 (mm)	Cpe
fréquence précipitations 1/6 au 31/8 (jour)	Fpe
Cumul de précipitations du 1/10/(n-1) au 1/4/n (mm)	CP(h-1)
ŒNOLOGIE	
Température des raisins à la récolte [°C]	T°C
Nombre jours de Fermentation Alcoolique (FA)	JFA
Vitesse moyenne de FA (en g/l/jour)	VFm
Vitesse de FA de Jour 1 à Jour 3 (en g/l/jour)	VFj1.3
Vitesse de FA de Jour 1 à Jour 5 (en g/l/jour)	VFj1.5
Vitesse de FA de Jour 5 à Jour 10 (en g/l/jour)	VFj5.10
ANALYSES DES MOUTS (après débourbage)	
Sucres D [g/l]	S d
Acidité TotaleD [g/l H_2SO_4]	AT d
S/AT D	S/AT d
pH D	pH d
Acide Malique D [g/l]	M d
Acide Tartrique D [g/l]	T d
Rapport TH2/MH2 D	T/M d
IPT D Jus	PP d
K+ D [g/l]	K d
turbidité (NTU) D	NTU

RESULTATS

L'AFD pas à pas est utilisée afin de sélectionner les variables analytiques les plus pertinentes pour la prédiction des classes aromatiques. La validation est réalisée par validation croisée de 23 blocs randomisés de 8 échantillons. Pour l'ensemble des échantillons, on observe deux optima, l'un pour 6 variables avec une erreur de validation de 22% et l'autre

pour 14 variables avec une erreur améliorée à 20%. Les pourcentages d'erreur sont très équilibrés entre 2nd ordre et 1er ordre ainsi qu'entre les classes <bon> et <faible>.

La sélection des variables discriminantes pour les classes aromatiques, donne également un optimal de 6 variables avec une nette amélioration des erreurs de classifications sur la série d'échantillons fiabilisés passant de 22% à 16%. Pour les deux classifications, les deux premières variables informatives sont <la vitesse de FA des 5 premiers jours > et <la quantité de cuivre> (figure1). <La quantité de potassium>, <l'Indice de Polyphénols Totaux>, <la fréquence des précipitations de juin à septembre> et <la quantité d'azote aminé> sont également sélectionnés. Ces variables faisaient partie de la sélection de 7 à 14 pour la classification précédente. A nouveau, les erreurs sont assez bien équilibrées. Les échantillons mal classés ne montrent pas de tendance particulière selon le millésime, excepté le fait que les échantillons 2008 présentent seulement 2 échantillons mal classés. Les erreurs globales sont : 21% en 2006, 23% en 2007 et 5% en 2008.

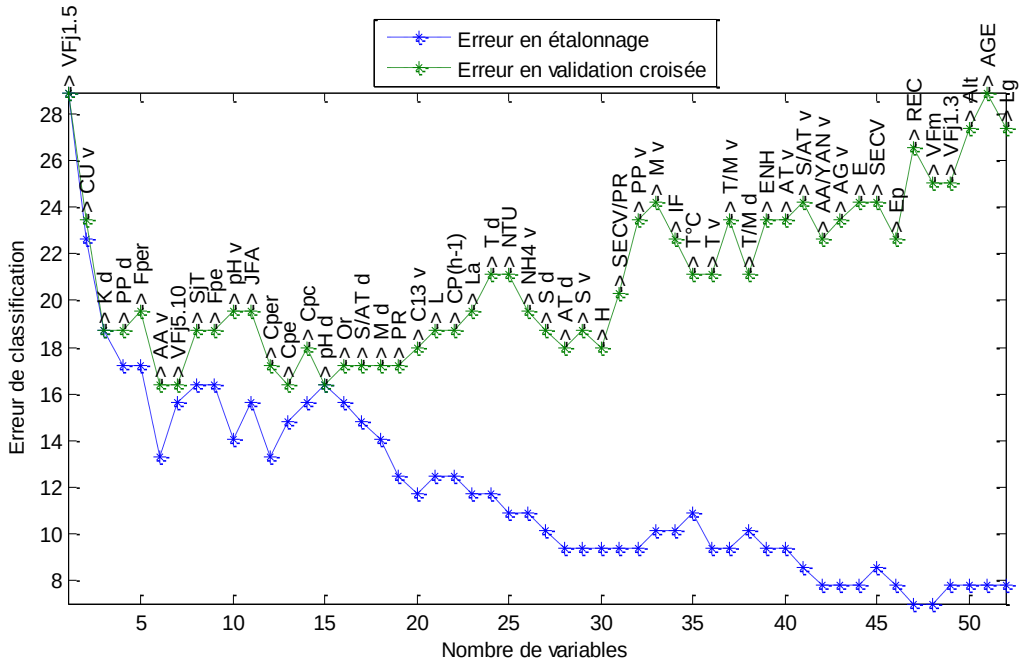

Figure 1 : hiérarchisation des variables par step-AFD pour la prédiction des classes aromatiques des vins de Colombard à partir des échantillons fiabilisés (128 échantillons / 52 variables)

La modélisation par PLS-AFD a donné des performances plus faibles que les méthodes pas à pas dans le cas de cette étude. En revanche, l'avantage est de pouvoir observer le poids de chaque variable dans la réalisation du modèle. Les variables les plus importantes sont similaires à celles obtenues par sélection pas à pas (figure 2). Comme pour la step-AFD, la sélection d'échantillons fiabilisés permet d'améliorer la classification. Les performances sont de 20% d'erreur en validation contre 28% pour l'ensemble des échantillons. Les coefficients de régression (b-coefficients) sont très semblables à ceux obtenus sur la classification de l'ensemble des échantillons.

Le tableau 2 indique les premières variables sélectionnées pour la prédiction des classes. On constate que <la quantité de cuivre> et <La vitesse de FA des 5 premiers jours> sont sélectionnés dans les deux analyses et les deux séries d'échantillonnage. <La concentration en potassium> ainsi que <la fréquence des précipitations de juin à septembre> sont sélectionnés trois fois. Enfin, <l'azote aminé> apparait deux fois.

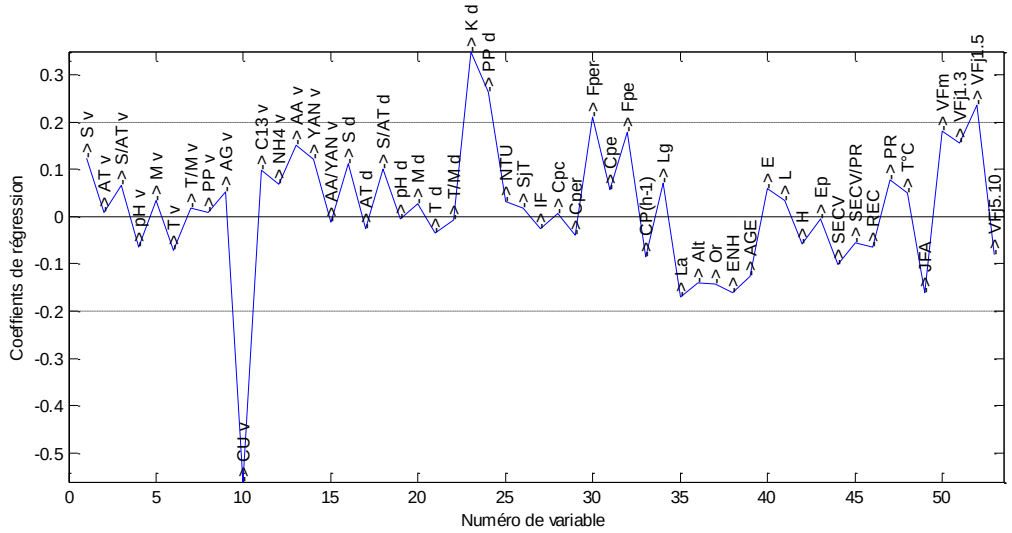

Figure 2 : coefficients de régression des variables issus de PLS-AFD pour la modélisation des classes aromatiques des vins de Colombard à partir des échantillons fiabilisés (128 échantillons / 52 variables)

Tableau 2 : variables informatives sélectionnées dans les différents traitements (Step-AFD, PLS-AFD) en fonction des séries d'échantillons (184 ou 128) et pour la prédiction des classes aromatiques des vins de Colombard

Analyses de données	Step-AFD	Step- AFD	PLS-AFD	PLS-AFD
échantillonnage	<tous les échantillons> (184)	<échantillons fiabilisés> (128)	<tous les échantillons> (184)	<échantillons fiabilisés> (128)
Nombre de variables optimal	6	6	2 variables latentes	3 variables latentes
Erreur en validation croisée	**22%**	**16%**	**28%**	**19%**
Variables informatives sélectionnées au moins deux fois	Cuivre (x4)	Vitesse FA jours 1-5 (x4)	Cuivre (x4)	Cuivre (x4)
	Vitesse FA jours 1-5 (x4)	Cuivre(x4)	Vitesse FA jours 1-5 (x4)	Vitesse FA jours 1-5 (x4)
		Potassium (x3)	Potassium (x3)	Potassium (x3)
		IPT en débourbage (x2)	Fréquence précipitations (juin-sept) (x3)	IPT en débourbage (x2)
		Fréquence précipitations (juin-sept) (x3)	Azote aminé (x2)	Fréquence précipitations (juin-sept) (x3)
		Azote aminé (x2)		

Quelques remarques sont à associer aux différentes variables sélectionnées. Tout d'abord, la **concentration en Cuivre des raisins au stade vendange e**st un facteur qui va influencer la production de thiols variétaux au cours de la fermentation (Hadzidimitriou et al, 1996). Bien que ce paramètre soit bien identifié par les acteurs de la production et peu employé en Gascogne, il apparait tout de même comme une variable influente majeure. On confirme ici la part prépondérante de cet élément pour expliquer par sa présence la conséquence négative de son influence sur le potentiel aromatique des vins de Colombard. Ensuite, le début de la fermentation est la phase où se produit la transformation en thiols variétaux (Subileau, 2008). La **Vitesse moyenne de fermentation alcoolique durant les 5 premiers jours** (à

température constante et souche constante) est une variable intéressante. Elle est corrélée à la quantité d'azote du moût et influence positivement la qualité aromatique des vins.
Les vins situés dans la classe <faible> sont significativement moins riche en c**oncentration en potassium du moût** avant fermentation. Croisé avec le millésime, ce résultat est observé sur les millésimes 2007 et 2008 mais pas en 2006. Aucune relation claire n'a été montrée à ce jour entre ce cation et la qualité aromatique en thiols variétaux des vins.

CONCLUSION

La compréhension des leviers et de leurs interactions, qui contribuent au potentiel aromatique des vins de Colombard, est un enjeu d'autant plus important que l'on cherche, comme c'est le cas en Gascogne, la surexpression de thiols variétaux dans le produit fini. A partir d'un ensemble de variables agronomiques, climatiques et œnologiques, il a été tenté une approche de modélisation ou de prédiction de deux classes de vins générées à partir de l'expertise sensorielle de ceux-ci et du dosage des deux composés thiols qui contribuent à la typicité attendue des vins. Les résultats obtenus nous montrent qu'on arrive à un niveau de performance dans la prédiction intéressant (16% d'erreur dans le meilleur des cas). Les variables influentes qui sont générées par les traitements statistiques doivent être replacées dans le contexte de l'étude c'est-à-dire tout d'abord dans le champ de variation des millésimes. Il est à considérer aussi si ces variables influent directement ou sont simplement la représentation mathématique la plus pertinente d'une cause indirecte. Enfin, ces résultats permettent de hiérarchiser des variables déjà connues pour leur influence et peuvent ainsi inciter les producteurs à agir pour optimiser le potentiel aromatique de leurs vins.

BIBLIOGRAPHIE

Dufourcq T., Charrier F., Poupault P., Schneider R., Gontier L., Serrano E, 2009. Foliar spraying of nitrogen and sulfur at veraison: a viticultural technique to improve aromatic composition of white and rosés wines. *Proceedings of the 16th International GiESCO Symposium, Davis (USA)* p379-383.

Dufourcq T., Charrier F., Schneider R., Serrano E., 2007. Effets de l'exposition des grappes sur le potentiel aromatique des raisins et des vins de Colombard B et Melon B. *8 Internationales ATW-Symposium, Technik im Weinbau, Stuttgart (Allemagne), KTBL ed.* p221-228.

Du Plessis, C. S. et Augustyn, O. P. H., 1981. Initial study on the guava aroma of Chenin blanc and Colombard wines. *S Afr J Enol Vitic 2,* p101-103.

Hadzidimitriou E., et al., 1996. Incidence d'une protection viticole anticryptogamique utilisant une formulation cuprique sur le niveau de maturité des raisins et l'arôme variétal des vins de Sauvignon. *J. Int. Sci. Vigne Vin*, 30, n°3, p 133-150.

Murat, M. L., Masneuf, I., Darriet, P., Lavigne, V., Tominaga, T. et Dubourdieu, D., 2001. Effect of Saccharomyces cerevisiae yeast strains on the liberation of volatile thiols in Sauvignon blanc wine. *Am J Enol Vitic* 52, p136-139.

Peyros Des Gachons C., 2000. Recherches sur le potentiel aromatique des raisins de *Vitis vinifera* L. Cv. Sauvignon blanc. *Thèse de Doctorat Sciences biologiques et Médicales, option Œnologie-Ampélologie, Université de Bordeaux II*, 176p.

Schneider R., Kotseridis Y., Ray J.L., Augier C., Baumes R., 2003. Quantitative determination of sulfurcontaining wine odorants at sub parts per billion levels. 2.

Development and application of a stable isotope dilution assay. *J. Agric. Food Chem.*, 51, 3243-3248.
Subileau M., 2008. Parameters influencing varietal thiol release by strains of *Saccharomyces cerevisiae* : from a controlled synthetic medium to the complexity of Sauvignon blanc must. *Thèse de doctorat de l'Ecole Nationale Supérieure d'Agronomie de Montpellier*, 154p.
Swiegers, J.H., Pretorius I.S., 2007. Modulation of volatile sulfur compounds by wine yeast. *Appl. Microbiol. Biotechnol*., 74, p954-960**.**
Tominaga, T., DarrieT, P. & Dubourdieu, D. (1996). Identification of 3-mercaptohexanol acetate, compound having a powerful odor reminiscent of box-tree, involved in the aroma of Sauvignon wines. *Vitis 35*, p207-210.
Tominaga T., Masneuf I., Dubourdieu D. (1995) A S-cysteine conjugate, precursor of aroma of white sauvignon. *J Int Sci Vigne Vin 29:*p227–232.

L’influence des bio-stimulateurs naturels sur la production des plants greffés de haute qualité

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abstract

Dans le laboratoire de la biochimie technique auprès de l’Institut de l’Horticulture, de la Viticulture et de la Viniculture nous avons effectué l’examen de l’extrait reçu de la rafle du raisin en tant que le bio-stimulateur.

La matière de greffage des espèces vignobles comme Rkatsiteli, Goruli Mtsvane et Tavkveri a été soumise à l’examen. La matière de greffage a été mise dans la solution du stimulateur pendant 45-50 heures, puis le greffage a été effectué. Les greffes produits ont été installés dans la serre avec les spécimens de contrôle pour la stratification. L’extrait des greffes de callus complet a été étudié sur les greffes stratifiés, ce qui s’élevait à 94%, volume des greffes à boutons ouverts à 78%, volume des racines à 31%, extrait du plant de première qualité à 59,8%.

Le stimulateur susmentionné cause le développement rapide du bouton et l’inhibition de la pousse ce qui conditionne l’accroissement de l’intensivité du développement de callus à l’endroit de l’union de la racine et du greffe, du réveil du bouton de greffage et de l’intensivité de la naissance des racines dans la zone de la racine et du fond. Le bio-stimulateur offert par nous assure la production dans un temps très court de la matière de plant biologiquement pure, propre du point de vue de sélection et de phytosanitaire, sans virus, saine et certifiée.

Die Prüfung vom Traubenspross bekommenen Extraktes als biostimulator wurde im technischen Biochemie-Laboratorium des Instituts für die Gartenbau, den Weinbau und das Wein-Bilden durchgeführt..

Es wurde Weinrebearte - Rkatsiteli, Goruli Mtsvane und Tavkveri wurden erforscht. Das Geproftematerials wurde in der Anreger-Lösung während 45-50 Stunden gehindert. Später das Pfropfen wurde zur Verfügung gestellt. Die Pfropfreiser zusammen mit den Überprüfungsproben wurden ins Treibhaus für die Stratifikation gelegt. Auf den geschichteten Pfropfreisern

erforschten wir Produktivität von ganzen-callused Pfropfreisern, die 94 % bestehen; die Menge der Pfropfreiser mit dem Knospen - 78 %, Menge von Wurzeln - 31 %, und der Produktivität der erstklassigen jungen Werke - 59.8 %. Der Anreger, der oben erwähnt ist, provoziert Hemmung der schnellen Entwicklung von Knospen und Zunahme, die das Wachstum der Intensität der sich entwickelnden Schwiele auf dem Verbindungsplatz des Bodens und der engraftment Abteilungen, gemmating von Engraftment-Knospen und Intensität der Wurzel-Bildung im Boden und den Kellerzonen verursacht.

Von uns abgebotene Biostimulator gewährleistet in der kurze Zeit biologisch, auswählend und phito-sanitarily saubere, gesunde, bescheinigte Pflanzen-Materialien ohne Viren in der kürzesten Zeitspanne zu erhalten.

Introduction

La formation de la plantation industrielle de haute productivité, l'intensification de la viniculture et de la viticulture et les rythmes de développement dépendent beaucoup à la production des matières de plant, à leur qualité et composition.

Pour grandir la production du plant greffé standard et améliorer sa qualité les substances biologiquement actives sont utilisées à très petite dose. Elles se caractérisent du large spectre de l'influence, de la régularisation convenable des étapes séparées de croissance et de développement, de la mobilisation des possibilités potentielles des organismes végétaux et de la croissance de productivité.

Il est connu des sources littéraires que le traitement des greffes de la vigne biologiquement active (auxine, hétéro-auxine, épine, caroténoïdes, aminoacides etc.) élève le développement des systèmes des racines.

Suite aux examens végétaux-pratiques depuis des années nous avons obtenu le bio-stimulateur du débris viticoles, notamment de la rafle du raisin. Il contient de l'ensemble des substances biologiquement actives : auxines, gibbérellines et cytokinins. Sauf les phytohormones il contient de soi-disant substances d'élévation secondaires : flavonoïdes, aminoacides, lipides, carbonates acides (par exemple les acides de gal et du café – inhibiteurs d'élévation), alcaloïdes, lactones non saturées, terpénoïdes et autres.

Matières et méthodes

Afin d'établir l'influence des bio-stimulateurs obtenus de la rafle et d'élaborer des technologies efficaces de l'augmentation de la production des plants greffés, les travaux d'examens ont été effectués à la base expérimentale de l'Institut de l'Horticulture, de la Viniculture et de la Viticulture.

Les objets de l'examen étaient l'espèce de la vigne Goruli Mtsvane, Berlandieri / Riparia Cober 5 BB pour la racine, la solution aqueuse de l'hétéro-auxine (contrôle) a été utilisée comme le bio-stimulateur et l'extrait de la rafle – bio-stimulateur d'essai obtenu par nous. Les espèces de greffage et de racine ont été travaillées jusqu'à la stratification dans la solution aqueuse de l'hétéro-auxine et dans des solutions de différentes concentrations du bio-stimulateur d'essai.

Les examens ont été effectués selon le schéma suivant :

1. Non travaillé

2. Dilution du stimulateur d'essai avec l'eau 1:10

3. Dilution 1:15

4. Dilution 1:30

5. Dilution 1:40

6. Solution aqueuse de l'hétéro-auxine (contrôle)

Pour chaque variante dans 3 expositions (24, 848, 72 heures) avec 5 fois de répétition.

Il a été réalisé par nous des analyses suivantes. Des indices physiologiques il a été déterminé :

- composition des pigments plastides - chlorophylle « a » et « b », des caroténoïdes dans les feuilles par la méthode colorimétrique ;

- l'intensivité relative de la photosynthèse a été définie par l'utilisation de la méthode fluorescente à l'appareil PAM 2100 (4).

Processus de régénération dans les conditions de la serre :

- germination du plant greffé dans la pépinière et indices biométriques ;

- production du plant greffé de première qualité ;

Pour l'établissement de la nature stimulatrice du bio-stimulateur d'essai il a été défini :

- phytohormones, auxines, gibbérellines, cytokinins (5, 6)

- flavonoïdes, albumines, aminoacides, terpènes.

Résultats et discussion

L'objectif de notre examen consistait au traitement de la nouvelle technologie parfaite de la production du plan greffé de la vigne en utilisant le bio-stimulateur naturel (végétal) obtenu des matières végétales, notamment de la rafle du raisin, car ce type des stimulateurs ont plus d'avantages vis-à-vis aux autres stimulateurs organiques ou minéraux ce qui s'exprime dans le suivant :

La nature bio-stimulatrice de l'extrait d'essai a été identifiée suite à l'observation effectuée sur le développement du callus et des racines sur les pousses et sur l'intensivité de leur élévation. La production du callus et des racines commencent 3-4 jours avant que des pousses y travaillées et du contrôle (solution de l'hétéro-auxine). Il est à noter également que dans l'objet d'examen le système des racines fortes se développe et l'élévation des pousses se passe activement (image 1).

Enraciner les greffes de la vigne traitées dans le stimulateur d’essai.

1. Contrôle (eau)
2. Espèce de la vigne Goruli Mtsvane
3. Espèce de la vigne Tavkveri
4. Hétéro-auxine (contrôle).

La concentration optimale du bio-stimulateur pour l’enracinement du plant est 1/40, exposition – 72 heures.

Il a été montré par les examens laboratoires que l’essai du bio-stimulateur obtenu de la rafle du raisin est convenable pour recevoir du plant greffé de haute qualité.

Il a été étudié par nous l'influence stimulatrice du bio-stimulateur, obtenu de la rafle du raisin, sur les greffes de racine et de greffage (Berlandieri / Riparia Cober 5 BB a été utilisé pour la racine, Goruli Mtsvane pour le greffage).

Sous l'influence du bio-stimulateur d'essai, les greffes, ainsi que les greffes plantées pour prendre des racines et les racines mêmes commencent la germination rapidement et avec une grande énergie. Il est donné dans le tableau l'influence du stimulateur sur l'intensivité du développement des calus, boutons et racines.

Les pousses greffées commencent à prendre rapidement des racines et à développer les boutons après leur plantation dans la pépinière.

Indices du développement des greffes traitées par les stimulateurs d'essai

variante	Comlet calus	La quantité de reins	La quantité de racines	La sortie haute qualité plant
Etudie bio-stimulateurs	94	78	31	60
Contrôle . Hétéro-auxine	78	75	28	52
Contrôle	80	70	6	46

L'avantage du stimulateur proposé par rapport aux stimulateurs présentés au marché géorgien consiste à la possibilité de la préparation d'activer les processus de changement des substances dans les organismes végétaux, d'élever leur vitalité et persistance contre les conditions défavorables ; il favorise à l'amélioration de l'alimentation minérale de la greffe vignoble, à la croissance du raccordement et de la cicatrisation des composants de greffage et au développement fort du systèmes des racines. Il renforce comme le système des racines ainsi le développement des parties au-dessus du sol. Les racines sont plus logues et ont plus de branches, les tiges deviennent plus grosse, mais les feuilles sont plus larges et la composition des chlorophylles est augmenté en elles ce qui accroit l'intensivité de couleur (image 1).

Conclusions :

Suite aux examens réalisés nous pouvons faire les conclusions suivantes :

1. La stimulation du callus s'effectue lors de la dilution du stimulateur d'essai avec l'eau 1:40.

2. Les substances biologiquement actives ne font aucune influence négative sur le déroulement du cycle vital des cellules. La pousse ainsi reçue, avec l'utilisation des substances biologiquement actives, est durable du point de vue de cytologie.

3. Les greffes dont la matière de greffage est travaillée par la solution du stimulateur d'essai se distinguent de l'énergie élevée de germination du plant greffée. Ils se dépassent beaucoup à la variante d'essai.

4. Il est remarqué l'élévation intensive de la pousse.

5. Les conditions favorables au développement du système des racines et des plants greffés sont créées à l'aide du traitement préalable des greffes.

6. La croissance de l'intensivité de la photosynthèse et de la constitution des pigments plastides par rapport au contrôle (hétéro-auxine) est conditionnée par le stimulateur d'essai.

7. La production du plant greffé standard est élevée et son prix coûtant est diminué.

Littérature :

1. V. I. KAPEL, R. KH. TURETSKAIA – détermination des auxines et inhibiteurs biologiquement actives, libres dans la matière végétale.
2. Chauvin P. Notes concernant L 'exuberone. Chauvin s.a. agrodistribution. – Catalaogue № 4, 2000. p. 46.
3. Passos J. R. S.,Pommer C.V. Vitis rodunitolia Miehx. Secao de Viticultura – J. AC, C.P.28, CEP: 300-970- Campinas. S.P>

SVILUPPO DI SAGGI DI RT-PCR REAL TIME IN MULTIPLEX PER LA DIAGNOSI DEI VIRUS DELLA VITE

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RIASSUNTO

La diagnosi dei virus è molto importante nei lavori di selezione clonale sanitaria e nella certificazione dei materiali di propagazione viticola. Lo scopo di questo lavoro è stato di mettere a punto saggi rapidi basati sulla RT-PCR *real time* in multiplo (multiplex) con sonde ad idrolisi per la diagnosi dei più importanti virus della vite (ArMV, GFLV, GLRaV-1, 2 e 3, GFkV, GVA, GRSPaV). I metodi sono poi stati confrontati con i saggi sierologici ELISA, ove possibile, su circa 150 campioni di vite. I saggi di RT-PCR *real time* in singolo (singleplex) hanno dimostrato di essere generalmente più sensibili dei saggi ELISA per tutti gli 8 virus. I saggi *real time* multiplex hanno invece dato risultati identici ai saggi singleplex, anche se il limite di rilevamento è risultato migliore nei saggi singleplex. In conclusione, i saggi messi a punto in questo lavoro hanno dimostrato di essere utili per la diagnosi dei virus della vite, poiché sono più sensibili e veloci dei metodi convenzionali e permettono un minor consumo di reagenti e di DNA stampo rispetto ai saggi singleplex.

SUMMARY: DEVELOPMENT OF MULTIPLEX RT-PCR REAL TIME ASSAYS FOR THE DIAGNOSIS OF GRAPEVINE VIRUSES

Diagnosis of viruses is very important in clonal sanitary selection and certification schemes of grapevine propagation materials. The aim of this work was to develop fast multiplex real time RT-PCR assays with TaqMan probes for the detection of some of the most important viruses of grapevine (ArMV, GFLV, GLRaV-1, 2 and 3, GFkV, GVA, GRSPaV). The assays were then compared with the ELISA serological test on approximately 150 grapevine samples. The real time singleplex RT-PCR assays developed for the 8 viruses showed to be generally more sensitive than the ELISA. The results of the multiplex real time RT-PCR assays designed always agreed with results obtained in the singleplex tests, saved for the limit of detection, which was better in the singleplex assays. In conclusion, the assays proved to be a useful tool for the diagnosis of grapevine viruses, as they are more sensitive and faster than the conventional diagnostic methods and they allow to reduce reagent and cDNA template consumption with respect to singleplex assays.

INTRODUZIONE

Le attività di controllo ai fini della certificazione dei materiali di moltiplicazione e della selezione clonale sanitaria della vite richiedono l'applicazione di metodi diagnostici per i virus in grado di fornire risultati rapidi, affidabili ed universalmente riconosciuti. Negli ultimi anni sono stati emanati, a livello europeo e nazionale, direttive e decreti che, da una parte, definiscono i criteri di lavoro per la selezione clonale ed i requisiti sanitari minimi per la certificazione e la selezione, dall'altra forniscono indicazioni sui tipi di saggi diagnostici da eseguire, implementando le nuove informazioni acquisite dalla ricerca negli ultimi 40 anni e colmando così una lacuna legislativa che permaneva dagli anni '70 (Direttiva Comunitaria n. 205/43/CE del 23 giugno 2005, Decreto MiPAAF del 7 luglio 2006; Decreto MiPAAF del 24 giugno 2008).

Esistono diversi metodi per diagnosticare le infezioni virali della vite. Lo sviluppo delle biotecnologie avvenuto negli ultimi anni ha permesso di utilizzare nuove tecniche diagnostiche sempre più affidabili: accanto ai tradizionali saggi biologici tramite indicatori sensibili ed ai saggi sierologici di tipo ELISA (*Enzyme Linked ImmunoSorbent Assay*), si sono affermati sempre più i metodi molecolari basati sulla PCR (*Polymerase Chain Reaction*). Le tecniche molecolari più usate per la diagnosi dei virus sono la RT-PCR (*Reverse-Transcriptase* PCR) convenzionale, specifica per ciascun virus, seguita da elettroforesi su gel di agarosio. Le tecniche di *real time* PCR, che si sono evolute negli ultimi anni, sono ancora più rapide e sensibili, ma al momento sono poco applicate nella diagnosi dei virus della vite (Osman *et al.*, 2007, 2008; Osman, Rowhani, 2008).

Lo scopo di questo lavoro è stato di mettere a punto saggi rapidi per la diagnosi di 8 fra i più importanti virus della vite, basati sulla *real time* RT-PCR, che possono essere utilizzati singolarmente per l'analisi di ciascun virus (singleplex) o in contemporanea con due sistemi da 4 virus ciascuno (multiplex).

MATERIALI E METODI

Sono state utilizzate più di 150 accessioni di vite, che si erano rivelate infette da uno o più virus in saggi precedenti. I campioni, costituti da talee legnose, sono stati analizzati sia con saggio ELISA sia con i saggi *real time* sviluppati in questo lavoro. I risultati analitici ottenuti sono stati poi confrontati tra loro per determinare le caratteristiche di sensibilità, specificità ed accuratezza secondo le procedure EPPO (AA.VV., 2010).

Il saggio ELISA è stato eseguito con *kit* commerciali (Agritest e Bioreba), adottando i protocolli consigliati dalle case produttrici.

Dagli stessi campioni utilizzati per il saggio ELISA sono stati estratti gli acidi nucleici (MacKenzie *et al*., 1997) e l'RNA è stato trascritto in cDNA per l'analisi molecolare (Bertazzon, Angelini, 2004). Nel caso del virus GRSPaV, per il quale non esiste un saggio sierologico affidabile, il saggio di PCR *real time* è stato confrontato con il saggio di RT-PCR convenzionale, condotto utilizzando 3 coppie di *primer*, complementari fra loro, che riconoscono i 3 principali gruppi filogenetici di questo virus (Rowhani, comunicazione personale).

Sono stati sviluppati saggi di *real time* PCR con sonde ad idrolisi per 8 virus della vite (Tab. 1). I *primer* e le sonde sono stati disegnati su regioni altamente conservate di sequenze *consensus* del gene del capside proteico per tutti i virus oggetto di studio, utilizzando i programmi informatici Primer 3 (http://www.broad.mit.edu/cgibin/primer/primer3.cgi) e Beacon Designer (versione 2.13, Biorad). Le sequenze *consensus* sono state ottenute tramite l'allineamento del maggior numero possibile di accessioni per ogni virus ricavate da GenBank (Tab. 2).

Tab. 1. Virus e virosi della vite per i quali è stata messa a punto la diagnosi in *real time* PCR.

Tab. 1. Grapevine viruses and viroses which were detected by real time PCR.

Virosi	**Virus**	**Acronimo**
Complesso della degenerazione Infettiva (*fanleaf*)	Virus del mosaico dell'Arabis	ArMV
	Virus dell'arricciamento fogliare	GFLV
Complesso dell'accartocciamento fogliare (*leafroll*)	Virus dell'accartocciamento fogliare 1, 2 e 3	GLRaV-1, 2 e 3
Maculatura infettiva (*fleck*)	Virus del fleck	GFkV
Complesso del legno riccio (*rugose wood*)	Virus della scanalatura del Kober	GVA
	Virus della butteratura della rupestris	GRSPaV*
Necrosi delle nervature (*vein necrosis)*	Virus della butteratura della rupestris	GRSPaV*

* GRSPaV è l'agente patogeno di due diverse malattie della vite. *GRSPaV is the etiological agent of two different grapevine diseases (*Bouyahia *et al.*, 2005; Borgo *et al.*, 2009*).*

Tab. 2. Accessioni di GenBank utilizzate per ottenere la sequenza *consensus* di ogni virus.

Tab. 2. GenBank accessions used for obtaining the consensus *sequence in each virus.*

Virus	**Sequenze (n. accessione di GenBank)**
ArMV	X55460; NC_006056; X81815
GFLV	AY821657, DQ526452, NC_003623, X16907, AY371023, AY371025, AY371026, AY464090, X60775, AF304013, AF304014, AF304015, U11768
GLRaV-1	EF103902; AB222850
GLRaV-2	Y14131; AF039204
GLRaV-3	DQ911148; DQ680142; DQ680142; NC004667; AJ603339; AJ603340; AJ603341; AJ603343; AJ603344; AJ603345; AJ603346; AJ603348; AJ603349; AJ603350; AJ603351; AJ603352; AJ603353; AJ603354; AJ603355; AJ603356; AY753208
GFkV	NC_003347; AJ309022
GVA	NC003604; DQ855086; DQ855087; DQ855088; DQ855082; DQ911145, AF441235, X75443, AF441236, AF494187, AY340581, AY244516, AF007415
GRSPaV	AY881626, AY881627, AF026278, AF057136; NC_001948, AY368590

Tutti i *primer* e le sonde sono state disegnati per amplificare frammenti di dimensioni inferiori a 100 pb, al fine di incrementare al massimo l'efficienza della reazione PCR. I *primer* sono stati scelti sulla base della temperatura di fusione, in modo da impostare un protocollo termico di PCR con una temperatura di appaiamento di 60°C, tale da garantire adeguate condizioni di specificità nei confronti dei vari virus. La compatibilità di tutti i *primer* e delle sonde per l'allestimento delle PCR *real time* multiplex è stata verificata preliminarmente con il programma AUTODIMER 1.0 (Vallone, Butler, 2004). Le sequenze di tutti i potenziali amplificati sono state poi confrontate con le sequenze nucleotidiche presenti in GenBank, utilizzando il programma BLAST, allo scopo di valutare preventivamente eventuali omologie con altri organismi diversi dai virus oggetto di studio.

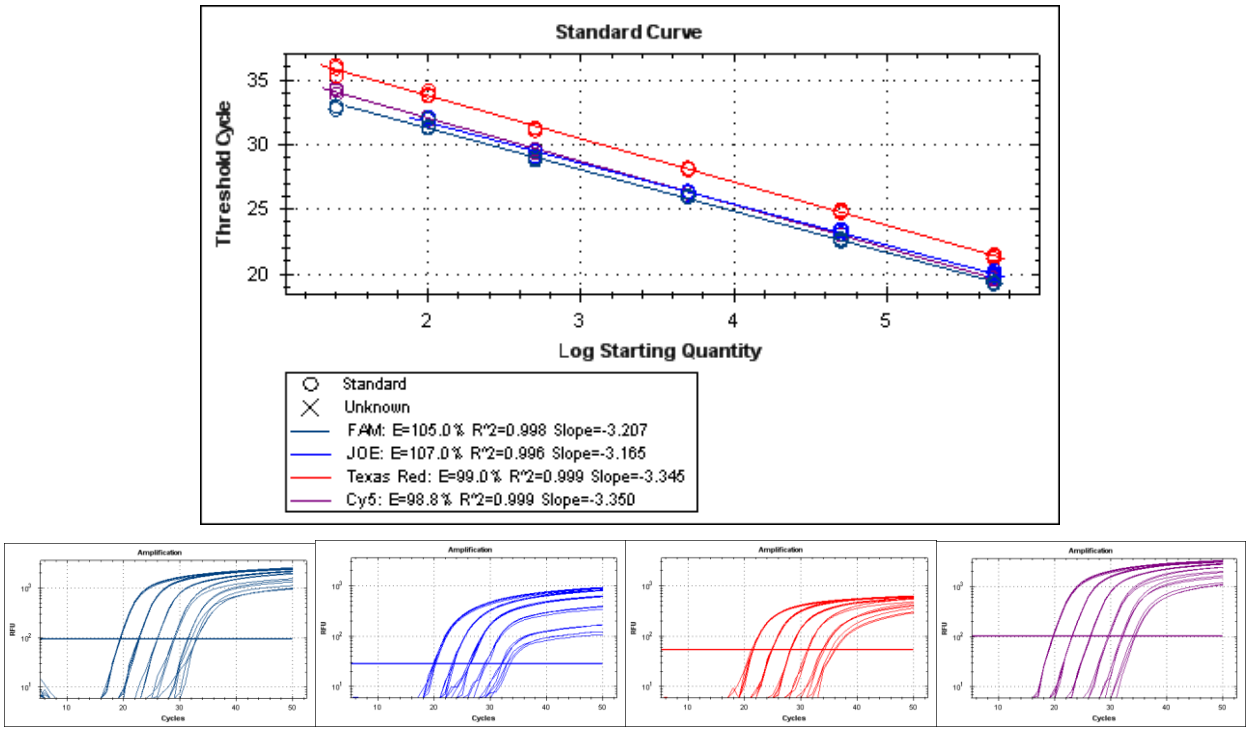
Le prove di PCR *real time* sono state effettuate presso i laboratori dell'ERSA di Pozzuolo del Friuli (UD) e del CRA-VIT di Conegliano (TV), al fine di valutare la ripetibilità e riproducibilità dei risultati in condizioni sperimentali e strumentali diverse. Le prove sono state eseguite utilizzando film ottici e piastre Bio-Rad a 96 pozzetti e tutti i campioni sono stati saggiati in doppio in prove ripetute almeno due volte. E' stato sempre utilizzato il seguente protocollo termico: denaturazione iniziale di 3' a 95°C, seguita da 50 cicli di reazione PCR con 5" di denaturazione a 95°C e 30" di appaiamento ed estensione a 60°C. Dapprima sono stati messi a punto gli 8 saggi di PCR singleplex, che sono stati poi riuniti in due sistemi di PCR *real time* in quadruplo (multiplex), capaci ciascuno di diagnosticare 4 virus in contemporanea, e cioè GLRaV-1, GLRaV-2, GLRaV-3 e GVA con il primo sistema, ArMV, GFLV, GFkV e GRSPaV con il secondo sistema.

Le prove di PCR *real time* singleplex sono state allestite in un volume finale di 25 µl, utilizzando 5 µl di cDNA e la Supermix Bio-Rad 2X. I *primer* e le sonde sono stati sempre utilizzati alla concentrazione finale rispettivamente di 0,3 µM e 0,2 µM. Le prove di PCR *real time* multiplex sono state realizzate con le stesse modalità, ma utilizzando la Powermix Bio-Rad 2X ed aumentando la concentrazione finale dei *primer* a 0,4 µM. I *primer* e le sonde ad idrolisi sono state fornite dalla ditta M-Medical. Tutte le sonde sono state inizialmente sintetizzate utilizzando come marcatore di emissione il fluoroforo FAM in posizione 5' ed il marcatore di assorbimento TAMRA in 3'. Per gli esperimenti di PCR *real time* multiplex tutte le sonde state marcate in 3' con il marcatore di assorbimento BHQ, mentre in 5' le sonde di GFkV e GLRaV-2 sono state marcate con FAM, le sonde di GFLV e GLRaV-3 sono state marcate con JOE, le sonde di ArMV e GVA sono state marcate con TEXAS RED e le sonde di GRSPaV e GLRaV-1 sono state marcate con CY5, in modo da distinguere i diversi virus grazie ai diversi fluorofori.

Tutti i sistemi di PCR *real time* sono stati saggiati in singleplex ed in multiplex per la determinazione del limite di rilevamento (LOD) e dell'efficienza della PCR mediante l'utilizzo di *standard* plasmidici, che sono stati prodotti amplificando il frammento di interesse con *primer* specifici per ciascun virus a partire da cDNA ottenuto da piante infette. Il prodotto di PCR è stato quindi recuperato da gel e inserito nel vettore pGEM-T (Promega) per la successiva trasformazione di cellule competenti di *Escherichia coli* JM101. I plasmidi così ottenuti sono stati estratti mediante il *kit* QIAprep Spin Miniprep (Qiagen) e quantificati allo spettrofotometro. La preparazione delle diluizioni contenenti l'opportuno numero di copie per ciascun plasmide è stata effettuata come descritto da Applied Biosystem (2003). Tutti gli esperimenti sono stati realizzati almeno in doppio, creando 8 diluizioni seriali del plasmide di riferimento, a partire da 500.000 copie e fino ad un limite inferiore di 5 copie. Ogni diluizione è sempre stata replicata in triplo ed ai fini del calcolo del LOD sono state considerate solo le diluizioni positive nelle tre repliche.

RISULTATI E DISCUSSIONE

In questo lavoro sono stati messi a punto saggi diagnostici con la tecnica PCR *real time* per l'analisi di 8 virus della vite. Gli 8 saggi singleplex sono stati poi riuniti in due sistemi di PCR *real time* in quadruplo, marcati con 4 colori differenti e capaci ciascuno di diagnosticare 4 virus in contemporanea, e cioè GLRaV-1, GLRaV-2, GLRaV-3 e GVA con il primo sistema, ArMV, GFLV, GFkV e GRSPaV con il secondo sistema.



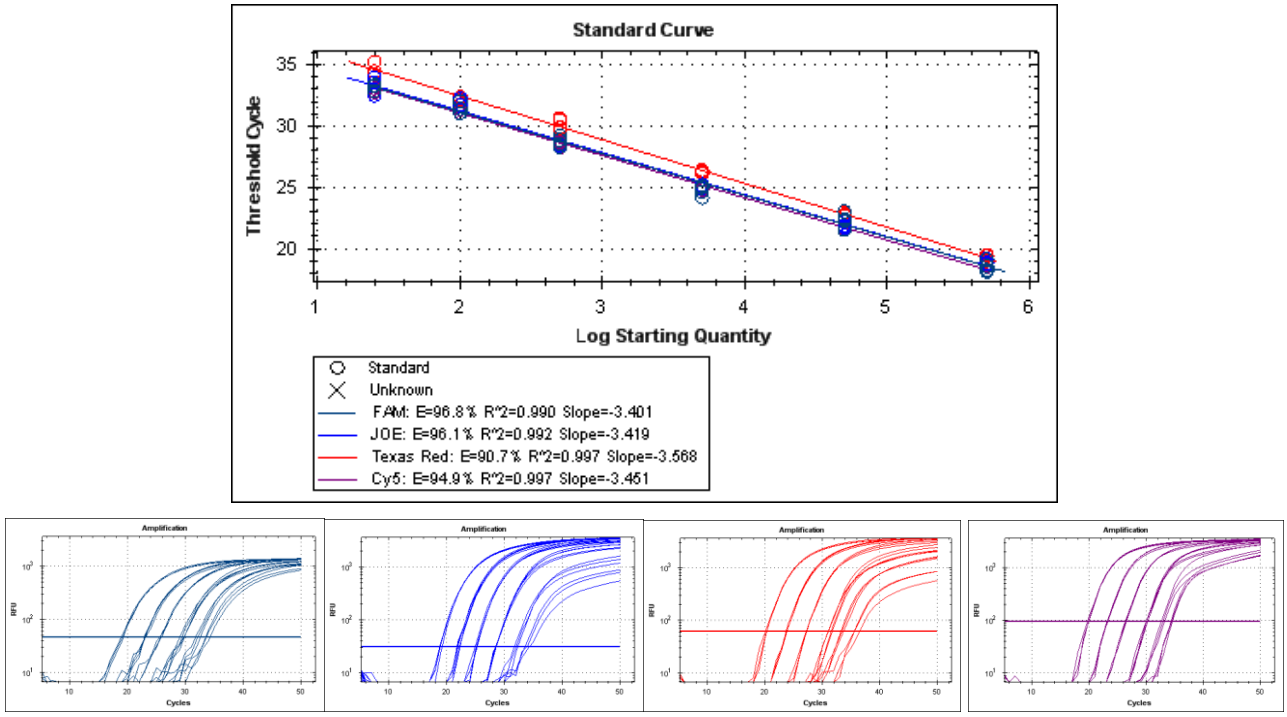
GLRaV-2 GLRaV-3 GVA GLRaV-1

Fig. 1. Determinazione del limite di rilevamento e dell'efficienza della PCR *real time* multiplex per l'analisi contemporanea di GLRaV-1 (marcato CY5), GLRaV-2 (marcato FAM), GLRaV-3 (marcato JOE) e GVA (marcato TEXAS RED).

Fig. 1. Analyses performed for assessing the limit of detection and the efficiency of multiplex real time PCR for the simultaneous analyses of GLRaV-1 (marked CY5), GLRaV-2 (marked FAM), GLRaV-3 (marked JOE) e GVA (marked TEXAS RED).

Per verificare le caratteristiche di ciascun metodo, prima sono state controllate le risposte agli 8 saggi singleplex, virus per virus; una volta accertata la validità dei saggi singleplex, si è proceduto a mettere a punto i due sistemi multiplex ed a verificarne l'affidabilità. Tutti i parametri tecnici intrinseci al sistema sono stati saggiati su campioni a concentrazione nota di virus, costruiti con *standard* plasmidici. I 150 campioni di vite infetti sono stati invece utilizzati per la comparazione con gli altri metodi, cioè con il metodo ELISA per 7 virus e con la RT-PCR convenzionale per GRSPaV.

Le prove effettuate con gli *standard* plasmidici per la verifica dell'efficienza dei vari metodi di *real time* non hanno evidenziato differenze significative tra gli esperimenti in PCR singleplex (dati non mostrati) e multiplex (Fig. 1 e 2). Tutti i saggi hanno evidenziato un'efficienza ottima, superiore al 94,9%, ad eccezione del saggio per GVA, la cui efficienza è risultata pari a 90,7%, valore ritenuto comunque buono ai fini dell'affidabilità del saggio. In tutti gli esperimenti il coefficiente di regressione R^2 è sempre risultato ottimo, uguale o superiore a 0,99.



GFkV GFLV ArMV GRSPaV

Fig. 2. Determinazione del limite di rilevamento e dell'efficienza della PCR *real time* multiplex per l'analisi contemporanea di ArMV (marcato TEXAS RED), GFLV (marcato JOE), GFkV (marcato FAM) e GRSPaV (marcato CY5).

Fig. 2. Analyses performed for assessing the limit of detection and the efficiency of multiplex real time PCR for the simultaneous analyses of ArMV (marked TEXAS RED), GFLV (marked JOE), GFkV (marked FAM) e GRSPaV (marked CY5).

Il limite di rilevamento (LOD) indica la sensibilità delle diverse PCR ed è stato calcolato come il numero più basso di copie di plasmide virale che ciascun saggio è capace di diagnosticare; minore è il numero di copie e quindi il LOD, tanto più sensibile è il metodo. Le prove hanno evidenziato in PCR singleplex risultati variabili da un minimo di 5 copie per GLRaV-2 e GRSPaV ad un massimo di 25 copie per tutti gli altri virus, ad eccezione di GLRaV-3, che si è attestato ad un LOD di 10 copie (Tab. 3). In PCR multiplex i risultati sono stati ottimi per 5 degli

Tab. 3. Determinazione del limite di rilevamento (LOD), espresso come numero di copie del plasmide contenente la sequenza virale, in esperimenti di PCR *real time* singleplex e multiplex.

Tab. 3. Limit of detection (LOD), expressed as number of viral plasmid copies, obtained in singleplex and multiplex real time assays.

	PCR *real time*	
Virus	**singleplex**	**multiplex**
Arabis Mosaic Virus	25	25
Grapevine Fanleaf Virus	25	25
Grapevine Leafroll associated Virus 1	25	25
Grapevine Leafroll associated Virus 2	5	25
Grapevine Leafroll associated Virus 3	10	50
Grapevine Fleck Virus	25	25
Grapevine Virus A	25	25
Grapevine Rupestris Stem Pitting associated Virus	5	25

8 virus, in cui il LOD è stato simile a quello dei saggi singleplex; nel caso di GLRaV-2 e GFkV la qualità è risultata leggermente inferiore, in quanto nessun dei due saggi è riuscito a rilevare con sufficiente ripetibilità meno di 25 copie; il saggio per GLRaV-3, infine, ha fornito i risultati meno buoni, con un valore di 50 copie.

Le analisi condotte su 150 accessioni di vite hanno dato risultati identici nei laboratori dell'ERSA ed in quelli del CRA-VIT, sia in sinlgeplex che in multiplex. Il confronto con i risultati ottenuti con il metodo ELISA, o con la RT-PCR convenzionale nel caso di GRSPaV, hanno permesso di evidenziare le caratteristiche di sensibilità, specificità e accuratezza dei sistemi di PCR *real time* (Tab. 4). Questi parametri, quindi, non misurano caratteristiche intrinseche dei saggi, ma li confrontano con un metodo di riferimento, ritenuto universalmente valido. Tali valutazioni non sono state estese ad ArMV per la disponibilità di soli 3 campioni positivi, che sono stati ritenuti insufficienti per la valutazione dei diversi parametri. Ugualmente, il valore di specificità di GRSPaV non è stato calcolato, dato il basso numero di campioni negativi analizzati, che inficerebbe la validità del dato. Tutti i valori di sensibilità misurati rispetto al test ELISA sono risultati superiori al 90% e, in particolare per GLRaV-1, GVA e GFKV, sono stati superiori al 95%.

Tab. 4. Valutazione della sensibilità, della specificità e dell'accuratezza dei saggi di PCR *real time* sviluppati in questo lavoro in confronto alla metodica di riferimento. PA: n. di campioni positivi in entrambi i metodi; NA: n. di campioni negativi in entrambi i metodi; PD: n. di campioni positivi in più rispetto al metodo di riferimento; ND: n. di campioni negativi in più rispetto al metodo di riferimento.

Tab. 4. Sensitivity, specificity and accuracy of real time PCR assays developed in the present work, in comparison with the reference method. PA. n. of samples positive with both methods; NA: n. of samples negative with both methods; PD: n. of samples positive only with the real time PCR; ND: n. of samples positive only with the reference method.

VIRUS	**PA**	**NA**	**PD**	**ND**	**Sensibilità**	**Specificità**	**Accuratezza**	**Metodo di riferimento**
GFLV	33	4	0	3	91,7%	100,0%	92,5%	ELISA
GLRaV-1	33	20	2	1	97,1%	90,9%	94,6%	ELISA
GLRaV-2	20	27	8	2	90,9%	77,1%	82,5%	ELISA
GLRaV-3	32	5	0	3	91,4%	100,0%	92,5%	ELISA
GFkV	11	25	2	0	100,0%	92,6%	94,7%	ELISA
GVA	21	14	8	1	95,5%	63,6%	79,5%	ELISA
GRSPaV	26	2	4	2	92,9%	-	82,4%	RT-PCR convenzionale

Ciò significa che solo pochi campioni positivi all'ELISA sono risultati negativi in *real time*; tali campioni sono stati per lo più quelli infetti con varianti virali divergenti. Questa differenza è da imputare alle diverse caratteristiche delle metodiche sierologiche e molecolari: infatti il metodo ELISA generalmente diagnostica uno spettro più ampio di varianti virali rispetto alle metodiche PCR, che sono invece basate sulle informazioni di sequenziamento delle banche dati, non sempre esaustive.

La specificità è stata caratterizzata da una maggiore variabilità tra i vari saggi, con differenze più marcate. In generale, i saggi di PCR *real time* hanno evidenziato un maggior numero di campioni positivi rispetto al totale dei campioni positivi in ELISA. Questo potrebbe essere dovuto alla maggiore capacità dei metodi molecolari, rispetto a quelli sierologici, di individuare i virus quando presenti a basse concentrazioni. I saggi *real time* per la diagnosi di GLRaV-3 e GFLV sono stati gli unici in cui gli stessi campioni sono risultati positivi sia in PCR *real time* che in ELISA e, per questo motivo, la specificità è risultata pari al 100%; dati precedenti ottenuti al CRA-VIT hanno sempre confermato l'alta affidabilità del saggio ELISA per questi due virus. Nei saggi per la diagnosi di GLRaV-2 e GVA ben 8 campioni, negativi in ELISA, sono risultati invece positivi in PCR; anche in questo caso, dati precedenti ottenuti dal CRA-VIT indicavano che il saggio ELISA per questi due virus non è sempre affidabile e varia moltissimo in funzione della matrice analizzata e del periodo di campionamento. Nel caso di GRSPaV sono stati identificati altri 4 campioni positivi, che erano negativi al saggio con la RT-PCR convenzionale. I valori di specificità sono quindi risultati bassi in alcuni casi (esempio 63% per GVA), ma ciò non implica forzatamente una minor validità dei nuovi saggi; indica piuttosto una discrepanza fra il metodo di riferimento ed i saggi *real time*, che verrà risolta inserendo nella comparazione anche i risultati di altri tipi di saggi, come la RT-PCR convenzionale ed i saggi biologici.

L'accuratezza, che è un parametro che unisce sensibilità e specificità, è risultata fra il 90 ed il 95% per GFLV, GLRaV-1, GLRaV-3 e GFkV; leggermente inferiore, intorno all'80%, per gli altri tre virus, a causa dei motivi già elencati.

CONCLUSIONI

Questo lavoro presenta la messa a punto e lo sviluppo di 8 saggi in PCR *real time* singleplex, che possono essere utilizzati anche in due sistemi di PCR *real time* multiplex, per la diagnosi dei principali virus della vite. I risultati ottenuti sono da considerarsi di base per ulteriori analisi, volte a valutare compiutamente alcuni valori dei parametri analizzati. I metodi sono stati saggiati con successo su un elevato numero di accessioni infette di vite, di svariata provenienza geografica, ed hanno evidenziato generalmente affidabilità molto buona rispetto ai metodi tradizionali basati sulla tecnica ELISA e, solo nel caso di GRSPaV, su RT-PCR convenzionale.

Le prove di confronto hanno evidenziato generalmente un maggior numero di campioni positivi rispetto alle analisi immunoenzimatiche, in particolare per GLRaV-2 e GVA e, seppure in misura minore, per GLRaV-1 e GFkV. I campioni discordanti dovranno essere analizzati nuovamente in condizioni ottimizzate, allo scopo di valutare se si tratta di falsi positivi in PCR *real time* o falsi negativi in ELISA, anche se il fatto che generalmente i metodi molecolari siano più sensibili di quelli sierologici fa propendere per la seconda ipotesi.

Nel caso di GRSPaV, in cui il confronto è stato fatto con un metodo convenzionale di RT-PCR di tipo qualitativo, sarà necessario continuare il confronto utilizzando un numero maggiore di campioni negativi, come per esempio materiale clonale risultato negativo al saggio biologico con gli indicatori cv. Rupestris du Lot e 110 Richter. Infine, il sistema *real time* per la diagnosi di ArMV, che però è un virus di diffusione molto limitata, potrà essere adeguatamente valutato solo in presenza di un maggior numero di accessioni positive da poter analizzare.

Tutti i saggi proposti hanno evidenziato ottime caratteristiche di efficienza e limiti di rilevamento molto bassi anche in condizioni di PCR *real time* multiplex. Per le analisi di *routine* ai fini certificativi e di controllo dei materiali di moltiplicazione della vite, i protocolli di PCR

real time multiplex sviluppati in questo lavoro si propongono quindi quale concreta alternativa alla tecnica ELISA tradizionalmente usata, grazie alle loro caratteristiche di rapidità, sensibilità e specificità. Purtroppo, al momento, i costi delle analisi effettuate con tecniche molecolari sono maggiori di quelli del saggio ELISA, ma tali differenze sono comunque destinate a diminuire in un prossimo futuro.

Inoltre, grazie alla disponibilità di *standard* plasmidici di riferimento, i nuovi sistemi di PCR *real time* sviluppati in questo lavoro possono essere utilizzati anche per studi e valutazioni di tipo quantitativo della carica virale.

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BIBLIOGRAFIA

AA.VV., 2010. Specific requirements for laboratories preparing accreditation for a plant pest diagnostic activity. *OEPP/EPPO Bull*, 40: 5-22.

Applied Biosystems, 2003. Creating standard curves with genomic DNA or plasmid DNA templates for use in quantitative PCR. https://www.appliedbiosystems.com/support/tutorials/pdf/quant_pcr.pdf.

Bertazzon N., Bazzo I., Repetto O., Borgo M., Angelini E., 2009. Advances in molecular detection of GLRaV-1. *Le progrès Agricole et Viticole*, hors série, spécial Congrès ICVG, 294-295.

Bertazzon N., Borgo M., Vanin S., Angelini E., 2010. Genetic variability and pathological properties of Grapevine Leafroll-associated Virus 2 isolates. *Eur J Plant Pathol*, DOI: 10.1007/s10658-010-9583-3.

Borgo M., Bertazzon N., Anaclerio F., Angelini E., 2009. Different variants of GRSPaV are associated to diverse diseases in grapevine. *Le progrès Agricole et Viticole*, hors série, spécial Congrès ICVG, 327-328.

Bouyahia H., Boscia D., Savino V., La Notte P., Pirolo C., Castellano M.A., Minafra A., Martelli G.P., 2005. Grapevine rupestris stem pitting associated virus is linked with grapevine vein necrosis. *Vitis*, 44: 133-137.

MacKenzie D. J., McLean M. A., Mukerji S., Green M., 1997. Improved RNA extraction from woody plants for the detection of viral pathogens by reverse transcription-polymerase chain reaction. *Plant Dis*, 81: 222-226.

Ministero delle Politiche Agricole Alimentari e Forestali, 2006. Recepimento della direttiva n. 2005/43/CE della Commisisione del 23 giugno 20005, che modifica gli allegati della direttiva n. 68/193/CE del Consiglio, relativa alla commercializzazione dei materiali di moltiplicazione vegetativa della vite. *GU Repubbl Italiana, Serie Generale*, 211: 17-20.

Ministero delle Politiche Agricole Alimentari e Forestali, 2008. Modifica del protocoolo tecnico di selezione clonale della vite. *GU Repubbl Italiana, Serie Generale*, 195: 5-7.

Osman F., Leutenegger C., Golino D., Rowhani A., 2007. Real time RT-PCR (TaqMan) assays for the detection of Grapevine Leafroll associated viruses 1-5 and 9. *J Virol Methods*, 141: 22-29.

Osman F., Rowhani A., 2008. Real time RT-PCR (TaqMan) assays for the detection of viruses associated with rugose wood complex of grapevine. *J Virol Methods*, 154: 69-75.

Osman F., Leutenegger C., Golino D., Rowhani A., 2008. Comparison of low-density arrays, RT-PCR and real time TaqMan RT-PCR in detection of grapevine viruses. *J Virol Methods*, 149: 292-299.

Vallone P.M., Butler J.M., 2004. Autodimer: a screening tool for primer-dimer and hairpin structures. *Biotechniques*, 37: 226-231.

Aspekte der Klimaänderung und Konsequenzen für den Pflanzenschutz im österreichischen Weinbau

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ABSTRAKT

Die Ausdehnung der Weinbauregionen ist durch die klimatischen Anforderungen von Wein begrenzt. Der Temperatursummenbedarf wird durch den Huglin-Index beschrieben, der erheblich zwischen verschiedenen Sorten variiert. Österreichs Wein wird im Osten in semi-ariden Regionen, teilweise in Hanglagen und im Flachland, sowie im Südosten im semi-humiden Klimagebiet angebaut. Die österreichischen Weinbaugebiete waren, historisch gesehen, in ihrer Ausbreitung immer durch niedrige Temperaturen limitiert. Risikofaktoren für die Traubenerträge in der Vergangenheit waren kühle und feuchte Sommermonate oder sehr tiefe Temperaturen im Februar. Klimamodelle prognostizieren einen Erwärmungstrend und wärmere Sommermonate, wodurch die wesentlichsten Risikofaktoren für Missernten seltener werden. Begünstigt durch den Klimawandel treten aber neue Krankheiten und Schädlinge auf, die wiederum neue Pflanzenschutzstrategien erfordern. In diesem Projekt wurden Warnmodelle mit den üblichen Pflanzenschutzstrategien verglichen und für den Einsatz im Risikomanagement geprüft.

The extension of winegrowing regions is confined by the thermal requirements of grapevine. Temperature requirements are characterised by the Huglin-index that varies significantly between different cultivars. In Austria viticulture is limited to the warmest regions in the Eastern and South-Eastern part of the country with semi-arid to semi-humid climates. Historically, in Austria grape cultivars with relatively low temperature requirements are grown in the majority of the winegrowing regions. An assessment of Austrian grape yields in past decades has shown that in years when February was unusually cold or when the summer months were both cool and wet, yield depressions have occurred. Therefore future climate scenarios that forecast a warming trend and drier summer months will reduce the vulnerability of viniculture because the weather extremes related to the yield reductions will occur less frequently. However, the risk of new pests and diseases has to be considered and may require new plant protection strategies. In this project disease models were tested and compared with the usual plant protection strategies to improve the phytopathological risk management.

La extensión de las regiones vitícolas está limitado por las exigencias climáticas del vino.
El índice de posibilidades heliotérmicas de Huglin es diferente según el tipo de cultivo y determina la temperatura idónea para cada caso. El vino austríaco se cultiva principalmente en dos regiones: en la zona semiárica del este (en terrazas y llanos) y en la zona semihúmeda del oeste. Las zonas vitícolas austríacas han estado siempre limitadas por las bajas temperaturas. Los factores de riesgo en el pasado han sido: veranos fríos y húmedos y temperaturas muy bajas en febrero. Los modelos climáticos pronostican un calentamiento y por tanto veranos más cálidos, lo que implica que los factores de riesgo sean menores. Pero por otra parte y debido a este cambio climático también aparecen nuevas enfermedades y nuevos parásitos

que exigen nuevas estrategias en el uso de los productos fitosanitarios. En este proyecto se han comparado modelos nuevos en la aplicación de los productos fitosanitarios con modelos normales. También se han probado dichos modelos nuevos en situaciones de riesgo.

EINFÜHRUNG

Regionale Klimamodelle prognostizieren für den Osten Österreichs in den nächsten 50 Jahren einen weiteren Temperaturanstieg und eine Verschiebung der Niederschläge vom Sommer- ins Winterhalbjahr bei ungefähr gleich bleibenden Jahressummen. Inwieweit diese Prognosen bereits anhand der Aufzeichnungen der vergangenen Jahrzehnte nachvollziehbar sind, gehörte zu den Untersuchungsthemen dieser Studie. Temperaturerhöhungen sind im globalen Mittel die bislang am eindeutigsten nachweisbaren Begleiterscheinungen des Klimawandels und zeigen sich im Weinbau durch deutliche Verfrühungen bei Blüh- und Ernteterminen (Bauer und Fardossi, 2008).
Trotz der nur relativ kurzen Auswertungsperiode der Wetterbedingungen von 38 Jahren zeigte sich für diesen Zeitraum eine hochsignifikante Temperaturzunahme, die bei Minimum-, Mittel- und Maximumtemperaturen ähnlich ausgefallen ist und etwa 1,0-1,5 °C beträgt (Abb.1). Die beobachteten phänologischen Veränderungen fanden somit in der gemessenen Temperaturerhöhung ihr Gegenstück. Die dadurch immer früher erfolgende Abdeckung des minimalen Wärmebedarfes der traditionellen Hauptsorten Riesling und Grüner Veltliner wirkt sich dadurch auf eine Verlegung der Traubenausreifung in eine Jahreszeit aus, welche durch höhere Nachttemperaturen und einen schnelleren Säureabbau gekennzeichnet ist.

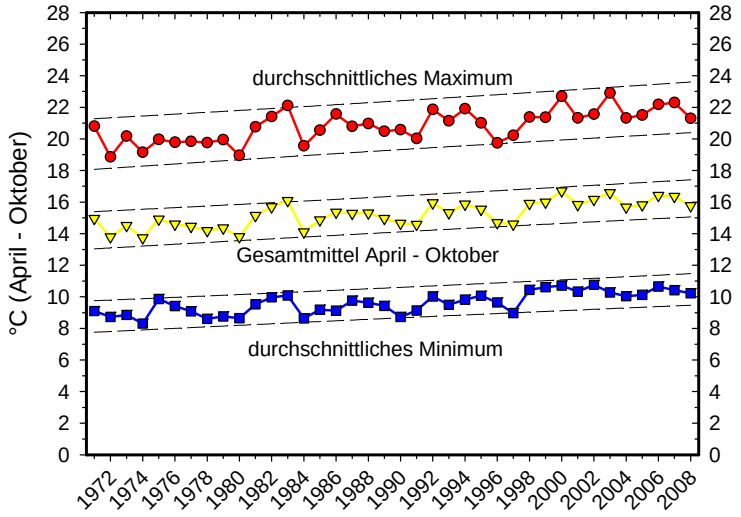

Abb.1: Temperaturtrends der meteorologischen Station Krems 1971-2008 für die Vegetationsperiode April bis Oktober. Jeder Parameter wird vom 95%-Vertrauensintervall der Einzelwerte umgrenzt. Homogenisierung der Daten auf Basis des HISTALP-Datensatzes (http://www.zamg.ac.at/histalp/; Böhm et al., 2009)

Die genauere zeitliche Auflösung des Temperaturzunahmetrends enthüllt eine ungleichmäßige Erwärmung zu verschiedenen Jahreszeiten.
Während die Temperaturszenarien regionaler Klimamodelle sich offenbar bereits in der jüngeren Vergangenheit realisiert haben, lassen sich aus dem Niederschlagsverhalten der letzen Jahrzehnte die erwarteten Veränderungen noch nicht ablesen. Die Niederschlagssummen während der Vegetationsperiode haben im Zeitraum 1971-2008 hingegen sogar einen schwachen, marginal signifikanten Trend der Zunahme erkennen lassen (Abb.2). Dargestellt ist hier nicht der Gesamtniederschlag, sondern der bodenhydrologisch wesentlich relevantere, um die oberirdischen Verluste (Abfluss, Dachwirkung der Blätter) bereinigte nutzbare Niederschlag. Der Wasserbedarf jeder für die Pflanzenproduktion

genutzten Fläche setzt sich aus der Verdunstung des Bodens und der Transpiration der Pflanzenblätter zusammen. Ist dieser als Evapotranspiration bezeichnete Wasserbedarf höher als die nutzbare Niederschlagsmenge, trocknet der Boden aus. Dieses Bodenwasserdefizit ist für den ostösterreichischen Raum nichts Unübliches und wird durch die Niederschläge des Winterhalbjahrs wieder aufgefüllt. Der in Abb.2 dargestellte Langfristtrend des Bodenwasserdefizits (untere Kurve) zeigt eine geringere Abnahme als die Zunahme des Niederschlags, da die Veränderungen sonstiger meteorologischer Parameter eine Erhöhung der Evapotranspiration fördern. Sollte sich somit der Trend zunehmender Niederschläge in den nächsten Jahrzehnten wieder umkehren (was auf Basis der regionalen Klimamodelle als nicht unwahrscheinlich anzusehen ist), ist daher eine Verschärfung der sommerlichen Trockensituation zu erwarten.

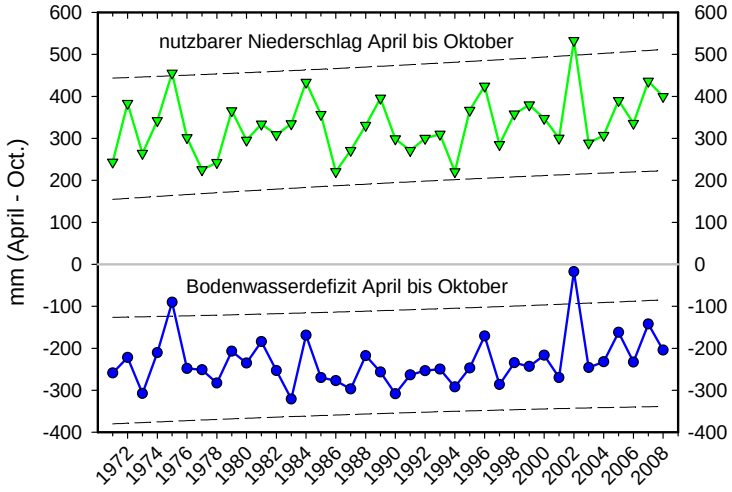

Abb.2: Verlauf der Niederschlagsentwicklung (April bis Oktober) in der Region Krems in den Jahren 1971-2008. Der nutzbare Niederschlag entspricht dem Gesamtniederschlag vermindert um Oberflächenabfluss und Interzeption durch die Vegetation. Das Bodenwasserdefizit wurde als nutzbarer Niederschlag minus Bestandes-Evapotranspiration (nach Allen et al., 1998) durchschnittlicher Weingärten (ohne Begrünung) berechnet.

Das Auftreten von Pilzkrankheiten hängt von den kleinräumigen Witterungs-Bedingungen ab, welche die Sporenverbreitung und Infektion beeinflussen. Veränderungen im Klima werden sich auf die Verbreitungsrisiken und den Einsatzbedarf von Fungiziden auswirken. Diese Abhängigkeiten wurden sowohl für Oidium (Willocquet und Clerjeau, 1998; Jailloux et al., 1999) als auch für Peronospora (Seem, 2004; Salinari et al., 2004) quantifiziert. Beide Pilzkrankheiten sind in Österreich relevant und verursachen erheblichen Pflanzenschutzmittelverbrauch. Die Modellierung der Infektionsgefahr hängt wesentlich von verlässlichen Daten der Blattfeuchte ab (Dalla Marta et al., 2005), welche in Abhängigkeit von den meteorologischen Umgebungsbedingungen für Warndienste eingesetzt werden können (Sentelhas et al., 2006).

Der Schwerpunkt der Studie lag in der Untersuchung der Nachhaltigkeit und der Einsparungsmöglichkeit von Pflanzenschutzmaßnahmen. Dabei sollten im Sinne eines integrativen Pflanzenschutzes insbesondere die Einsatzmöglichkeiten eines Frühwarnsystems und in Folge eine Verringerung der Fungizidanwendungen untersucht werden. Wünschenswert ist eine höhere Treffsicherheit des Erkennens von Anlassfällen. Durch das Auftreten von Schädlingen und Krankheiten wie Peronospora, Oidium und Botrytis ist der Pflanzenschutz eine große Herausforderung und kann nur schwer auf chemische Hilfsmittel verzichten. Aus Umweltschutzgründen und den hohen Produktionskosten sind viele Betriebe dazu bereit, den Betrieb auf Nachhaltigkeit auszurichten. Das zunehmende ökologische Wissen, ein härteres ökonomisches Umfeld und die Forderungen der Konsumenten nach einer

weiteren Reduktion des Pflanzenschutzes zwingen den heutigen modernen Weinbaubetrieb, sein Pflanzenschutzprogramm mehr und mehr zu optimieren. Eine Reduzierung des Pflanzenschutzes bedeutet für die Winzerinnen und Winzer eine genaue und optimierte Spritzung beim Auftreten des Schädlings oder der Krankheit. Um beim Abbau der Bekämpfungsintensität die Gefahr von Ertragsverlusten zu vermindern, sind moderne Vorhersagen über den Zeitpunkt und die Stärke des Auftretens von Schadorganismen notwendig. Diese Prognosen sind durch Informationen über die Überwachungsmethoden und Empfehlungen angepasster Bekämpfungsmaßnahmen zu ergänzen.

MATERIAL UND METHODEN

Die Methodik der regionalen Erfassung eines vorhandenen Befallsdruckes beruhte auf einer empirischen Erhebung mittels Fragebogen (n= 100). Damit wurden die im Vordergrund stehenden Pflanzenschutzprobleme, die angewendeten Pflanzenschutzmaßnahmen und das diesbezügliche Fachwissen der Winzerinnen und Winzer abgefragt. Für den Vergleich der Warnprognosen und der Spritzungen wurden ein biologisch geführter Weingarten und ein Weingarten, der nach integrierter Produktion bewirtschaftet wird, herangezogen. Die Erhebungen erfolgten über 3 Jahre. Die Wetterstationen mit den programmierten Warnmodellen wurden in der Nähe der beiden Weingärten platziert, die Daten konnten verglichen werden, ohne einen Einfluss unterschiedlicher Witterung zu befürchten. Die Wetterstationen wurden von der Firma Adcon Telemetry zur Verfügung gestellt und nach vorgeschriebenen Standardwerten der Prognosemodelle aufgestellt. Über diese Station wurden die einzelnen Wetterdaten gemessen und an das Programm addVANTAGEPRO 5.0 gesendet.

ERGEBNISSE

Bei der Befragung der Winzerinnen wurden als häufigsten Schaderreger, die regional und saisonal auftreten, Peronospora, Oidium und Botrytis, genannt. Ein geringeres Problem stellen Roter Brenner und Phomopsis dar (Abb.3). Diese Schaderreger werden im Durchschnitt mit 7 bis 8 Spritzungen bekämpft, wobei die Botrytisspritzung hauptsächlich separat unter besonderer Berücksichtigung der Traubenzone und besonders häufig beim Traubenschluss durchgeführt wird.

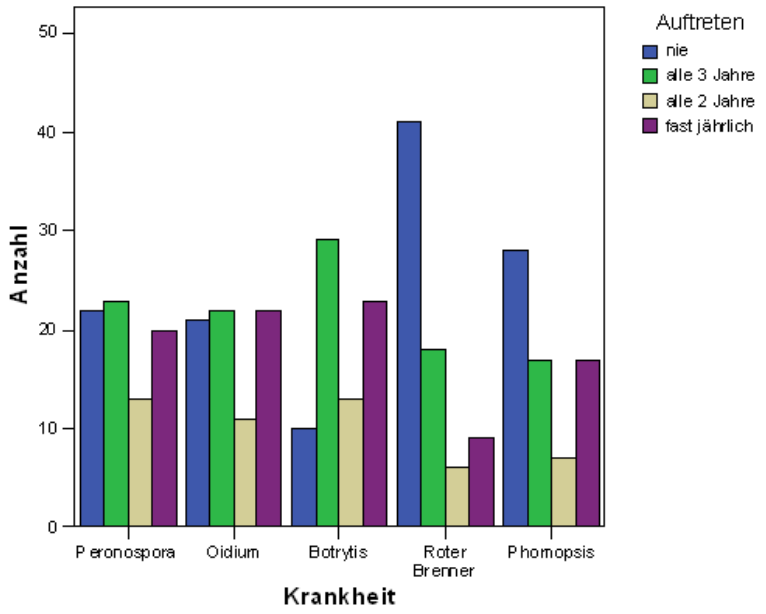

Abb.3: Umfrageergebnisse über das Auftreten von Pilzkrankheiten in Traisentaler Weingärten.

Zwecks Prüfung eines Frühwarnsystems wurden verschiedene Warnmodelle in Kombination mit den jeweiligen Pflanzenschutzmaßnahmen über drei Jahre kontrolliert. Es zeigte sich, dass der Einsatz der Maßnahmen sehr wetterabhängig ist und weniger eine Reduzierung, sondern eher eine zeitliche Optimierung des Pflanzenschutzmitteleinsatzes genau zum Zeitpunkt des Auftretens möglich ist (Abb.4).

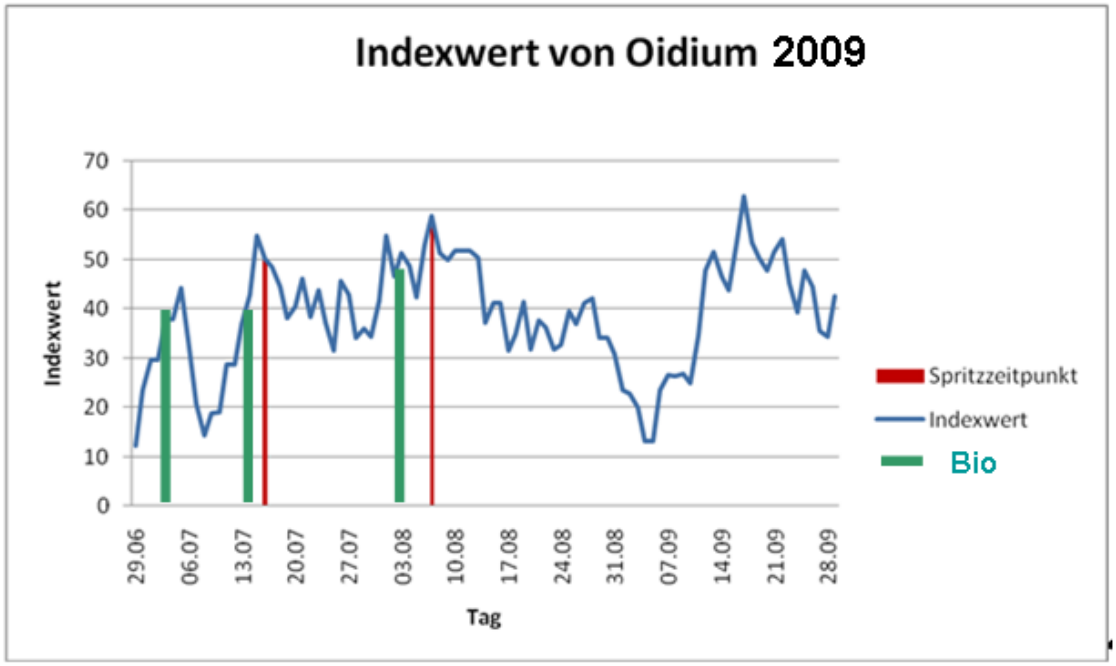

Abb.4: Verlauf der Oidium-Infektionsgefahr und der durchgeführten Pflanzenschutzmaßnahmen 2009 im Traisental.

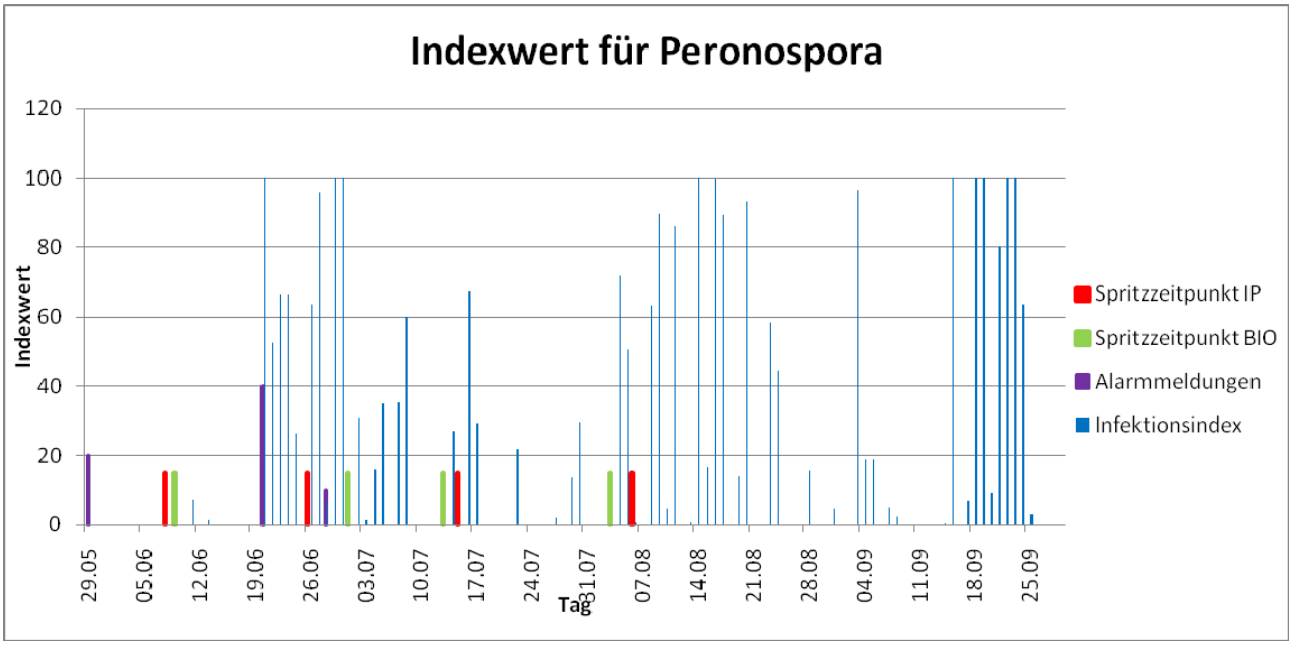

Abb.5: Verlauf der Peronospora-Infektionsgefahr und der durchgeführten Pflanzenschutzmaßnahmen 2009

Als ein Beispiel, dass für die gleiche Methode der anderen Jahre stehen soll, wird Abb.5 herangezogen, das die Prognose des Peronospora-Auftretens zeigt, die eingetragenen Spritzzeitpunkte von IP und Bio und die Alarmmeldungen bei hohem Infektionsrisiko. Am 29. Mai 2009 ging von der Prognose eine Meldung der Oosporenkeimung aus und somit eine

Gefahr der Infektion. Am Beginn vom Juni wurde im IP sowie im BIO eine Spritzung durchgeführt. Hier war keine Gefahr einer Infektion gegeben und daher ist die Wirkung des Wirkstoffes nicht gezielt ausgebracht worden. Erst am 20. Juni 2009 empfiehlt das Programm eine Behandlung. Hier war der Indexwert sehr hoch und die Spritzungen hätten hier angelegt gehört um eine Wirkung zu erzielen. Die nächsten Spritzungen IP und BIO wurden um die Alarmmeldung vom 28. Juni 2009 ausgebracht. Der Indexwert im August war sehr hoch und die Gefahr einer Infektion war gegeben. Die Spritzungen IP und BIO wurden in dieser Zeit gut angelegt, um eine Infektion zu behandeln.

DISKUSSION

Jedes Jahr bringt andere Extreme mit sich. In manchen Jahren gibt es vermehrt Probleme mit Oidium und in anderen Jahren mit Botrytis oder Peronospora. Je nach Stärke und Ausprägung der Witterung verhalten sich die Krankheitsanfälle anders. Aufgrund der Ergebnisse der Prognosen im Vergleich zu der Witterung in den drei Jahren, kann davon ausgegangen werden, dass Warnmodelle den Befallsdruck anhand von Wetterdaten prognostizieren können und dadurch die Möglichkeit für den Winzer besteht eine gezielte Maßnahme gegen die Krankheit vorzunehmen. Es ist aus dem Vergleich der Prognosen von 2007, 2008 und 2009 ersichtlich, dass bei optimalen Bedingungen für die Krankheit sich auch der Indexwert entsprechend stark ändert.
Die Pilzkrankheiten sind sehr stark von den Witterungsverhältnissen abhängig. Der Echte Mehltau (Oidium) zeigt hohe Indexwerte und daher einen hohen Befallsdruck bei einer hohen Temperatur und Luftfeuchtigkeit. Ab dem Zeitpunkt einer Nässe sind die Bedingungen für eine Vermehrung des Oidiumpilzes schlecht. (Kast, K. W. 2009) Das warme und trockene Jahr 2007 war ein Beispiel für die optimalen Bedingungen. Die warmen und niederschlagsarmen Monate Juni Juli und August waren für die Entwicklung des Oidiumpilzes gute Bedingungen. Der Falsche Mehltau (Peronospora), weist hingegen erst optimale Bedingungen bei Nässe auf. Das niederschlagsreiche und warme Jahr 2008 war ein Peronospora-Jahr. Die Indexwerte für Sporulation und Infektion zeigten eindeutig hohe Werte im Jahr 2008. Botrytis kam in diesem Jahr bei niedrigeren Temperaturen und bei hoher Blattnässe verstärkt vor. Das Jahr 2009 brachte viele verschiedene extreme Situationen bezüglich der Witterung sowie den Krankheitsauftreten mit sich. In den Herbst hinein wurden die Niederschläge wieder schwächer.
Die Warnmodelle sind auf die klimatischen Bedingungen angewiesen und berechnenden tatsächlichen Infektionsindex mathematisch (Lind K. 2005). Die klimatischen Bedingungen werden von den Modellen aufgenommen und einberechnet, um den ersten Pollenflug zu prognostizieren. Die Prognosefenster zeigen wie klein das Zeitfenster für das Auftreten der Krankheit und für eine Bekämpfung ist. Je nach Witterung ist das Zeitfenster unterschiedlich groß. Die Prognosen befinden sich ca. in einen Zeitrahmen von 4 -5 Monaten. Dies ist in etwa der Zeitraum zwischen Blüte der Gescheine und somit den Zeitpunkt der Primärinfektion und der Ernte. Eine erste Sporulation oder eine erstes Anzeichen für einen Infektionsdruck einer Krankheit ist der Zeitpunkt einer Warnmeldung. Infektionen nach der Ernte haben keine wirtschaftliche Bedeutung mehr und werden daher auch nicht berücksichtigt.
Der Vergleich der Prognosen mit den Spritzungen aus den Versuchsanlagen zeigte, dass viele Spritzungen in einer Zeit getätigt worden sind, wo kein hoher Infektionsbefall vorherrschte. Es kann daraus verdeutlicht werden, dass die Spritzungen nicht alle exakt zu einem richtigen Zeitpunkt eingesetzt wurden. Durch den Einsatz eines Warnmodells könnten die Zeitpunkte für den Einsatz von Pflanzenschutzmitteln bestimmt werden. Dadurch könnten Fungizidspritzungen optimiert beziehungsweise dezimiert werden, die zu einer Verringerung der Umweltbelastung führen. Zum Anderen könnte der Winzer finanzielle Einsparungen bei

der Produktion haben. Betriebe mit ökologischer Produktion haben nach den Richtlinien eine eingeschränkte Verwendung von Pflanzenschutzmitteln zu arbeiten.
Diese Betriebe dürfen bei bestimmten Mitteln die Höchstmenge nicht überschreiten und haben somit die Möglichkeit durch die Wetterstation genau dezimierte Spritzungen durchzuführen ohne die höchst zugelassene Menge zu überschreiten. Der Integrierte Pflanzenschutz verfolgt verstärkt die Ziele der Nachhaltigkeit im Weinbau. Nur die chemischen Pflanzenschutzmaßnahmen, die wirklich nötig sind könnten durch den Einsatz von solchen Warnsystemen ergänzt werden. Dies wäre die Basis für eine erfolgreiche und effiziente Bekämpfung von Schaderregern.
Der Einsatz der Wetterstation und der Prognosemodelle verlangt Erfahrung und nimmt viel Zeit in Anspruch. Für die Praxistauglichkeit nimmt die Wetterstation viele Ansprüche wie die Wartung der einzelnen Sensoren, um genaue Werte und dadurch die richtige Prognose zu erhalten, mit sich (Hopmann D, Dannecker H.W, 1992) Für die Darstellung und Warnmeldungen müssen die Warnmodelle für die jeweilige Krankheit am Programm erstellt und immer wieder kontrolliert werden. Die einzelnen zugelassenen Pflanzenschutzmittel müssen in die Chemikalienliste eingetragen werden um bei einer Behandlung dieses Mittel bei der Prognose mit einzuberechnen. Werden Behandlungen nicht eingetragen so können auch keine genauen Berechnungen in Bezug mit den Prognosewerten genommen werden. Dies in die Praxis umzusetzen wird noch einige Anfangsprobleme mit sich bringen, dadurch die Ergebnisse nicht klar sind, ob die einzelnen Spritzungen genau zur gleichen Zeit ausgebracht werden oder diese immer einzeln bekämpft werden müssen.

BIBLIOGRAPHIE

Bauer, K., Fardossi, A. (2008) Herausforderung der Zukunft. Auswirkungen des Klimawandels, Teil 1. Der Winzer, 4.5.2008.
Böhm, R., Auer, I., Schöner, W., Ganekind, M., Gruber, C., Jurkovic, A., Orlik, A., Ungersböck, M. (2009) Eine neue Webseite mit instrumentellen Qualitäts-Klimadaten für den Großraum Alpen zurück bis 1760. Wiener Mitteilungen Band 216: Hochwässer: Bemessung, Risikoanalyse und Vorhersage.
Hopmann, D und Dannecker H.W(1992), Wetterautomaten im Weinbau: Anspruch und Wirklichkeit, Der Deutsche Weinbau 04/1992, S.390-393
Jailloux F, Willocquet L, Chapuis L, Froidefond G (1999) Effect of weather factors on the release of ascospores of Uncinula necator, the cause of grape powdery mildew, in the Bordeaux region. Canadian Journal of Botany-Revue Canadienne de Botanique 77: 1044-1051
Kast W (2009) Oidium-Bekämpfung mit EDV-Unterstützung, Der Deutsche Weinbau 03/2009, S.28-31
Lind K (2005) Pflanzenschutz-Prüfeinrichtungen und Wetterstationen, Technik im Obst- und Weinbau, S.36
Salinari F, Giosue s, Tubiello FN, Rettori A, Rossi V, Spanna F, Rosenzweig C, Gullino ML (2006) Downy mildew (Plasmopara viticola) epidemics on grapevine under climate change. Global Change Biology 12: 1299-1307
Seem RC (2204) Forecasting plant disease in a changing climate: a question of scale. Canadian Journal of Plant Pathology-Revue Canadienne de Phytopathologie 26: 274-283
Sentelhas PC, Gillespie TJ, Gleason ML, Eduardo J, Monteiro BM, Pezzopane JRM, Pedro MJ (2006) Evaluation of a Penman-Monteith approach to provide “reference” and crop canopy leaf wetness duration estimates. Agricultural and Forest Meteorology 141:105-117
Willocquet L, Clerjeau M (1998) An analysis of the effects of environmental factors on conidial dispersal of Uncinula necator (grape powdery mildew) in vineyards. Plant Pathology 47: 227-233

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Biophysical Methods for Express Diagnosis of the Physiological Status of Plants and it`s Aplication for the Technological Improvement in the Replanting Materials Production

ABSTRACT

It has been studied and experimentally proved the bioelectric characteristics of plants (on the example of grapevines and citrus) in relation to their physiological and pathological state, external factors, time, biostumulation and curative dosages. Defined is also the correlative dependence of plants' activity on the level of diseases and frost damage, level of elecrtophysical influence, change of vegetation period and external factors. It has been defined experimentally and proved theoretically that a correlative indicator of physiological status of plants is a constant (invariable) figure and does not depend on the time of measuring or vegetation period. Defined is interrelation of biophysical indicators of vine graftining components with their anatomical peculiarities, levels of maturity and damage from frost. On the example of citrus plants elaborated is the bioelectric potential and impedance defining method for plants under vegetation, their root system, separated grafting components and seedlings.

Biophysische Methoden für Expressdiagnostik des physiologischen Zustandes der Pflanzen und ihre Anwendung für technologische Vervollkommnung von Herstellung der Pflanzlinge von Weinreben

Zusammenfassung

Experimental sind bioelektrische Kennwerte von Pflanzen unter Berücksichtigung von ihren physiologischen und pathologischen Zustanden, externen Effekten, Biostimulation und Behandlungsdosen studiert. Nachgewiesen ist der Korrelationszusammenhang der bioelektrischen Aktivität der Pflanzen zum Grad von Verletzung infolge Erkrankung und Frost, mit den Dosen von elektrophysischer Wirkung, mit Vegetationsperiode und mit dem Wechsel des Aussenfaktoren. Nachgewiesen ist das Verhältnis der bioelektrischen Kennwerte von Propfkomponenten der Weinrebe zu ihren

anatomischen Eigenschaften, zur Tauglichkeit des Gutes zum Impfen, zu Kennwerten von Reife und Verletzung durch Frost. Erarbeitet sind die Methoden für Bestimmung der Biopotenziale und Impedanz von vegetierenden Pflanzen, Wurzelsystem, Impfkomponenten und der Pflanzlinge.

Introduction

Contemporary plant growing, like specific technologies as a whole, require accurate definition of physiological and pathological status of plants without destroying integrity of bioobjects exactly within the vegetation period and identifies the necessity for elaboration of express diagnostic experimental methods. Noteworthy is the fact, that whilst the existing express diagnostic methods are based on visual observations, they are so far the only simple way to control the technological process, though rough and producing not quite objective results.

Relatively accurate biochemical, cytological and other methods require complex laboratory equipment and involvement of highly qualified specialists and therefore are not suitable for field work and for obtaining information from bioorganisms in conducting express analysis. For the assessment of plants' physiological and pathological status in laboratory practice it is often used the electrical method (chemiluminescence's method, etc.) for the data receipt and control. However, due to various reasons (non transportability of equipment and complexity of their application, etc.) utilization of this equipment in agrarian plant cultivation process is rather limited.

Material and Methods

As of today, it is defined that biophysical indicators (biopotentials and impedance), as effective means for objective express assessment of physiological condition of plants can provide information directly from plants without destroying the wholeness of the organism. Correspondingly, it is essential to elaborate such diagnostic methods and implementation of portative electro technical equipments in science researches and practical utilization.Their application will speed up diseases diagnostics and selection process, simplify definition of effective dosing for healing and feeding materials of new generation, promote flexible operation of plant protection and quarantine services, improve abilities of automation of

experimental research and microclimate regulation systems (in which the plant itself act as a sensor).

Based on our conducted researches and literature data we have elaborated and improved common methodology for measuring bioelectrical activity of vegetative plants and their organs, which minimizes measurement errors and diagnostic inaccuracy.

Results and Discussion

We have studied experimentally the bioelectric characteristics of plants (on the example of grapevines and citrus) in relation to their physiological and pathological state, external factors, time, biostumulation and curative dosages. Defined is also the correlative dependence of plants' activity on the level of diseases and frost damage, level of elecrtophysical influence, change of vegetation period and external factors.

We have defined experimentally and proved theoretically that a correlative indicator of physiological status of plants is a constant (invariable) figure and does not depend on the time of measuring or vegetation period.

On the example of citrus plants elaborated is the method defining bioelectric potential and impedance of plants under vegetation, their root system, separate grafting components and seedlings. We have started definition in the relation between biophysical indicators (bioelectric potentials, response bioelectric reaction and impedance) of vine graftining components with their specific anatomical peculiarities, levels of maturity and damage from frost.

Technical requirements for portable field equipment for measuring bioelectricity of plants have been specified and justified are possibilities to provide longer control over the express analysis of plants by means of these equipment. We also elaborated original experimental smaples of equipment and transmitters.

We have elaborated technical requirements for the impedance frequency dispersion depicting device, method for frequency dispersion definition, determined algorithm determining active and reactive components of impedance, constructed experimental sample of the device.

We have also developed automatic electrophitodiagnostic information and measuring system (registration and control of electrophysiological parameters of plants, environment and leaf diagnostics) and multifactor research method, which allows receipt of complex information from plants and environment simultaneously, selection and elaboration of express diagnostic

appropriate method for information parameter (or combination of parameters) in various technological process.

Electrical methods for technological improvement of vine sibling production is based on original methods of bioelectrical activity (biopotencials responsively reaction on irritation through electric impulse, impedance frequency disperse within wide range from 100hz to 100 Mhz) of rut system and separate parts of plants and their modification taking into consideration specific anatomic and other peculiarities of vine plant, the research object.

Conclusions

Results received based on our experimental researches on vine seedlings and subtropic plants, guarantee that elaboration and utilization of express diagnostic method and portative equipments in production of vine seedlings (determination of physiological condition of grafting components and engrafts, phyto-sanitary control, determination, prophylactics and therapy of virus infection, also determination efficiency of new generation biostimulators for raising reliability of seedlings) will significantly improve moden vine production technology, have a positive impact on the solution of existing problems in the sector and reduction of attacks from activated viruses and other diseases. In the future, the technology worked out under the proposed project will help farmers to produce high quality competitive product, enter new markets for seedlings and ensure sustainable development of their business activities.

Introduction of elaborated methods will speed up process for establishing effective dosage of mineral feeding, prophylactic, therapeutic and biostimulative influence of plants.

Bibliography

Bichiashvili T, Canava V, Solovev E, Marichev G (1990) Set of electrophysiological and electrochemical diagnostic methods for diagnosing physiological condition of subtropics plants. Collection – Biophysics of Plants and Phytomonitoring. Agrophysics Research Institute, Sankt-Peterburg, pp 128-139

Bichiashvili T, Tvalashvili O (1990) Frequency Dispersion Impedance Measuring Device and algorithms for calculating its components. Collection – Biophysics of Plants and Phytomonitoring. Agrophysics Research Institute, Sankt-Peterburg, pp 230-235

Bichiashvili T (1990) Method for defining damage of citrus plantation by *Phoma tracheiphila.* Invention, Copyright Certificate №1589435. Moscow

Bichiashvili T (1990) Equipment defining physiological state of plants. Invention, Copyright Certificate №1617685, Moscow

Bichiashvili T, Didebulidze K (1986) Device removing bioelectric potential from plant organisms. Invention, Copyright Certificate №126929, Moscow

Bichiashvili T, Didebulidze K (1986) Method for defining Damage of Plants. Invention, Copyright Certificate №1436934. Moscow.

Bichiashvili T (1990) Method for defining the condition of repair-trimming work for tea-plant. Invention, Copyright Certificate № 1584792. Moscow.

Bichiashvili T (2003) „Production, Demonstration and spreading of Tavkveri and some indigenous varieties seedlings in Mukhrani Valley".Methodology Instructions, Tbilisi, 24p

Bichiashvili T, Mchedlishvili Z (2004) Recomendation for some principles of production of grafted wine. Brochure, Tbilisi, 22 p

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SELECTION CLONALE DE L'UGNI BLANC POUR LA PRODUCTION DU COGNAC

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RÉSUMÉ

L'Ugni blanc représente 98 % de l'encépagement du vignoble destiné à la production du Cognac. Les clones d'Ugni blanc actuellement disponibles en France, sélectionnés dans les années 1970, ne présentent que peu de diversité. En 2003, un programme destiné à accroître la diversité disponible au sein de ce cépage, a été initié. Une prospection a été conduite dans le vignoble de Cognac, puis dans d'autres vignobles français. Un nouveau conservatoire a été implanté dès 2006. Il regroupe aujourd'hui environ 700 accessions. A ce jour, la prospection se poursuit dans différents vignobles hors de France, en particulier en Italie. Parallèlement, un travail de phénotypage est en cours sur l'ancien conservatoire en vue d'évaluer la diversité disponible et de mettre au point des méthodes de screening rapides.

INTRODUCTION

L'Ugni blanc est un cépage originaire d'Italie, plus précisément de Toscane, où il est connu sous le nom de « Trebbiano toscano ». Avec environ 170 000 ha implantés, l'Ugni Blanc est l'un des principaux cépages cultivés dans le monde. On le retrouve majoritairement en Europe, particulièrement en France et en Italie, mais aussi en Bulgarie, Grèce, Portugal, Roumanie et dans d'autres vignobles hors d'Europe : Afrique du Sud, Australie, Californie, Argentine…. En France, il représente notamment plus de 98% des 75000 hectares du vignoble destiné à la production du Cognac.

Alors qu'il était déjà cultivé dans le Midi et le Sud-ouest de la France, l'Ugni blanc n'a été implanté à grande échelle dans le vignoble de Cognac qu'après la crise phylloxérique, il y a un siècle environ (Ravaz, 1900). Il va devenir vers le milieu du XX^{ème} siècle le cépage majoritairement utilisé en Charentes, où il s'est rapidement imposé pour la production du Cognac en raison de ses qualités : productivité, débourrement tardif, production d'un vin acide et peu alcoolisé, particulièrement adapté à la production d'eaux-de-vie de qualité.

La multiplication de l'Ugni blanc a, dans un premier temps, été réalisée par sélection massale. Les nouvelles parcelles étaient implantées à partir de greffons prélevés dans des parcelles plus anciennes, sur des souches sélectionnées pour leurs caractéristiques de vigueur et de production. A partir des années 1960, un important travail de sélection clonale a été entrepris par la Station Viticole du BNIC, aidée dans cette démarche par des pépiniéristes et viticulteurs de la région de Cognac, qui avaient déjà repéré et multiplié les origines les plus intéressantes. En effet, il peut exister une certaine diversité entre les clones d'un même cépage (Bouquet, 2008).

Entre 1960 et 1964, environ 800 souches, choisies d'après leur état sanitaire et leur comportement agronomique, sont prélevées dans différentes parcelles du vignoble de Cognac, et implantées, franches de pied, dans les sables de l'Ile de Ré, au domaine de Halda (Courlit 1995). Un travail de sélection, basé sur des critères agronomiques et d'état sanitaire des accessions, aboutit en 1976 à l'agrément de 8 clones inscrits par le CTPS au catalogue officiel

des variétés et clones de vigne cultivés en France sous les numéros 479 à 486 (ENTAV *et al*, 1995).
Plusieurs parcelles de comportement de ces clones, implantées sur des sites correspondant à des situations naturelles très différentes, ont été suivies depuis leur agrément. Les résultats de plus de trente années d'expérimentation portant sur les huit clones d'origine charentaise sont concordants : les caractéristiques de ces clones sont très proches. La figure 1 illustre par exemple la moyenne de production des clones en Charentes (Courlit, 1995).

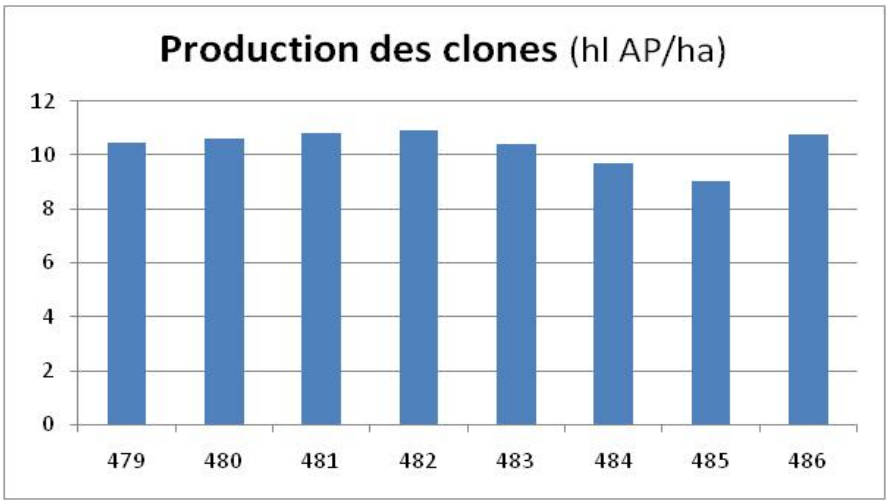

Figure 1 : Production en hectolitre d'alcool (AP) pur à l'hectare des clones charentais (moyenne 1992-1994, Courlit, 1995)

La proximité génétique de ces clones a également fait l'objet d'une étude mettant en œuvre la technique des microsatellites, utilisée notamment par l'INRA de Colmar (Lefort, Pelsy *et al*, 2003) et susceptible de révéler des différences génétiques au sein d'un cépage. La comparaison des clones d'Ugni blanc agréés, mettant en œuvre 32 microsatellites choisis pour leur capacité à différencier les clones d'autres cépages, n'a montré aucune différence dans leurs profils génétiques.

En 1992, afin de prévenir la perte de la variabilité naturelle, la Station Viticole du BNIC a décidé, en concertation avec les organismes en charge de la sélection (ENTAV, INRA, ONIVINS) de mettre en place en Charente le conservatoire national de l'Ugni blanc (Desaché, 1995). Les accessions rassemblées au sein de ce conservatoire sont les suivantes : six clones d'origine Charentaise parmi les onze agréés en France, un large échantillon (171) des accessions rassemblées dans la parcelle de sélection originelle de l'Ile de Ré, une vingtaine de clones issus de différentes régions françaises (Armagnac, Hérault…) transmis par l'ENTAV. Ce conservatoire a été implanté dans le vignoble de la Fondation Fougerat, à Graves-Saint Amant (16120 - France), avec 8 plants par accession. Il a été utilisé pour tester des méthodes d'évaluation de la diversité.
En 2004, compte tenu de la faible diversité constatée dans ce premier conservatoire et de son vieillissement, un nouveau projet de conservatoire, issu de nouvelles campagnes de prospection, a été initié pour constituer une population présentant davantage de variabilité et susceptible de pouvoir mieux répondre aux attentes actuelles.

MATERIEL ET METHODES

Prospection dans différentes régions

La prospection consiste à repérer des souches d'aspect particulier dans de vieilles parcelles, dans des régions où le cépage est depuis longtemps implanté. La méthodologie suivie est la « méthode d'installation de gestion et d'étude des conservatoires de clones de vigne », mise au point par L'ENTAV, l'INRA et la CTNSP en 2004, (Lacombe *et al*, 2004).

Le nombre de sites prospectés a été privilégié par rapport au nombre de souches repérées par site, ce qui a permis de marquer des souches dans les sites recensés. On pense en effet que, du fait du mode de multiplication majoritaire de la première moitié du XX^{ème} siècle (greffage avec du matériel végétal sélectionné localement), les souches d'une même parcelle peuvent provenir du même matériel végétal, mais que des parcelles différentes peuvent présenter de la diversité, a fortiori si elles sont éloignées géographiquement.

Dans chaque parcelle, le travail consiste à parcourir les rangs au cours de la période végétative, en particulier entre la véraison et la récolte pour observer les grappes. Le repérage des souches se fait sur les critères suivants :

- cépage Ugni blanc,
- vieux cep d'origine (certaines parcelles ont connu de nombreuses entreplantations),
- absence de virose manifeste,
- diversité d'aspect végétatif.

Ce dernier point porte sur l'aspect général du cep (vigueur, production, port…), l'aspect des feuilles (bullure, villosité, couleur, taille, forme, forme des sinus pétiolaires et latéraux, aspect des dents,…), l'aspect des grappes (compacité, taille, forme, taille et couleur des baies…).

Ce travail est d'autant plus délicat que les parcelles en place présentent parfois un état dégradé (figure 3). A l'extrême, les souches « ensauvagées », non taillées ni traitées depuis des années, présentent un aspect très différent de ce qu'elles donneraient dans une parcelle entretenue. De ce fait, l'observation des souches en place est réduite au minimum, et le choix des souches est parfois réalisé de façon aléatoire, ce qui rejoint la méthode employée par A. Martins au Portugal (Martins, 2003).

Installation du conservatoire

Les principales viroses sont recherchées par tests sur bois et racines des plants constitués. Les accessions atteintes de court-noué ou d'enroulement de type 1 ou 3 sont éliminées. Les accessions atteintes d'enroulement 2 sont conservées dans une parcelle isolée du reste du conservatoire. Le greffage des 200 à 400 assemblages annuels doit être particulièrement rigoureux pour assurer la traçabilité de ces petits lots : il a été réalisé par le Centre de Pré multiplication de la Station Viticole du BNIC. Des observations sont faites en pépinière pour confirmer l'identité du cépage (éventuellement complétées par des tests génétiques) et pour repérer des caractères morphologiques originaux.

Afin de respecter la méthodologie décrite par Lacombe *et al*, 2004, une parcelle n'ayant jamais porté de vigne a été choisie pour créer le nouveau conservatoire situé sur le domaine du Lycée agricole de l'Oisellerie (La Couronne, 16000 - France). Les accessions (plants provenant d'une même souche d'origine) sont plantées par placettes de 5 souches. Pour cela, 15 plants sont greffés par accession. Des références (clones agrées d'Ugni Blanc) sont également incluses dans le dispositif.

Analyse des raisins

Echantillonnage : 5 grappes saines (moins de 5% de pourri visuel) sont prélevées par placette, en choisissant toujours la première grappe du sarment le plus près du cep, sur les ceps présentant un état normal. Les grappes sont placées dans un sachet-filtre (Atlantic Labo), qui est ensuite introduit directement dans un malaxeur à palettes (« Bagmixer® 3500 Jumbomix® » d'Interscience, distribué par Atlantic labo) pour extraire le jus (2 min, fréquence maxi). 40 accessions ont été évaluées en 2008 et 196 en 2009.

Analyse des moûts : les principaux paramètres analytiques des moûts (TAVP, acidité totale, pH, acide tartrique acide malique, potassium, azote aminé, azote ammoniacal) sont déterminés par IRTF (infra rouge à transformé de Fourié), par le laboratoire de la Station Viticole du BNIC, accrédité par le COFRAC pour le programme 78 (analyse des vins et des moûts). Les composés volatils des moûts sont dosés quantitativement par chromatographie en phase gazeuse du distillat obtenu par la technique de micro distillation du moût en conditions hydro alcooliques (10%) et acides (pH=1), décrite par Galy (1998).

RESULTATS ET DISCUSSION

Prospection

Les principales régions prospectées en France sont le vignoble de Cognac, le Midi méditerranéen, la Corse (où l'Ugni blanc est appelé Rossola), le Sud-ouest (Gers, Landes, Pyrénées Atlantiques). Dans le Midi, en Corse, et le Sud-Ouest, ce travail a été mené en 2004 en partenariat avec des techniciens régionaux de Chambres d'Agriculture, de coopératives agricoles, ….

Dans la région de Cognac, la prospection a été menée avec l'aide du Conservatoire du Vignoble Charentais. Une enquête postale, adressée à tous les viticulteurs de l'aire d'Appellation Cognac, et complétée par de nombreux contacts directs, a permis de recenser environ 200 parcelles âgées de plus de 50 ans, certaines de plus d'un siècle, dont 126 ont été retenues et visitées. La figure 2 illustre la couverture de l'aire de production par la campagne de prospection. Elle montre que de nombreux secteurs du vignoble charentais comportaient encore de vieilles parcelles en 2003. En moyenne une dizaine de souches ont été marquées par parcelle. La prospection a aussi porté sur des treilles, des parcelles abandonnées ou même des souches « ensauvagées » comme sur l'île de Ré où les souches d'anciennes vignes plantées dans les dunes continuent à pousser, alors que le sable tend à les recouvrir. Trouver 15 yeux greffables a parfois été difficile sur ces vieilles souches, en particulier sur les vignes ensauvagées dont l'aoûtement est souvent imparfait. Une prospection a dans le même temps été conduite au Portugal par le professeur A. Martins de l'Université Technique de Lisbonne.

Le tableau 1 présente le nombre de parcelles et de souches repérées par année et par région.

Tableau 1 : sites et souches repérés par région de prospection

	Région prospectée	Nombre de parcelles	Nombre souches marquées
2004	Midi-Corse-Sud ouest	54	408
2005 - 2006	Vignoble de Cognac	126	1142
2008	Vendée	9	26
2007-2008	Portugal	14	71
2009	Gironde	4	23
Total		207	1670

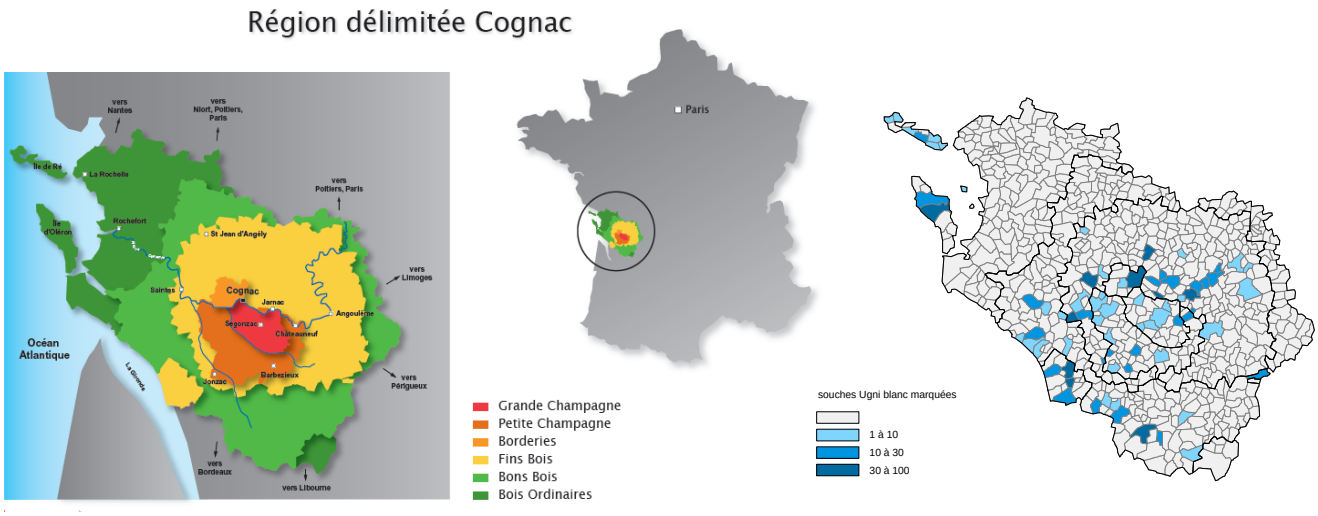

Figure 2 : Carte du vignoble de Cognac et localisation du nombre de souches d'Ugni blanc marquées par commune

Pour les vignobles français, la prospection est aujourd'hui considérée comme achevée, à quelques exceptions près. Certains vignobles d'autres pays où l'Ugni blanc est implanté de façon significative restent encore à prospecter : l'Italie en premier lieu, mais aussi la Bulgarie et la Grèce.

Figure 3 : Prospection dans une parcelle âgée (a), repérage d'un cep (b) et implantation du nouveau conservatoire au Lycée de l'Oisellerie (c)

Installation du conservatoire

Le tableau 2 récapitule le nombre d'accessions plantées pour les différentes tranches. Environ la moitié des souches marquées ont du être éliminées, principalement à cause de l'enroulement dans le Midi et en Corse, et principalement à cause du court-noué en Charentes. Cette répartition n'est pas homogène : certaines parcelles voient toutes leurs souches éliminées, d'autres aucune. Aucune technique d'assainissement pour un grand nombre d'accessions n'est utilisable à ce jour, ce qui explique le faible pourcentage des accessions saines implantées dans le conservatoire, par rapport aux souches repérées au vignoble.

Des références (clones agréés) sont incluses dans le conservatoire pour pouvoir comparer dans les mêmes conditions les accessions aux clones connus. En 2010, 10 clones de

Trebbiano toscano, agréés en Italie (Tamai, 2009), ont également été implantés dans le conservatoire.

Tableau 2 : Nombre d'accessions plantées dans le nouveau conservatoire

Années	Accessions saines	Avec enroulement 2
2006	78 + références	91 + références
2007	143 + références	44 + références
2008	250 + références	80 + références
2010	10 + références	
Total	481	215

Une fois achevée l'implantation du conservatoire, il s'agira d'observer le comportement des différentes accessions pour en choisir les plus intéressantes, qui seront implantées dans des collections d'étude avec répétitions et observations plus complètes. Les nouveaux clones candidats à l'agrément seront issus de ces collections d'étude.

Premières observations sur la variabilité

Dans l'attente de l'entrée en production du nouveau conservatoire (Oisellerie), deux campagnes d'observations ampélographiques, assorties de notations diverses (précocité, maladies du bois), ont été réalisées en 2008 et 2009 sur l'ancien conservatoire de la Fondation Fougerat (Graves), qui comporte environ 200 accessions. Elles ont été complétées par l'analyse d'échantillons de raisins récoltés à maturité ainsi que par une évaluation de la composition des raisins en composés volatils (figure 5).

L'objectif de ces observations était double : d'une part, préciser les méthodologies à mettre en œuvre dans le cadre des futurs programmes de sélection, d'autre part, disposer d'une première évaluation du niveau de diversité disponible au sein de ce conservatoire. Ces premières observations constituent une base méthodologique qui sera mise à profit pour optimiser le processus de sélection, qui débutera en 2010 sur le conservatoire du Lycée de l'Oisellerie.

Une certaine diversité ampélographique a pu être observée entre accessions (figure 4).

Figure 4 : Différence de couleur d'apex observée au débourrement entre deux accessions du conservatoire de Graves.

Sur le plan analytique, les résultats préliminaires portent sur 40 accessions en 2008 et 196 en 2009. Une analyse en composante principale (ACP) des résultats analytiques a été réalisée : l'axe 1 montre une tendance liée à la maturation (opposition entre le TAV potentiel et l'acidité totale), les norisoprénoïdes (trimethyl-1,1,6 dihydro-1,2 naphtalène, β damascenone, vitispirane 1 et 2) étant plus en relation avec la richesse en sucres qu'avec l'acidité. Ces résultats montrent une faible diversité globale de cette population (figure 5), qui est toutefois légèrement supérieure à celle observée pour les clones agrées. La confirmation de ces premiers résultats par les récoltes à venir, et son élargissement aux 700 accessions du conservatoire du lycée de l'Oisellerie devrait permettre d'identifier certaines accessions présentant des caractéristiques différentes qui seront alors mises en collection d'étude.

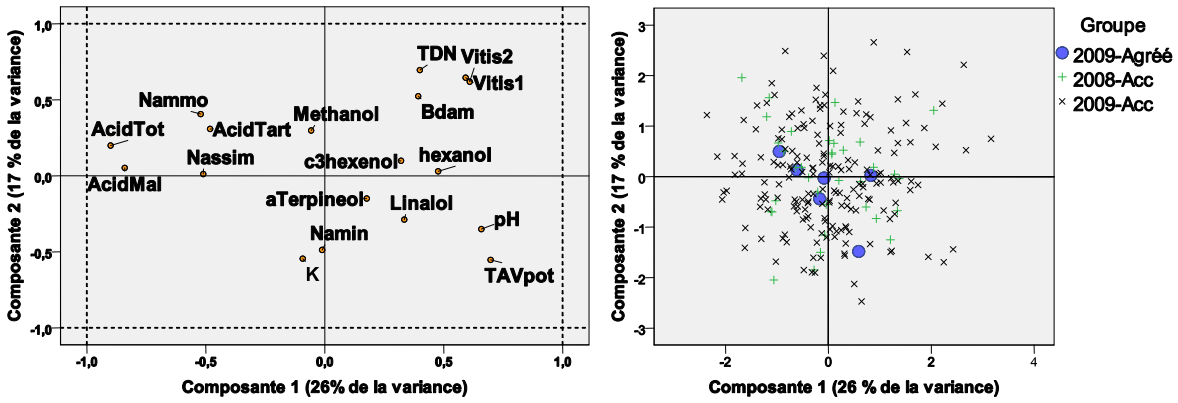

Figure 5 : Analyse en composante principale des résultats analytiques 2008 et 2009. Les clones agrées sont représentés par les points bleus.

CONCLUSION

La prospection menée en France par le BNIC a permis l'implantation d'un nouveau conservatoire de près de 700 accessions, qui fera encore l'objet de compléments dans les prochaines années. Ce conservatoire devrait avoir permis de sauvegarder une part importante de la diversité de l'Ugni blanc présente dans le vignoble français. L'objectif est maintenant de rechercher de la diversité dans d'autres vignobles, et notamment en Italie, berceau de ce cépage, où il est présent dans de nombreuses régions (Storchi, 2008 ; Tamai, 2009).

Le nombre important d'accessions à caractériser implique de faire appel à de nouveaux outils d'analyse rapide. Les essais réalisés en 2008 et 2009 sur le conservatoire de la Fondation Fougerat ont permis une première approche de la diversité disponible, et de tester des méthodes d'échantillonnage et de caractérisation rapide des raisins. De nouveaux outils de tri seront néanmoins recherchés, aussi bien sur le plan agronomique (mesure rapide du rendement, de la maturité…) que génétique. Les travaux récents sur le génome de la vigne (Adam-Blondon *et al*, 2007) ouvrent à cet égard d'intéressantes perspectives de mise au point d'outils innovants, utilisables dans le cadre de nouvelles stratégies de sélection.

Figure 6 : Grappe d'Ugni blanc dans le vignoble de Cognac

BIBLIOGRAPHIE

ADAM-BLONDON A.F. *et al*, 2007. The grapevine genome sequence suggests ancestral hexaploidization in major angiosperm phyla. Nature, Volume 449, Issue 7161, pp. 463-467 (http://dx.doi.org/ 10.1038/nature06148).

BOUQUET A., 2008. La vigne et sa diversité génétique. Revue bibliographique. Progrès Agricole et Viticole, 125, n°6, 91-102.

ENTAV, INRA, ENSAM, ONIVINS, 1995, Catalogue des variétés et clones de vigne cultivés en France, ENTAV éditeur, 30240 – Le Grau du Roi (France).

COURLIT Y., 1995. La sélection de l'Ugni blanc : clones agréés. Journée Technique de la Station Viticole, pp 9-11.

DESACHE F., 1995. Mise en place en Charentes d'un conservatoire pour le cépage Ugni blanc. Nouveaux objectifs pour la sélection de l'Ugni blanc. Journée Technique de la Station Viticole, pp 13-17.

DI VECCHI STARAZ M., THIS P., BOURSIQUOT J-M., LAUCOU V., LACOMBE T. *et al*. 2007. Genetic structuring and parentage analysis in grapevine: kingroup and origin of cv. « Sangiovese » revealed, The Journal of the American Society for Horticultural Science, 132(4):514-524.

GALY B., 1998. Mise en évidence de la trituration dans la chaîne d'extraction : un marqueur le TDN. Journée Technique Régionale, EAUZE, Septembre 1998, ITV Midi-Pyrénées. 15-21.

LACOMBE T., BOURSIQUOT J-M., AUDEGUIN L., 2004. Prospection, conservation et évaluation des clones de vigne en France. Bul. O.I.V., 885-886, 799-809.

LEFORT F., PELSY F., SCHEHRER L., SCOTT K.D., 2003. Evaluation des deux loci microsatellites hautement polymorphes dans 103 accessions du genre Vitis. J. Int. Sci. Vigne Vin. 37 (2) : 67-75.

MARTINS A., 2003. Protocole de sélection de la vigne et résultats de son application au cépage grenache. Groupe expert sélection. OIV, avril 2003.

STORCHI P., BANDINELLI R., PISANI P.L., SCALABRELLI G. 2008. Il germoplasma viticolo in Toscana: i vitigni ad uva bianca, *"Il germoplasma toscano"* **11:** 13-37, ARSIA.

RAVAZ L., 1900. Le Pays de Cognac. L. Coquemard, Angoulême.

TAMAI G. 2009. Catalogo dei cloni, varietà di uva da vino, Edagricole.

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AGRO-BIOLOGICAL AND TECHNOLOGICAL VALUE OF SOME GRAPE VARIETIES (VITIS VINIFERA L.) FROM CAUCASUS, IN THE AGRO-PEDO-CLIMATIC CONDITIONS OF NORTH-EAST ROMANIA

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ABSTRACT

Caucasus grape varieties are among the oldest in culture. According to the polycentric theory and to the polyphyletic origin of grape varieties it is possible that some of these varieties come either from wild vine (Vitis silvestris Gmel.), found commonly in this area, either through their own reproduction towards European varieties.

This paper aims at studying three varieties with origin in Georgia: Dodrelabi, Rkațiteli and Saperavi, compared to Chasselas doré, the cosmopolite grape variety, cultivated in the Ampelographic Collection of the Horticulture Faculty in Iasi. The viticultural area of north-east Romania is restrictive towards vines especially when taking into consideration the absolute minimal temperatures during winter. Climatic accidents often occur as the values of these temperatures (4 years out of 10) make necessary the semi-protected vine culture and promoting the vine varieties with a higher resistance to freeze. The studied varieties were considered under the following aspects: freezing behavior, vegetation phenophases, fertility and productivity, quantity and quality of grapes during 2007-2009.

INTRODUCTION

Starting to cultivate the wild vine *Vitis silvestris* Gmel. And forming the *Vitis vinifera* L. happened around 7000-9000 BC. The place where the wild wine was transferred into human culture cannot be specifically set. History mentions that the oldest culture centre is in Small Asia, in the Transcaucasia, due to the large spreading of *Vitis silvestris* Gmel. while in some areas it is hard to trace a limit between wild and human bred vines. This phenomenon of interpenetration between wild and cultivated vines is found, at a smaller scale, in other regions, as: the Rhein valley in Germany, Mosselle's valley in France, valea Mosselei în Franța, Neretva's valley in eastern part of the Adriatic basin, Kopet Dag region on Central Aisa, Tian-Șan region in China, Olt river valley, Prut river valley, Danube Delta in Romania (Iacob M., 1990). It must be noticed that the forms of wild vines that exist in Europe are dioic plants, while the fruit-bearing vines from the *Vitis vinifera* L. are hermaphrodites. As it is generally known, hermaphroditism in plants is generated by the multiple allels of H gene, met only in cultivated vines. It has been found our, nevertheless, that wild vines also have hermaphrodite forms, that provoked through introgression in local dioic populations, the apparition of hermaphrodite forms that are basis of European noble grape varieties. All of the above are proof that cultivated vines (*Vitis vinifera* L).originate from wild ones.

MATERIAL AND METHOD

Evaluating the climatic favourability was analysed as a synthesis of climatic factors that have an impact on the bioproductive behaviour and qualitative factors of the vine. Within the viticultural ecosystem of Iasi vineyard, the caucasian vine variaties (cv) Dodrelabi, (cv) Rkațiteli and (cv) Saperavi, were compared to (cv) Chasselas dore, largely known.

The study has been done and analysed in the Ampelographic Collection of the Horticultural Faculty. The used rootstock was (cv) Berlandieri x Riparia Oppenheim 4, planting was done in 1995 at planting distances of 2,2/1,2 m (3787 trunks/ha). The cutting was done in bilateral cordon, semi-tall, with 2-3 buds. During 2007-2009 analyses were done concerning: freeze behaviour, vegetation pheno-phases development, fertility and productivity of the vine varieties, quantity and quality of grape production.

RESULTS

The problematic of climatic change on vineyards is one of maximum importance for researchers in different areas. These climatic changes are of interest to the vine growers, considering the fact that premium wines are produced in vineyards very sensible to any changes in the pedo-climatic temperatures.

Modernising the vineyards by introducing tall and semi-tall forms of cutting, with partial or no protection during winter of the vine trunks led to establishing adequate technologies and vine varieties for each vineyard and viticultural center.

In this context come the studies and observations that established the pedoclimatic disponibility of the viticultural area of Iasi vineyard taking into consideration the ecological, bio-productive and qualitative needs of vines; evaluating the behaviour of vines originated from the Caucasus area, the biological, quantitative and qualitative potential, in order to underline their use and manipulation the existent resources of the viticultural ecosystem as alternative for a sustainable viticulture.

Through its actions, except the normal limits and according to the vine's climate, the climatic risk causes violent destructions, having as final result partial or total loss of biological capacity.

A viticultural ecosystem is a functional block created and controlled by man in order to obtain a high quality grape production, in social and economically viable conditions. The viticultural ecosystem is directly influenced by the global climate changes. The existence of extreme climatic phenomena plus many more major climatic changes imposed on the vine growers the necessity of finding new approach methods in establishing their impact on the viticultural ecosystem. In order for a sustainable viticulture to exist, climatic changes must be take into consideration, evaluated and monitored, especially now, when, we deal with unknown climatic phenomena. Global climatic changes changed the frequency of rains, leading to draughts expansion and desertification.

The viticultural north-east area of Romania is restrictive for viticulture especially because of the absolute minimal temperatures during winter. Climatic accidents can often appear, the values (-20 .... - 22^0C) and frequency (4 years out of 10) of them making compulsory the culture of vines in a semi-protected manner and promoting vine varieties that are resistant to freezes. By analysing table 1, one can notice that in the last 50 years (1961-2010), for 14 years, absolute minimal temperatures in the air of -20^0C were registered the average being of -$18,4^0$C, at the top limit of resistance in table grapes. Lower temperatures values were registered in 1985, 1987, 1996 and 2006. Compared to these years, during 23 – 27.01.2010 temperatures were situated between -22°C ....-25°C for more than 3 days.

Table 1

Minimal absolute temperatures registered in Copou viticultural center Iaşi (1961 - 2010)

Year	In the air			At soil surface		
	month	day	t ºC	month	day	t ºC
1961	I	27	-20,6	I	28	-22,1
1962	XII	29	-17,0	XII	29	-22,4
1963	I	20	-25,2	I	23	-32,5
1964	I	11	-21,0	I	11	-24,6
1965	II	6	-17,1	II	10	-19,2
1966	II	5	-18,5	II	5	-20,4
1967	I	31	-20,3	II	1	-24,7
1968	I	10	-16,8	I	10	-25,5
1969	I	28	-19,0	II	13	-27,3
1970	I	31	-16,1	I	1	-18,9
1971	I	9	-14,8	I	8	-25,5
1972	I	14	-24,0	I	15	-28,2
1973	I	26	-15,0	I	25	-26,1
1974	I	15	-16,8	I	16	-22,1
1975	II	17	-10,0	II	18	-12,0
1976	II	8	-22,0	II	9	-31,6
1977	XII	14	-15,0	I	6	-21,6
1978	XII	8	-17,0	XII	8	-23,5
1979	I	3	-15,6	I	8	-20,5
1980	I	7	-18,2	I	18	-28,0
1981	I	8	-14,6	I	8	-24,6
1982	II	22	-14,3	II	11	-23,0
1983	XII	15	-14,0	II and XII	24 febr	-17,0
1984	II	18	-16,0	II	18	-23,0
1985	I	14 and 16	-25,5	I	14	-30,4
1986	XII	28	-18,8	II	28	-29,0
1987	I	9	-25,0	I	9	-33,4
1988	II	2	-17,4	II	2	-28,1
1989	XII	11	-13,0	XII	11	-16,0
1990	I	5	-14,0	I	6	-20,6
1991	II	1	-19,2	I	31	-17,8
1992	I	22	-15,2	I	22	-22,4
1993	I	3	-18,2	I	4	-24,0
1994	II	13	-20,8	II	14	-17,2
1995	XII	30	-16,0	XII	31	-21,2
1996	XII	28	-27,2	XII	28	-33,5
1997	XII	17	-19,2	I	7	-22,0
1998	XII	3	-19,0	XII	24	- 24,0
1999	XII	24	- 13,0	II	20	-19,3
2000	I	25	- 15,9	I	26	- 22,2
2001	XII	18	- 20,4	XII	18	- 24,5
2002	XII	26	- 19,8	XII	26	- 21,0
2003	I	13	- 21,6	I	13	-30,6
2004	I	31	-17,0	I	31	-19,0
2005	II	8	- 19,4	II	6	-27,6
2006	I	23	- 25,1	I	25	- 29,0
2007	II	24	-19,6	II	24	- 25,0
2008	I	5	-19,5	I	5	-24,2
2009	XII	19	-17,0	XII	21	-29,0
2010	I	26	-27,0	I	26	-35,0
average			**-18,4**	**average**		**-24,2**

Resistance to freezing. From table no. 2, it is noticed that lowest viability of winter buds, 25-30% in the case of main buds of the winter eye, when the absolute minimal temperatures were below the resistance limit of table grapes varieties(-18^{0}C). The vine variety Rkațiteli shows a much better behaviour towards resistance to freezing, with main bud viability of 92-95%. A satisfactory behaviour was noted in Saperavi variety for red wines, where main bud viability was between 63-66%. Both grape varieties for wine had superior values compared to the control, concerning winter eyes viability.

Table 2

Bud viability during 2007, 2008 and 2009

Grape variety	Viable buds					
	2007		2008		2009	
	main	total	main	total	main	total
Dodrelabi	28	49	25	57	30	76
Rkațiteli	92	91	94	98	95	100
Saperavi	64	89	66	88	63	90
Chasselas doré-witness	34	61	40	77	45	91

Vegetation pheno-phases development (table 3). According to the genetic nature of the grape varieties and the climatic conditions, the studied grape varieties start vegetation in the second half of April, the same time with Chasselas doré, being thus less exposed to late frosts that are quite often in the area. The blooming period is in the beginning of June, all of the studied varieties showing lateness compared to the control. While grape mellowness takes part in the first half of August, grape maturation is complete in different periods, according to their genetic origin. The first grape variety that is suitable for consumption is Rkațiteli, which is fully mature during 15-20 IX, followed by Saperavi, which matures during 20-25 IX. The latest maturing grape variety is Dodrelabi, in the first ten days of October. All the studied grape varieties are tardier than the control variety Chasselas doré.

Table 3

Vegetation pheno-phases development

Grape variety	Vegetation start	Blooming	Grape mellowness	Grapes maturation	Leaf fall
Dodrelabi	18-28 IV	4-15 VI	6-15 VIII	1-10 X	25-29 X
Rkațiteli	15-23 IV	1 -7 VI	1-8 VIII	15 -20 IX	25-29 X
Saperavi	15-25 IV	1 - 8 VI	1-10 VIII	20 -25 IX	25-29 X
Chasselas doré-witness	15-28 IV	1-6 VI	25 VII-15 VIII	1-15 IX	25-29 X

Fertility and productivity of vine varieties (table 4). In general, the studied grape varieties have an average fertility, except Rkațiteli, where the percentage of fertile shoots is closer to the control of 83,7%, respectively 74,2%. The lowest fertility is found in Dodrelabi, with 63,5% fertile shoots. Saperavi grape variety has 69,8% fertility shoots. The absolute fertility index had supraunitary values in all vine varieties except Dodrelabi, where an average of one inflorescence is formed per fertile shoot. The control vine variety had the highest number of inflorescences formed per trunk, compared to the studied varieties. The productivity indices were higher in the studied grape varieties than the control vine Chasselas doré, as the weight of a grape is higher. The highest values are registered in Dodrelabi grape variety, where the average weight of a one grape is 412g.

Table 4

Fertility and productivity of vine varieties for table grapes in Iasi vineyard

Grape variety	% fertile shoots			Absolute fertility index	Relative fertility index	Absolute prod. index	Relative prod. index
	%	Dif. To control	Significance.				
Dodrelabi	63,5	-20,2	00	1,00	0,62	412,00	255,44
Rkațiteli	74,2	-9,5	0	1,10	0,74	270,60	182,04
Saperavi	69,8	-13,9	00	1,09	0,85	277,95	216,75
Chasselas doré-control	83,7	-	-	1,31	1,10	265,93	223,30

DL 5% = 17,2%;
DL 1% = 23,8%;
DL 0,1% = 31,3%

Quantity and quality of grape production (table 5). The average weight of a grape is superior to the control variety in all of the studied varieties and significantly different in Dodrelabi.

The highest productions are registered in Rkațiteli of 6,10 kg/trunk. Dodrelabi and Saperavi had average productions/trunk smaller than Chasselas doré. The production/hectare was highest in Rkațiteli as well, Saperavi and Dodrelabi having sufficient productions concerning their category.

From a qualitative point of view, the accumulated sugars of the studied grape varieties (Rkațiteli and Saperavi) are higher than in the control variety Chasselas doré, indicating the chance of obtaining table wines from them within Iasi vineyard. In the case of Dodrelabi variety, the quantity of sugars was smaller, taking into consideration their tardiness, as well as their variety specificity as table grapes.

The total acidity of must, at full maturity was equilibrated, between 4,5 g/l H_2SO_4 at Rkațiteli and 5,3 g/l H_2SO_4 at Dodrelabi. Saperavi grape variety registered 4,8 g/l H_2SO_4, allowing the obtaining of dry red table wines.

Table 5

Quantity and quality of grape production

Grape variety	Average weight of a grape			Mass 100 berries(g)	Grape production				Sugars (g/l)	Total acidity (g/l H_2SO_4)
	(g)	Dif.	Signif.		kg/trunk	Dif.	Signif.	t/ha		
Dodrelabi	412	+209	***	486	4,60	-1,20	00	17,4	144,3	5,3
Rkațiteli	246	+43	*	277	6,10	+0,30	*	23,1	177,2	4,5
Saperavi	255	+52	*	261	5,20	-0,60	0	19,7	186,9	4,8
Chasselas doré-witness	203	-	-	235	5,80	-	-	21,9	175,0	4,6

DL 5% = 29,8 g;
DL 1% = 66,9 g;
DL 0,1% = 143,4 g

DL 5% = 0,3 kg;
DL 1% = 1,1 kg;
DL 0,1% = 2,1 kg

CONCLUSIONS

1. In the north-eastern part of Romania, concerning the freezing resistance, the best behaviour was registered in Rkațiteli, where losses of main buds were the smallest. Dodrelabi proved more sensible than Chasselas doré.

2. The varieties that adapted well to the eco-climatic conditions if Iasi vineyard are Rkațiteli and Saperavi. They start vegetation in the second part of April, being saved of late frosts, while maturation of grapes takes place during the V-th era, after Chasselas doré, but in the specific time frame of grape varieties for wine.

3. The grapes' varieties fertility is average, except Rkațiteli that has 74,2% fertile shoots. Considering productivity, all studied varieties had productivity indices superior to Chasselas doré.

4. The grape productions are generally high, Rkațiteli having a bigger productivity index than Chasselas doré, another motive for extending its cultivation in Iasi vineyard.

5. Sugar accumulations are specific to the genetic characteristics of the varieties. Therefore, the quantity of sugars accumulated in the studied grape varieties allows the obtaining of white table wines (Rkațiteli) or red table wines (Saperavi). Dodrelabi, a table grape variety, is not valued at its total capacity, being a tardy maturing variety, that accumulates smaller quantity of sugars, its glucoacidimetric index (27,2) being far from the optimal values.

BIBLIOGRAPHY

1. Cotea, V.D., Barbu, N., Grigorescu, C. C., Cotea, V. V., 2005 - Vignobles et vins de Roumanie, Ed. Academiei Romane, Bucuresti.
2. Indreaş, Adriana, Vişan, Luminița, 2001 – Principalele soiuri de struguri de vin cultivate in România. Edit. Ceres, Bucuresti.
3. Rotaru, Liliana, 2009 - Soiuri de viță de vie pentru struguri de vin. Editura "Ion Ionescu de la Brad", Iaşi.
4. Simion, Cristina, Contoman , Maria, 1998- Des solutions techniques integrees pour la culture du cepage Rkațiteli, Lucrări ştiințifice, Anul XXXXII, vol.41, Seria Horticultura, Ed.Ion Ionescu de la Brad, Iaşi.
5. Zamfir, Cătălin, 2009 - Studiul autenticității şi tipicității vinurilor roşii obținute din soiuri autohtone. Teză de doctorat. Univ. de Stiințe Agric. şi Med. Vet. Iaşi.

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Effect of cluster thinning on the phenolic composition of cv. Syrah cultivated in Portugal.

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ABSTRACT

Three intensities of thining, 0% (Control - T), 30% (P30) and 60% (P60) and two growth stages,- berries pea-sized (BPS) and berries at veraison(BR) - were studied. The reduction in crop load is not equal to the thinning intensity; it is always a little slighter. Thinning at veraison allows better crop load compensations performances, than thinning at berries pea-sized. The pH value diminished when thinning was made at veraison . Acidity increased when intensifying and delaying thinning. Brix degree has not shown variation, neither with the intensity or the date of thinning. The content of total proanthocyanidins as well as their mean degree of polymerisation shows the tendency to be lower in the grapes from thinning essays.

Effets de la grappe éclaircissage sur la composition phénolique du cv. Syrah cultivée au Portugal

Trois intensités d' éclaircissage, 0% (Control - T), 30% (P30) et 60% (P60) et de deux stades de croissance,-les baies de taille d'un petit pois (BPS) et à la véraison (BR)- ont été étudiés.
La réduction du rendement n'est pas égale à l'intensité d'éclaircissage, il est toujours un peu plus légère. L'éclaircissage à la véraison permet une meilleure compensation dês rendements. La valeur du pH diminue quand éclaircissage a été faite à la maturation. L'acidité accrue lorsque l'on intensifie et on retarde l'éclaircissage. Le degrée Brix n'a pas montré changement avec l'intensité ou le moment de l'éclaircissage. Le contenu de proanthocyanidines totales ainsi que leur degré moyen de polymérisation montrent la tendance à être plus faible dans les raisins avec éclaircissage

INTRODUCTION

The viticulturist's goal is to keep healthy vineyards, for as many years as possible, producing good quality wines. The quality depends on many external interacting factors, many of which are complex to evaluate; but, essentially, there are two kinds of factors which can influence quality/crop level relationship: the external ones which are not manageable (such as sun light, climacteric conditions and so on) and the ones which the winemaker can act on (irrigation, fertilization, canopy management); the best way to act on this relationship is through canopy management and irrigation (Holzapfe e Rogiers, 2002).
In a vineyard, the balance between fruit weight and exposed leaf area is important to determine the production's quality and quantity (Reynolds *et al.*, 1994). It is a known fact, vines with too much load have shorter central roots, less knots and distance between then and smaller leafs (Edson *et al.,* 1993). In order to achieve this correct balance the winemaker has available many crop management operations such as leaf thinning or cluster thinning.

Cluster thinning is described as the suppression of flowers or clusters before full maturation (Palliotti e Cartechini, 2000). Therefore, cluster thinning has a direct effect on the source/sink ratio; having less sinks (fruits) photosynthetic assimilation might be improved, increasing grape quality (Reynolds *et al.*, 1994). It inducts physiological adjustments in the plant, improving the maturation's kinetics. Plus, this operation improves canopy sanitary conditions as thinning allows more enlightenment and fresh air penetration in the vegetation and clusters (Smithyman *et al.*, 1998).

Cluster's thinning most evident effect is apparently crop load reduction, but its decrease is not equal to thinning's intensity. Martins (2007) found that, for the same intensity of thinning, in two consecutive years, the decline in production has been uneven. In fact, the vine compensates the stack lost, increasing the berry's volume and weight (Rubio, 2002). Consequently, cluster thinning is an operation which is eventually void by the plant; actuality, after a few years of consecutive thinning its effects are no longer visible (Lavezzi *et al.*, 1994). For this reason, Clímaco (*et al.*,2005) support that cluster thinning must only be executed in the years when vineyards have such a fertility that may possibly condemned production's quality.

Production reduction is least notated when the thinning intensity is short, allowing a better production lost compensation to the plant and/or when thinning is made at an early growth stage (Dumartin *et al.*, 1990; Pita 2006; Martins, 2007).

The complex interactions, among factors involved in production and quality of wine grapes, account for some of the conflict findings regarding optimal crop load (Reynolds *et al*., 1994). Rubio (2002) admits that a thinning intensity till 40% has less influence than irrigation in the grapes' maturation and the vine final production.

The moment of thinning is important for the possible effects on the relationship between vegetative growth and maturation. But studies are not conclusive and continue to take place. Lukácsy (2006) admits that thinning is to be completed between berries establishment and cluster closure. Aires *et al.*, (1997) found that there was an increase in alcohol content, regardless thinning has taken place at the clusters closure or at ripening. Conduct thinning long before ripening seems to trigger the plant compensatory effects on vegetative growth, thus avoiding the targeting of thinning on improving the quality and ripeness of the grapes (Dumartin *et al.*, 1990). Unanimity seems to be the deadline for thinning completion: after ripening there are no recorded increases in alcohol content (Dokoozlian e Hirschfelt, 1995; Silva *et al.*, 2008).

The wine chemical variation is due to a redistribution of the photosynthesis assimilates caused by thinning (Noar *et al.*, 2002). Even though some results indicate that is possible to manipulate wine phenolic composition by modifying production practices using cluster thinning, the mechanism throughout phenolic compounds are affected by cluster thinning remains unclear (Prajitna *et al*., 2007).

Phenolic compounds are responsible for bitterness, astringency and colour intensity of wine, therefore this plays a major role in wine sensory quality.

Cluster thinning often induces an increase in alcohol content, in polyphenols and anthocyanins (Reynolds, 1989; Aires *et al.*, 1997; Palliotti e Cartechini, 2000; Boubals, 2001; Noar *et al.*, 2002; Rubio, 2002; Prajitna *et al.*, 2007). It also causes a decrease in acidity and consequent increase in pH, although it is not its objective (Reynolds, 1989; Gao e Cahoon, 1998; Boubals, 2001; Noar *et al.*, 2002; Rubio, 2002; Ó-Marques *et tal* 2005, Pena-Neira *et al.* , 2007; Prajitna *et al.*, 2007; Martins *et al.*, 2008).

Even thought what was said before, cluster thinning is a very controversial operation; there are many discrepancies between studies and authors' conclusions. Some authors defend that

the meteorological conditions of a certain year, such as temperature and soil moisture, are more relevant to a crop quality than cluster thinning (Keller *et al.,* 2005). For example, Prajitna (2007) demonstrated that cluster thinning is an essential ripening and quality tool that must be practiced in red winegrape cultivars grown in cool-climate and short growing season regions, which are not Portugal's conditions.
At last, it is important to state that whichever the thinning intensity, growth stage or technique there must occur an adjustment to grapevine variety, crop management and climatic conditions of the wine region (Jackson e Lombard, 1993).
The present research aimed to relate the intensity and time of thinning with the productivity and phenolic composition in the cv. Syrah.

MATERIAL AND METHODS

This study was conducted in Portugal, at Quinta do Monte d' Oiro, during the 2008 vegetative cycle. Three thinning intensities, 0% (Testimony – T), 30% (P30) and 60% (P60) and two growth stages, according to BBCH scale, berries pea-sized (BPS) and berries ripening (BR) were studied. Thinning was hand-made.
When thinning was performed while berries were pea-sized, the lower order cluster was kept in the vine. When thinning was made at ripening, it was possible to make a qualitative pruning, removing the unhealthier clusters or physiologically delayed. If the same sanitary or development characteristics were found in all grapevines' clusters, as in the previous mode BPS, the lower order cluster was removed. P60 left always a cluster per vine.
The berry volume and weight evolution was registered, sampling 100 berries, every 10 days during the campaign.
Total acidity, pH and Brix degree were determined using standard OIV methodology. To determine the total anthocyanins (Ribéreau – Gayon and Stonestreet 1965), total phenols (Ribéreau – Gayon ,1968), the separation and measurement of procyanidin according to their polymerization degree (Sun *et al.*, 1998) and their average polymerization degree (Monagas *et al.*, 2003) the samples were prepared according to the Carbonneau e Champagnol (1993) methodology.

RESULTS AND DISCUSSION

Berry volume and weight was increased by cluster thinning (tables 1 and 2), specifically when was 30% pruned and at ripening.

Table 1: Berry weight (g/berry) and Berry volume (ml/berry) at harvest.

	T	*P30*	*P60*	*BPS*	*BR*
Berry weight (g/berry)	1,940	2,245	2,025	2,215	2,315
Berry volume(ml/berry)	1,935	2,055	2,07	2,055	2,165

The reduction in crop load is not equal to the thinning intensity; it is always a little slighter.
Another interesting fact is, when comparing thinning dates, the results show that thinning at ripening allows better crop load compensations performances, than thinning at berries pea-sized (table 1).

Table 2: Crop load reduction (% comparing to Testimony)

	T	*P30/ BPS*	*P60/ BPS*	*P30/BR*	*P60/BR*
Crop load reduction	0	- 19,8	- 42,4	- 17,65	- 27,75

A linear relation was found between production/ha and thinning intensity (Figure 1). This equation was calculated only with three values (T, P30 and P 60) and only for a year; so it is not a good predict tool as still, the equation is here presented as it shows a slight image of a possible relation between the referred variables.

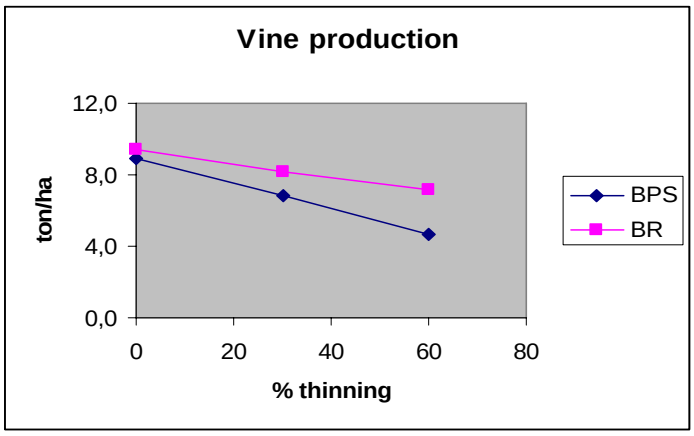

Figure 1: correlation between production/ha and thinning intensity

The pH value diminished when thinning was made at ripening (table 3); this is contradictory with the available references (Reynolds, 1989; Gao and Cahoon, 1998; Boubals, 2001; Noar *et al.*, 2002; Rubio, 2002; Pena-Neira *et al.*, 2007; Prajitna *et al.*, 2007; Martins *et al.*, 2008), which have all confirmed a pH increase when thinned. It is possible that weather conditions have played a far greater role than canopy management (Van Leeuwen, *et al.,* 2004).
Acidity increased when intensifying and delaying thinning. Puertas (et al., 2003) saw the same in Syrah cultivar when 50% thinned. It is interesting to note that pH value is not consistent along with acidity at P60, as it is at BR; there might have occurred acidity salification.
Brix degree, and consequently alcoholic content, have not shown a response, neither to intensity or time of thinning (table 3). Although there are slight differences, these values are not significantly diverse. It is important to remember this vineyard is usually thinned. The results here shown may translate the plant's sugar accumulation ability to adapt to thinning (Lavezzi *et al.,* 1994). Authors whose works have in common thinning at ripening have not found great improvement in sugar content (Ough e Nagaoka, 1984; Bravdo and Bravdo 1985; Boubals 2001; Keller *et al*., 2005). In fact, Van Leeuwen (*et al.,* 2004) considers that 88% of sugar content is mainly due to grapevine cultivar, soil and weather conditions of the specific year.

Table 3: Effect of intensity and stage of thinning on pH, Total acidity (g/l) and Brix degree;

	T	*P30*	*P60*	*BPS*	*BR*
pH	3,9a	3,8a	3,8a	3,9a	↓3,7b
Total acidity (g/l)	3,3a	3,4a	↑3,7b	3,2a	↑3,8b
Brix Degree	25a	24,5a	24,9a	25,45a	23,9a

values with the same letter, in the line, indicate there is no significant differences $p < 0,05$.

On Table 4 it is notable an effect of thinning intensity in the aroma precursors content, noticeable in the 60% intensity. Some authors, which did not specify the thinning intensity, also notated an increase of the aromas on the thinned grapevines (Reynolds, 1989; Ribéreau-Gayon *et al.*, 1998; Palliotti e Cartechini, 2000; Boubals, 2001; Noar *et al.*, 2002; Rubio, 2002; Prajitna *et al.*, 2007).

Table 4: effect of intensity and stage of thinning in aroma precursors

		BPS		*BR*	
	T	P30	P60	P30	P60
G-G µmoles/g of berry	n.d..	n.d.	2,37	n.d.	0,83

n.d. – not detected.

Thinning seems to have had no influence, with statistic significance, on the phenolic composition (Table 5). The reason to this no-response-to-thinning seems to be, as said before, the vineyard habituation to thinning and, therefore, self regulation to produce phenolic compounds (Lavezzi *et al.,* 1994); or, it is also possible, the specific terroir conditions during 2008/2009 have overcome thinning (Ough e Nagaoka, 1984; Bravdo *et al*., 1985; Boubals 2001; Van Leeuwen *et al.,* 2004; Keller *et al*., 2005).

Table 5: effect of intensity and stage of thinning in the phenolic composition of the berries

	T	*P30*	*P60*	*BPS*	*BR*
Total anthocyanins (mg/l)	1638,5a	1251,5a	1107,7a	1204,4a	1154,8a
Total phenols index	54,1a	44,8a	46,3a	55,3a	↓38,1b
Total Proanthocyanidins (mg/l)	1696,1a	1211,3a	1416,5a	1375,5a	1227,7a
Monomeric Proanthocyanidins (mg/l)	4,1a	4,3a	4,8a	5,4a	3,6a
Oligomeric Proanthocyanidins (mg/l)	20,6a	↑42,9b	↑62,2b	20,9a	54,6a
Polymeric Proanthocyanidins (mg/l)	873a	737,2a	942a	967a	712,2a
Average tannins polymerization degree	2,8a	3a	3,1a	3,3a	2,9a
Gallate % in tannins	6,9a	8,2a	8,5a	8,3a	8,4a
Prodelfinifin % in tanins	24,2a	27,1a	19,5a	20,5a	27a

values with the same letter, in the line, indicate there is no significant differences $p < 0,05$.

CONCLUSIONS

There were found compensations in the plant, in fact, both berry weight (g/berry) and berry volume (ml/berry) tended to augment in pruned samples, when compared with the unthinned testimony.

As the study was set in a vineyard usually thinned, many results are not correspondent with the available references, stating a benefit in the phenolic composition, for the great part of the vineyards. There must have been, in the studied vinegrapes, a habituation to thinning, visible in the analytical data. Consequently, when comparing with studies with the same characteristics, many resemblances were found, to this so called habituation to thinning. It is not anymore noticeable an improvement in wine quality. So, it is the authors' conclusion, thinning must be punctual and only made in years when excessive crop load compromises wine quality; a qualitative thinning in order to produce wines of character, removing the clusters of poor quality or delayed maturation, allowing a better leaf / fruit ratio and a better microclimate for the remaining clusters that are more accessible during the harvest, is preferable.

Since this is a one year study to understand the effect of thinning in the phenolic composition, it would be interesting to perform the same study over a longer period and greater number of repetitions in field.

REFERENCES

Aires, A., Neves, M., Almeida, C., Castro, R., 1997. Influência do controlo da produção na relação rendimento/qualidade (*Vitis Vinifera* L. cv Baga). Actas de Horticultura, III Congresso Ibérico de Ciências Hortícolas, 4:217-222.

Boubals, D., 2001. L'éclaircissage manuel de grapes (vendage en vert). *Progrés Agricole et Viticole*, 118 (17), 372-374.

Bravdo, B. , Hepner, Y. , Loinger, C. , Cohen, S., e Tabacman, H., 1985. Effect of crop level and crop load on growth, yield, must and wine composition, and quality of cabernet sauvignon. *Am. J. Enol. Vitic.* 36(2): 125-131.

Clímaco, P., Teixeira, K., Ferreirinho, Mª.C., 2005. Efeitos da monda de cachos no rendimento e qualidade da cv. Alicante Bouschet. Vinea, *Revista Viticultura Alentejo*, Abril-Junho, pp.13-16.

Dokoozlian, N., Hirschfelt, D., 1995. The influence of cluster thinning at various stages of fruit development on flame seedless table grapes. *Am. J. Enol. Vitic.*, 46 (4): 429-436.

Dumartin, P., Lemoine, B., Marcovelles, S., 1990. Les travaux en vert de la vigne. *Progrès Agrícole e Viticole*, 107, nº.6: 143-144.

Edson, C. E., Howell, G. S., Flore, J. A., 1993. Influence of crop load on photosynthesis and dry matter partitioning of seyval grapevines I. Single leaf and whole vine response pre- and post-harvest. *Am. J. Enol. Vitic.* 44: 139-147

Holzapfe, B., Rogiers, S., 2002. Ripening grapes to specification: identifying manageable factors determining grape composition & quality through carbohydrate sink-source relationships. Final Report to Grape and wine research & development corporation.

Jackson, D. I., Lombard, P. B., 1993. "Environmental and management practices affecting grape composition and wine quality- a review." *American Journal for Enology and Viticulture*, 44(4), 409-430.

Keller, M., Mills, L.J., Wample, R.L., Spayd, S.E., 2005. Cluster Thinning Effects on Three Deficit-Irrigated Vitis vinifera Cultivars. *Am. J. Enol. Vitic.* 56:2:91-103.

Lavezzi, A., Ridomi, A., Pezza, L., Intrieri, C., Silvestroni, O., 1994. Effects of bunch thinning on yield and quality of Sylvoz-trained cv. Prosecco (Vitis vinifera L.). GESCO , v. 48(2) p. 35-40, Valladolid.

Lukácsy, G., 2006. The effect of time and the severity of the Cluster thinning on the vegetative and generative performance of the ‘furmint‘ and the ‘hárslevelű’ cultivars in Tokaj. Dissertação da Tese de Doutoramento, Corvinus University of Budapest.

Martins, S., 2007. Monda de cachos na casta ‘Touriga Nacional. Efeitos no rendimento e qualidade. Tese Mestrado em Viticultura e Enologia, Universidade Técnica de Lisboa, Universidade do Porto.

Monagas, M., Gómez-Cordovés, C., Bartolomé, B., Laureano,O. e Ricardo-da-Silva, J.M., 2003. Monomeric, Oligomeric, and Polymeric Flavan-3-ol Composition of Wines and Grapes from Vitis vinifera L. Cv. Graciano, Tempranillo, and Cabernet Sauvignon. *Journal of Agricultural and Food Chemistry* 51, *6475-6481.*

Monagas M., Garrido, I., Bartolomé, B. e Gómez-Cordovés, C., 2006. Chemical characterization of commercial dietary ingredients from *Vitis vinifera* L. *Analytica Chimica Acta, Volume 563, 1-2, 401-410.*

Naor, A., Gal, Y., Bravdo, B., 2002. Shoot and cluster thinning influence vegetative growth, fruit yield, and wine quality of 'Sauvignon blanc' grapevines. *Journal of the American Society for Horticultural Science* 127: 628-634.

Ó-MARQUES, J.; R. REGUINGA; O. LAUREANO & J. M. RICARDO-DA-SILVA (2005). Changes in grape seed, skin and pulp condensed tannins during berry ripening: effect of fruit pruning. *Ciência Téc. Vitiv.,* 20(1):25-52

Ough, C. S. e Nagaoka, R., 1984. Effect of Cluster Thinning and Vineyard Yields on Grape and Wine Composition and Wine Quality of Cabernet Sauvignon. *Am. J. Enol. Vitic.* 35:1:30-34.

Palliotti, A. e Cartechini, A. 2000. Cluster thinning effects on yield and grape composition in different grapevine cultivars. Acta Hort. (ISHS) 512:111-120.

Pena-Neira, A. Caceres, A. Pastenes, C., 2007. Low molecular weight phenolic and anthocyanin composition of grape skins from cv. syrah (Vitis vinifera L.) in the maipo valley (Chile): Effect of clusters thinning and vineyard yield. Food Science and Technology International, vol. 13, n°2, pp. 153-158.

Pita, N., 2006. Influência da monda de cachos nas características analíticas de uvas e vinhos da casta Syrah. Relatório de trabalho de fim de curso em Engenharia Agronómica. Universidade Técnica de Lisboa, Instituto Superior de Agronomia.

Prajitna, A., Dami, I., Steiner, T., Ferree, D., Scheerens, J. e Schwartz, S., 2007. Influence of Cluster Thinning on Phenolic Composition, Resveratrol, and Antioxidant Capacity in Chambourcin Wine. *Am. J. Enol. Vitic.* 58, 346-350.

Puertas, B., Cruz, S., Serrano, M. J., Valcárcel, M. C., García de Luján, A.2003. Incidencia de la práctica del aclareo de racinos en la concentración de antocianos y taninos y en el color de los vinos de las variedades Cabernet Sauvignon, Merlot, Syrah y Tempranillo. X Congresso Brasileiro de Viticultura e Enologia 55-68.

Reynolds, A.G., 1989. Riesling' grapes respond to cluster thinning and shoot density manipulation. *Journal of the American Society for Horticultural Science* (USA), 114(3) 364-360.

Reynolds, A., Price, S., Wardle, D. & Watson, B., 1994. Fruit Environment and Crop Level Effects on Pinot noir. Vine Performance and Fruit Composition in the British Columbia. *Am. J. Enol. Vitic* 45, 452-459.

Ribéreau-Gayon, P., Dubourdieu, D., Donèche, B., Lonvaud, A., 1998. Handbook of Enology – Volume one – The Microbiology of wine and vinifications. pp. 254. Wiley Editions.

Rubio, J. A., 2002. Riego y aclareo de racimos: efectos en la actividad fisiolofica, en el control del rendimiento y en la calidad de la uva del cv. tempranillo (Vitis vinifera L.) Universidad Politécnica de Madrid, Escuela de Agrónomos.

Silva, L., 2008. Load levels in vineyards of Malbec cv. and their efects on evolution and fenolic maturation. *Rev. Bras. Frutic.* vol.30, n.3, pp. 675-680.

Smithyman, R. P., Howell, G.S., Miller, D.P.,1998. The use of competition for carbohydrates among vegetative and reproductive sinks to reduce fruit set and botrytis bunch rot in seyval blanc grapevines.*Am. J. Enol. Vitic.* 49: 163-170 .

Sun, B., Leandro, C., Ricardo-da-Silva,J.M. e Spranger, I.' 1998. Separation of Grape and Wine Proanthocyanidins According to Their Degree of Polymerization. *J. Agric. Food Chem. 46*, 1390-1396.

Van Leeuwen, C., Friant, P., Choné,X., Tregoat,O., Koundouras,S., Dubourdieu, D., 2004. Influence of Climate, Soil, and Cultivar on Terroir. *American Journal of Viticulture and Enology*, 55:3, pp.207-217.

TOTAL GRASS COVER IN VINEYARDS: AN INNOVATING AND PROMISING SOIL MANAGEMENT ALTERNATIVE TO REDUCE THE USE OF HERBICIDES

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RESUME

Dans une optique de limitation des intrants herbicides, l'enherbement total (entre les rangs et sur la ligne des souches) de la vigne est une alternative envisageable à condition de contrôler les impacts quantitatifs et qualitatifs sur la production. Depuis 2007, dans le sud-ouest de la France, l'enherbement total est comparé, sur les plans agronomique et œnologique, aux désherbages chimique et mécanique du rang. Différents types de couverts végétaux à base de graminées pérennes faiblement concurrentielles sont étudiés.

Après trois années d'étude, l'enherbement total induit une réduction du rendement et de la vigueur, une réduction de la teneur en azote des moûts, et une augmentation du degré potentiel et de la teneur en polyphénols. L'intensité de ces impacts, reliés à une contrainte azotée, est très variable selon les caractéristiques pédoclimatiques des parcelles expérimentales et les modalités testées. Les mélanges à base de *Koeleria macanthra* se distinguent notamment par leur impact le plus faible sur la vigueur et le rendement. Quant à l'enherbement naturel, son impact sur la vigne est fortement dépendant des espèces qui le composent ainsi que du taux de recouvrement sous le rang.

ABSTRACT

In order to reduce the use of herbicides, total grass cover (between and under the rows of vine) could be an interesting solution if the competition for nitrogen and water supply is controlled.

Since 2007, in South-West of France, natural and sown grass covers under the row have been compared, on experimental plots, to chemical and mechanical weeding. Several grass cover species were sown on each site, and data were collected on grapevine production and quality.

After three years of study, total grass cover induced yield and vigour reductions, fall in the nitrogen content of the musts, and increase in the sugar and polyphenolics contents. The intensity of these impacts was not the same on all the experimental fields (pedoclimatic conditions) and on all the studied treatments (grass cover species). Among all the treatments, the mixes containing in a major proportion the graminaceous *Koeleria macanthra* induced the weakest competition on the vine. The impact of the natural grass cover treatment was both dependent on its soil covering rate and on the competition level of the flora.

INTRODUCTION

In the South-West of France, pedoclimatic conditions (deep soils, regular rainfalls) are favourable to the setting up of a grass cover crop in non-irrigated situations. This technique is now widely spread but limited to inter-rows alleys. New regulations and environmental issues lead to a decrease in the list of herbicides that can be used by the winegrowers. As an alternative, the mechanical weeding of the row has already proved its good efficiency but unfortunately the technique cannot be adapted to all the situations and vineyards (Gaviglio, 2007). Total grass cover (row and inter-row), natural or sown using specific species, could be an complementing interesting alternative from an economical and technical point of view.

This new technique is not so easy to handle as it involves a good control of the competition between the grass cover and the vine in order to limit the negative consequences on yields and on grape quality.

During this research work, natural and sown grass covers were studied. The natural one, easier to setup has the drawback to present a wide variety of species in the grass cover, which can involve a significant water and nitrogen stress. The sown one, more difficult to setup, allows a better control of the water and nitrogen status, by the right choice of species, varieties or mixes (Delabays *et al.*, 2006).

Other qualities for the sown species to consider are their resistance to settlement by indigenous flora and their limited growth ability in order to minimize the number of cuttings to be performed.

MATERIALS AND METHODS

The study was started in 2007 in three areas of the South West of France presenting different production characteristics (Tab. 1.) : (a) AOP Cahors, production of red wine, variety Malbec, on clayey soils without water stress; (b) AOP Fronton, production of red wine, variety Négrette on silty soils inducing a temporary water stress in summer; (c) IGP Gascony, production of white wine, variety Colombard on clayey-limestone soils with a small water reserve.

Table 1.Experimental fields characteristics

Field name	Vineyard	Variety	Spacing (m)	Number of vinestock per hectare	Soil type	Production objective
Anglars	AOP. Cahors	Malbec	2.00 x 1.25	4000	clayey-siliceous	10 T/ha
Fronton	AOP. Fronton	Négrette	2.20 x 1.00	4545	silty soil	10 T/ha
Mons	IGP Gascony	Colombard	2.65 x 1.00	3774	clayey-limestone	15 T/ha

On all the fields, grass cover was the inter-row soil management practice chosen for several years (65% of the field’s whole surface).

On each area, several sown grass covered treatments were compared to a natural grass cover, to chemical and mechanical weeding treatments of the row. Each treatment was replicated four times in a randomized block design.

The different treatments are described in the table 2.

Table 2. Description of the studied treatments

Field name	Treatment code	Treatment description
Anglars	CHIM	chemical weeding
	MECA	mechanical weeding
	NATU	natural grass cover
	KOEL	sown grass cover : 10% *Lolium perenne*, 10% *Festuca rubra trichophylla*, 30% F*estuca ovina*, 50% *Koeleria macrantha*
	DACT	sown grass cover :100% *Dactylis glomerata* L. subsp. *hispanica*
Fronton	CHIM	chemical weeding
	MECA	mechanical weeding
	NATU	natural grass cover
	FETUO	10% *Lolium perenne*, 10% *Plantago coronopus*, 15% *Poa pratensis*, 65% *Festuca ovina*
Mons	CHIM	chemical weeding
	MECA	mechanical weeding
	NATU	natural grass cover
	FETUR	sown grass cover : 10% *Lolium perenne*, 10% *Plantago coronopus*, 15% *Poa pratensis*, 65% *Festuca rubra rubra*
	KOEL	sown grass cover : 10% *Lolium perenne*, 10% *Festuca rubra trichophylla*, 30% *Festuca ovina*, 50% *Koeleria macrantha*

The observations realized refer to three main aspects:

1. Cover crop performances in vineyard by measuring the rate of establishment, the grass cover persistence and the evolution of weed species.
2. Vine plants performances (when combined with different cover crops) by measuring grape yield, average weight per cluster, parameters related to plants growth and strength (budburst rate, pruning wood weight). Water stress has also been studied by the measurement of stem water potential.
3. Grape quality by analyzing must composition: sugar, acidity, anthocyanins and nitrogen content.

Figure 1.Illustration of total grass cover: Fronton experimental field, “FETUO” treatment, July 2007.

RESULTS AND DISCUSSION

Grass cover species behavior

The frequent weed species follow up under the row showed a good soil covering by the sown cover crop on all the experimental fields. The sown grass cover managed to limit the settlement of indigenous flora (Figure 2).

Dactylis hispanica (DACT) is the most efficient specie to control the growth of indigenous flora, which just represents some few percents of the grass cover. In term of efficiency, it is followed by the KOEL and FETUO mixes that showed a good behavior respectively on the Anglars and Fronton fields (average contamination by the indigenous flora of 15% and 23%). On the Mons field, the KOEL and FETUR performances were weaker and just limited the contamination of indigenous flora species to 35% of the surface under the row.

The natural grass cover, after several months of experiment, didn't reach a soil covering rate as important as the sown grass cover treatments.

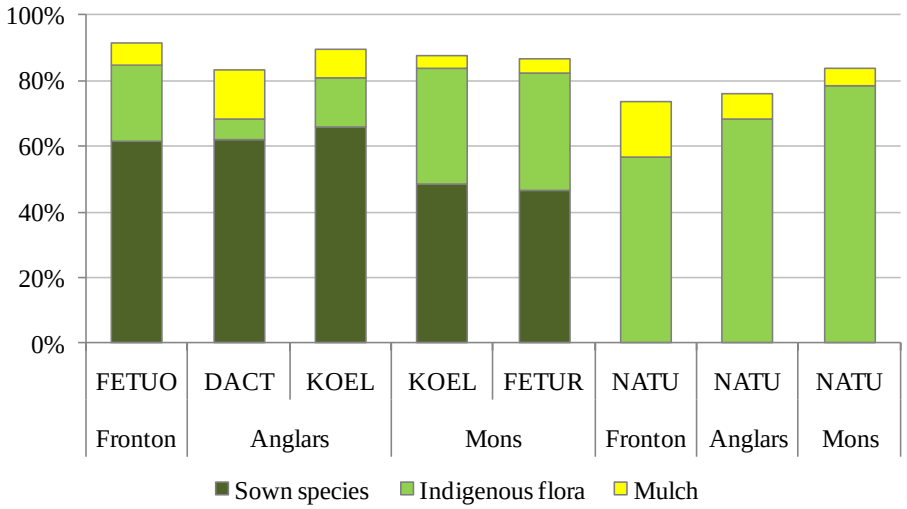

Figure 2. Settlement of the vegetation species under the row. Sown species, indigenous flora, mulch and bare soil's average covering rates (2008-2009)

Vine plants summer water stress

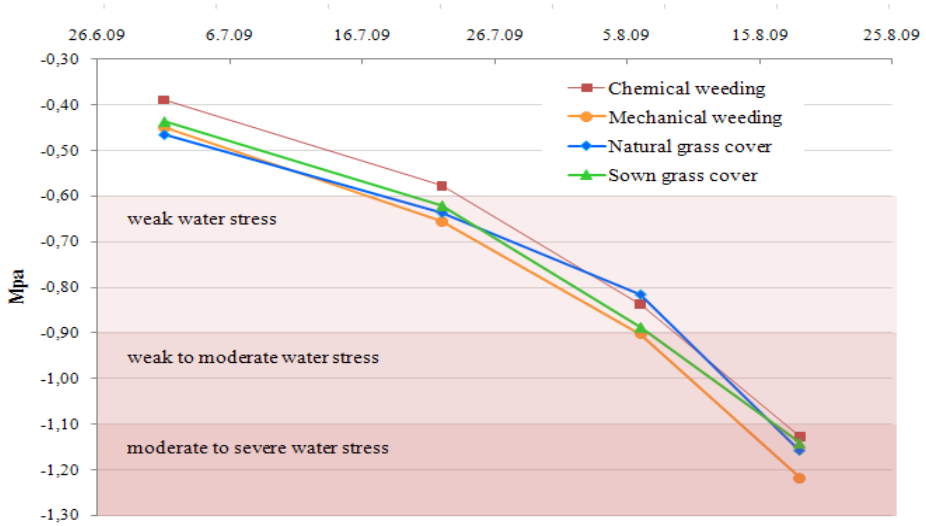

Figure 3. Stem water potentials' evolution on Fronton field - summer 2009

Summer water stress has been studied by the measurement of stem water potential. In the three cases of the study, the summer water stress was not affected by the increase of the grass covered surface (Figure 3).

Vine plants growth and strength

Grape yield

After three years of study, the effect of total grass cover on yields is not the same on all the experimental fields and depends on the studied treatments (Figure. 4).

In the Mons field case ("medium" production objective), yield were severely impacted by total grass cover. The decrease in comparison to the chemical weeding reference treatment varies between -29% and -39%: the production goal is not reached.

On the Fronton and Anglars fields ("controlled" production objective), total grass cover had a more limited impact that allows the production goal achievement.

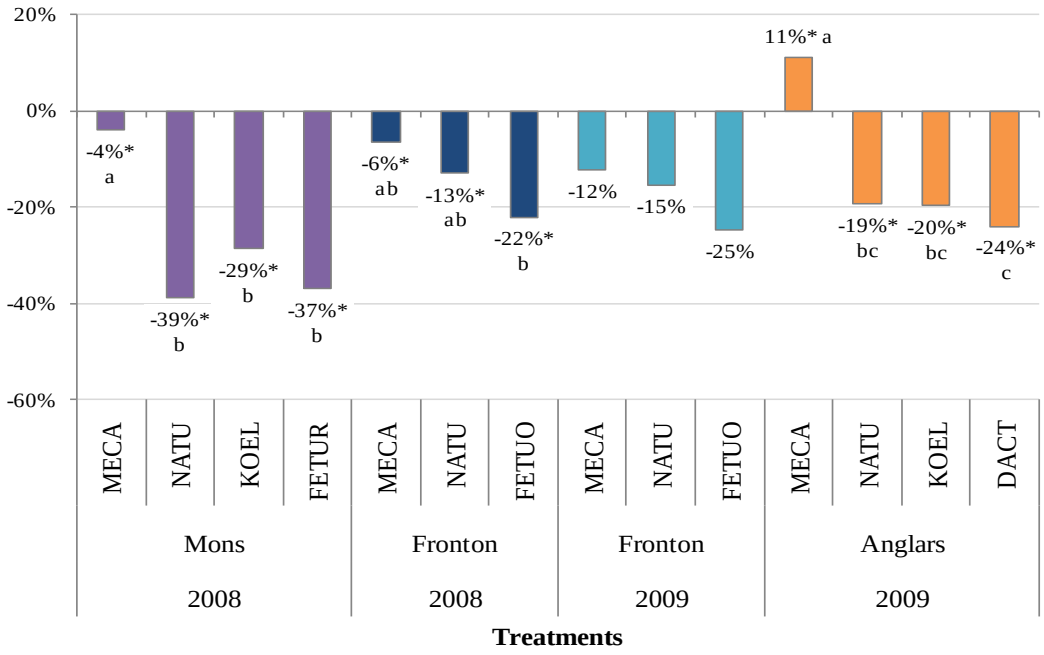

Figure 4. Yield (kg per vine): difference (%) to the chemical weeding reference treatment
ANOVA *: significant at P < 0.05; a, b, c: separation of means using the Tukey test
Due to severe hail damages in Mons in 2009, data are not available for this experimental field.

In all the situations, yield impact is a result of the decrease of both average cluster weight and average number of clusters per vine (consequence of the decrease in the budburst rate, results are not shown).

Among all the treatments, the mixes containing in a major proportion *Koeleria macanthra* ('KOEL', studied on Anglars and Mons fields) tend to induce the weakest competition on the vine. Natural grass cover's impact varies with the competition level of the spontaneous flora. On the Fronton field, the low impact of natural grass cover was more associated to a weak soil covering rate than to a low competitiveness of the present species (Figure 2).

Vigour

The vigour of the vine was estimated by the measurement of pruning wood weight. Pruning woods were counted and weighted separately. The same trends than those observed on grape yields were noticed.

The species with the most significant impact on vigour were *Dactylis hispanica* ('DACT') and *Festuca rubra rubra* ('FETUR'). The mixes containing in a major proportion *Koeleria macanthra* ('KOEL') induced the weakest competition on the vine.

On the Mons and Anglars fields, the "NATU" treatment has one of the biggest impacts on vigour. On these two fields, mechanical weeding also impacted the vigour. Mechanical weeding may have caused a destruction of the topsoil roots and disturbed the plant nutrition (Heinzlé, 2002).

Table 3: Mean cane weights (2008)

ANOVA *: significant at P < 0.05 and separation of means using the Newman & Keuls test.

Treatment		CHIM	MECA	NATU	FETUO	FETUR	KOEL	DACT
Field								
Anglars	Pruning weight (g)*	119 (a)	106 (ab)	94 (b)	-	-	103 (ab)	91 (a)
	Difference to reference (%)		-11%	-21%	-	-	-13%	-23%
Fronton	Pruning weight (g)	51	48	48	45	-	-	-
	Difference to reference (%)		-6%	-7%	-13%	-	-	-
Mons	Pruning weight (g)*	49 (a)	36 (b)	31 (b)	-	34 (b)	38 (b)	-
	Difference to reference (%)		-27%	-37%	-	-31%	-22%	-

Grape quality

Nitrogen content of the musts

Total grass cover – whatever the treatment – provoked a fall in the nitrogen content of the musts. A fall of 20% occurred on the Mons field and of about 60% on the Anglars field, the weakest impact was observed in Fronton.

A 10 kg nitrogen per hectare foliar application at veraison on the "NATU" treatment helped correct the nitrogen status of the musts to a level equivalent to the one of a chemical weeding treatment.

Table 4. Free Assimilable Nitrogen content of the musts

	Free Assimilable Nitrogen ($mg.L^{-1}$)		
Field	**Anglars** (2009)	**Fronton** (2009)	**Mons** (2008)
Treatment			
CHIM	93	85	122
MECA	50	76	94
NATU	41	76	95
FETUO	-	73	-
FETUR	-	-	91
KOEL	35	-	97
DACT	35	-	-
NATU + foliar nitrogen spraying (10 U)	-	-	119

Grape quality at harvest

On the Négrette red wine variety, on the Fronton field, a significant increase of the anthocyanins (+12%) and of the total phenolics (+ 120%) contents was noticed on the two total grass cover treatments. The same trends were also recorded on the Anglars field.

On the Colombard white wine variety, on the Mons field, must analysis (Tab. 6) showed an increase in the sugar content on the total grass cover treatment whereas Total Acidity was hardly affected.

Table 5. Grape quality at harvest 2009, Fronton experimental field
ANOVA *: significant at P < 0.05 and separation of means using the Tukey test.

Treatments / **Parameters**	**CHIM**	**MECA**	**NATU**	**FETUO**
Sugar content ($g.L^{-1}$)	203	207	204	214
Total acidity ($g.L^{-1}$ H_2SO_4)	3.2	3.0	3.1	3.0
Malic acid ($g.L^{1}$)	2.6	2.3	2.4	2.3
Tartaric / malic ratio	1.4	1.8	1.6	1.8
Total phenolics (OD 280 nm)	82 (b)	85 (ab)	91 (ab)	92 (b)
anthocyanins ($mg.L^{-1}$)	1127 (b)	1214 (ab)	1270 (a)	1249 (ab)

Table 6.Grape quality at harvest 2008, Mons experimental field

Treatments / **Parameters**	**CHIM**	**MECA**	**NATU**	**FETUR**	**KOEL**
Sugar content ($g.L^{-1}$)	168	173	200	190	183
Total Acidity ($g.L^{-1}$ H_2SO_4)	6.9	6.8	6.2	6.8	6.6
Malic acid ($g.L^{1}$)	1.9	2.1	1.8	1.7	1.8
Tartaric / malic ratio	0.3	0.3	0.3	0.3	0.2

CONCLUSIONS

After three years of study, the total grass cover of the vine induced the same agronomical and enological impacts than those observed some years ago during studies conducted on the grass cover of the inter-row: yield and vigour reduction, fall in the nitrogen content of the musts, increase in the sugar and polyphenolics contents (Maigre, 1996; Spring, 2001; Coulon et Prud'home, 2003). The intensity of these impacts was not the same on all the experimental fields (water reserve of the soils, variety) and on all the studied treatments (species sown).

Among all the treatments, the mixes containing in a major proportion *Koeleria macanthra* induced the weakest competition on the vine. *Dactylis glomerata* L. subsp. *hispanica* could be another interesting option in term of soil covering even if it appeared to be a more competitive specie.

It is important to notice that all the sown grass cover enabled to limit the growth of indigenous flora. Some of the studied species are very promising as they achieved the quality standard required for a total grass cover.

Nevertheless this study, which is very dependent on climatic conditions, needs to be continued during several extra years in order to obtain some more significant results. In addition it is interesting to analyze the long-term effects of this soil management practice.

The crucial element in the success of this experiment is the choice of the right specie and that's why we have decided to enlarge the range of species since autumn 2008. A multi-variety trial that aims at comparing 16 species (sown separately or in mixes with leguminous) has just been set up on the IFV experimental estate.

LITERATURE CITED

DELABAYS N., SPRING J.L., MERMILLOD G, 2006. Essai d'enherbement de la vigne avec des espèces peu concurrentielles: aspects botaniques et malherbologiques. *Revue suisse Vitic. Arboric. Hortic.*, 38 (6) : 343-354.

COULON T. and PRUD'HOMME P.Y., 2003. Effets d'un enherbement permanent sur la physiologie de la vigne dans les vignobles du Bordelais. *Progrès Agricole et Viticole*, 120 (7) : 154-161.

GAVIGLIO C. 2007. Alternatives au désherbage chimique : quelles stratégies pour l'entretien mécanique des vignes ? 20ème conférence du COLUMA, International Meeting on Weed Control, Dijon, 11-12 décembre 2007.

HEINZLE Y. 2002. Travail du sol et désherbage thermique. Colloque MONDIAVITI, Bordeaux, décembre 2002.

MAIGRE D. 1996. Comportement viticole et physiologique de la vigne soumise à différents modes d'entretien du sol. *Revue suisse Vitic. Arboric. Hortic.*, 28 (5) : 303-312.

SPRING J.L. 2001. Influence du type d'enherbement sur le comportemanet de la vigne et la qualité des vins. Résultats d'un essai sur Chasselas dans le bassin lémanique. *Revue suisse Vitic. Arboric. Hortic.*, 33 (5) : 253-260.

“Relación entre la superficie foliar expuesta, el nivel de radiación interceptado y el rendimiento global de la planta.”

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SUMMARY

The relationship between leaf surface area, solar radiation, sunlight exposure and its interception by the plant was studied under very warm climate conditions at Malpica de Tajo – Toledo -. For two consecutive years three training systems were evaluated: vertical shoot positioned (VSP) with 12 shoots per meter of row, and two non-positioned systems with different crop load namely Sprawl1, with 12 shoot per meter of row, and Sprawl2, with 18 shoots per meter of row. The influence of training system caused differences in dry matter and must composition. The results showed the higher leaf exposure the higher both daily PAR interception, dry matter (31.4%) and must soluble solids (5 -10%). Sprawl training system determined an increase in surface area by 40 -70 %. The higher crop load caused 40% increase in PAR interception. No differences in must composition were recorded for the first year while higher acidity levels were obtained in VSP for the second year. Berry weight was not affected neither by training system nor crop load.

RESUMEN

La relación entre la superficie foliar externa, radiación, exposición solar y su intercepción por la planta y por tanto, el consiguiente incremento de su rendimiento vegetativo y reproductivo fue estudiado en condiciones de clima muy cálido en Malpica de Tajo – Toledo - .La influencia del sistema de conducción causó diferencias en la producción de materia seca y en la composición del mosto. Tres sistemas de conducción fueron evaluados durante dos años consecutivos: sistema de posicionamiento vertical (VSP) con 12 pámpanos por metro de fila y dos sistemas no posicionados con diferente carga, Sprawl1 con 12 pámpanos por metro de fila y Sprawl2, con 18 pámpanos por metro de fila. Los resultados obtenidos muestran que la mayor exposición foliar, determinada por el incremento surpeficie foliar expuesta (40-77%) del sistema abierto (sprawl) y, por supuesto la carga mayor , causaron niveles superiores de intercepción de luz en sistemas abiertos (40%) comparado con sistemas fijos verticales para el balance diario de la radiación total acumulada (Ri- Rt; mol/h) y que fue resultado del incremento en el contenido de materia seca (31,4% más sobre el peso total de hojas y pámpanos del principal) y por tanto, en los sólidos solubles totales (5-10%más durante maduración) de la composición del mosto.

KEYWORDS

Training system, real leaf surface area (SFEr), solar radiation, must composition, sprawl, vertical shoot positioned

PALABRAS CLAVE

Sistema de conducción, superficie expuesta real (SFEr), radiación, composición del mosto, sprawl, espaldera..

INTRODUCCIÓN

La geometría de la planta determina la distribución espacial de los órganos aéreos de la cepa. Afecta, por un lado, a la actividad fotosintética y al comportamiento estomático de las hojas debido a que modifica, entre otros factores, la radiación interceptada, la temperatura y el estado hídrico de la planta (Patakas et al. 1997) y por otro, a la composición de la baya y el rendimiento, pues debido al modificarse el microclima de la planta, se verán afectados los procesos de maduración de la baya (Carbonneau y Costanza, 2004). El sistema de conducción actúa modificando la intercepción de radiación, la temperatura (Kliewer 1977; Spayd, et al. 2002), los procesos de ventilación y aireación interna en la zona de racimos y la fertilidad de las yemas (Goma-Fortin, 1998; Kliewer, 1980).

La determinación de la superficie foliar expuesta conjuga la actividad fotosintética de la planta con el rendimiento vegetativo y la forma de la vegetación; La forma de la cubierta vegetal puede modificar la eficiencia fotosintética y, por tanto, afectar a los parámetros de rendimiento y calidad (Schultz, 1995). La superficie externa (SA) tiende a sobredimensionar el valor de la superficie foliar fotosintéticamente activa al no tener en cuenta la porosidad. La superficie foliar expuesta real (SFEr) es un buen estimador de la superficie realmente expuesta a la luz, que resulta del producto del porcentaje de radiación interceptada por la cubierta y la superficie foliar total (LAI) (Carbonneau, 1995). Esta medida indirecta es un índice de la eficiencia de la superficie foliar (Peláez, 1999).

La correcta gestión de la vegetación tiene importantes efectos en el rendimiento global de la planta. En este sentido, muchos autores han comentado la importancia de la relación entre superficie foliar y el rendimiento como uno de los factores claves para el equilibrio de la planta y garantizar la correcta maduración de las bayas (Jackson y Lombard, 1993; Stewart et al, 1996; Murisier y Zufferey, 1997). Dokoozlian y Kliewer (1995) proponen alcanzar 7-15 cm^2/g fruto; Bonnisseau y Dufourcq (2004) obtuvieron que el color en los vinos tintos, en Burdeos, aumentó sensiblemente con valores superiores a 15 cm^2/g hasta un óptimo de 20 cm^2/g para ciertas variedades (Dufourcq et al, 2005). Kliewer y Dokoozlian (2005) en un ensayo con distintos sistemas de conducción y variedades, proponen 14,4 cm^2/g para sistemas verticales y posicionados y 15,5 cm^2 /g para sistemas abiertos tipo V-trellis.

Por otro lado, el rendimiento reproductivo de la planta se verá incrementado por efecto del rendimiento vegetativo y, del equilibrio de estos rendimientos dependerá por tanto, la calidad y cantidad de la cosecha. Dentro de los estimadores que mejor pueden reflejar estas relaciones están, sin duda, el balance de materia seca, el porcentaje de radiación interceptada por la planta y sus interacciones, que darán una fiel aproximación de la cantidad de biomasa generada, ya sea racimos, pámpanos, raíces, hojas o racimas.

La materia seca producida por la planta es un estimador directo de la fotosíntesis neta anual. Hay una relación lineal positiva entre la intercepción de radiación y la síntesis de materia seca (Miller y Howell, 1998; Howell 2001).

Diversos autores han reflejado el efecto del sistema de conducción y la carga en la productividad total del sistema (Peláez et al. 1995; Miller y Howell, 1998; Hunter, 2000; Wolf et al. 2003; Petrie et al, 2004). Un aumento de la carga implica un aumento en la productividad global de la planta, por lo que el rendimiento reproductivo se verá incrementado. Miller y Howell (1998) demostraron que el aumento de carga provocaba un incremento en la materia seca global de la planta; este aumento se debió a una mayor cantidad de materia seca destinada a racimos frente a la destinada a tallos y hojas, por lo que en cargas bajas había una mayor proporción de la parte vegetativa frente a la materia seca total que en cargas altas, debido, principalmente, al aumento del peso de los tallos, siendo la materia seca de las hojas más estable.

Por último, cabe destacar la importancia de este equilibrio en el crecimiento (vegetativo *vs*. reproductivo) para la correcta maduración de la baya. Tanto la carga como el sistema de conducción modifican las condiciones fisiológicas y microclimáticas de la cepa, alterando, a su vez, los componentes de la pulpa (sólidos solubles, acidez, pH) y del hollejo (antocianos, compuestos aromáticos volátiles de naturaleza fenólica y polifenoles en general…) que conllevan efectos visibles en el color, aroma, sabor y características organolépticas del mosto y, por ende, del vino, en general.

El presente ensayo persigue un doble objetivo, por un lado comparar y evaluar la respuesta de dos sistemas de conducción en zona muy cálida y, por otro, determinar el efecto del incremento de carga dejada en los sistemas libres y no posicionados (*sprawl*) acorde con las condiciones del medio, y cuantificar sus efectos sobre composición de la baya y del mosto y en el rendimiento productivo de la planta.

MATERIAL Y MÉTODOS

El ensayo fue realizado durante las campañas 2006 y 2007 en una parcela experimental (Latitud: 44º 15' y Longitud: 3º 59'y altitud 488 m) en Malpica de Tajo – Toledo - . El suelo era tipo arcillo-arenoso fino y el clima mediterráneo seco. La variedad empleada fue Syrah/110R a un marco de 2,7 x 1,2 m. La orientación de las líneas era NO – SE. El riego se realizó por goteo, con emisores de 3 L/h de caudal y situados a 1.2 m de distancia en el ramal, siendo idéntica la cantidad de agua aportada entre tratamientos.

El ensayo consta de tres tratamientos distribuidos en cuatro bloques al azar y cada parcela experimental consta de veinte plantas control separadas por filas y cepas borde.

Los tres tratamientos estudiados fueron:

- E1: Espaldera (VSP) con una carga de 12 pámpanos/ml.
- S1: Sprawl con una carga de 12 pámpanos/ml.
- S2: Sprawl con una carga de 18 pámpanos/ml.

El sistema de poda fue poda corta a pulgares de dos yemas en cordón Royat bilateral a 1,40 m. del suelo. El sprawl tuvo un par de hilos de vegetación a 40 cm sobre el portor separados 60cm entre sí. La espaldera tenía un par de hilos de vegetación a 30 cm del portor y un hilo superior a 1,5m del mismo.

Las condiciones climáticas del 2006 y 2007 (Tabla 1 y

Tabla 2) fueron muy diferentes, siendo 2006 una campaña extremadamente calurosa mientras que la del año 2007 fue más suave, pudiéndose observar diferencias en la acumulación de grado-día desde brotación hasta 31 de octubre (GDD) y en la evapotranspiración de referencia (Eto).

Tabla 1. Valores climáticos del año 2006

Mes	Tº Media mensual (ºC)	GDD[1] (ºC/mes)	Precipitación Efectiva (mm/mes)	ET_0[2] (mm/mes)
Enero	4,9	0,0	8,6	25,0
Febrero	6,1	0,0	17,5	41,8
Marzo	10,6	44	21,2	81,1
Abril	14,3	173	0,0	115,4
Mayo	19,6	471,3	0,0	163,3
Junio	23,6	879	4,9	184,1
Julio	27,3	1417	0,0	193,3
Agosto	25,1	1886	0,0	171,2
Septiembre	21,6	2235	0,0	115,7
Octubre	16,9	2449	115,8	67,5
TOTAL		**2525**	**168,00**	**1158,4**

Tabla 2. Valores climáticos del año 2007

Mes	Tº Media mensual (ºC)	GDD[1] (ºC/mes)	Precipitación Efectiva (mm/mes)	ET_0[2] (mm/mes)
Enero	5,2	0,0	8,6	16,3
Febrero	9,2	14	17,5	43,8
Marzo	10,2	38	21,2	87,9
Abril	12,5	125	0,0	101,6
Mayo	16,9	329	0,0	142,5
Junio	20,7	635	4,9	158,6
Julio	24,8	1084	0,0	182,8
Agosto	23,7	1526	0,0	163,8
Septiembre	21,1	1863	0,0	111,0
Octubre	14,5	2016	115,8	56,3
TOTAL		**2030**	**242,40**	**1064,61**

[1] GDD: grado día acumulado (growing degree day; ºC/mes)
[2] Et0: Evapotranspiración de referencia (mm).

Para evaluar la productividad global de los tratamientos estudiados se ha medido la cantidad y el reparto de materia seca de las partes renovables: hojas, tallos, racimos y racimas. La metodología aplicada fue la siguiente:

Se tomaron 8 muestras de pámpanos representativos en cada tratamiento. Se etiquetaron y se embolsaron para evitar pérdidas de humedad. En el laboratorio se descompusieron los pámpanos en: racimos, hojas principales, tallo principal, hojas de nietos y tallos de nietos. Se contó el número de nudos del pámpano principal, racimos, hojas principales, nietos, hojas de nietos y se midió la longitud del pámpano principal.

Se pesó por separado cada una de las partes separadas en una balanza COBOS® S.A. modelo C-600-SX de 0,01g de sensibilidad, expresando el resultado del peso fresco en g.

De entre los 8 pámpanos por parcela elemental muestreados, se escogió una sub-muestra de 2 pámpanos (25% de los pesados en fresco) para determinar el porcentaje de materia seca en cada una de las partes en que fueron descompuestos. Se secaron en una estufa modelo SELECTA® a 80ºC donde permanecieron hasta alcanzar peso constante, momento en el que se pesaron y se calculó el % de peso seco de cada una de las partes (tallo, hojas, racimos, tanto en principal como en nietos), para aplicarlo a cada una de las partes respectivas de los pámpanos muestreados. Se cuantifico la materia seca en hojas, tallos, racimos y racimas y su reparto (%) en cada una de las partes renovables respecto al total del pámpano. La materia seca por cepa se obtuvo multiplicando la materia seca total del pámpano medio por el número de pámpanos desarrollados por cepa.

Por otro lado, se calculó la superficie expuesta real o S.F.E.r (Carbonneau, 1995) como el resultado de multiplicar el porcentaje de radiación interceptada por la cubierta y la superficie foliar total desarrollada (L.A.I.) por la planta. Se tomaron datos de radiación a lo largo del periodo de maduración en diferentes horas del día (8:00 12:00 y 16:00 hora solar) para el cálculo de dicha variable. El estudio de radiación se realizó en un día despejado en el periodo de maduración. Se realizó un balance diario horario en el periodo de maduración en los dos años de estudio según el método propuesto por Varlet-Grancher et al. (Peláez, 1989), en base a la en el que quedan definidos cuatro zonas de cuantificación de radiación en el balance (R_a) que son:

R_i = radiación incidente.

$Rr_{(s)}$ = radiación reflejada por el suelo.

R_t = radiación transmitida por la cubierta al suelo.

$R_{(sp)}$ = radiación reflejada por el conjunto suelo-cultivo.

$$R_a = R_i - R_t + (Rr_{(s)} - R_{(sp)})$$

Las radiaciones transmitidas y reflejadas por el suelo y el conjunto planta-suelo son muy pequeñas respecto a la incidente y transmitida por la cubierta (3-5% del valor total) por lo que Ra se puede reducir a la diferencia (Ri - Rt) (Gonzalez-Padierna, 2003).

Las medidas de la radiación se realizarón con un sensor lineal de PAR (LI-191SA, LICOR®, Lincoln, E.E.U.U.) provisto de un detector fotovoltaico de Silicio de alta sensibilidad, con un área sensible al PAR de 1000 x 12,7 mm y un tiempo de respuesta de 10 µs. Con una unidad portátil de registro y almacenamiento de datos (Datalogger, LI-1000, LICOR®, Lincoln, E.E.U.U.) permite registrar las lecturas en $\mu mol \bullet m^{-2} \bullet s^{-1}$. La superficie receptiva es plana y los rayos que no inciden perpendicularmente son corregidos en función del coseno del ángulo con el que llegan. Detectan quanta de luz, y generan una señal eléctrica proporcional al número de fotones recibidos.

En cada tratamiento se colocaron unas barras metálicas perfectamente niveladas a 50 cm de altura del suelo atravesando el ancho de calle (Figuras 1 y 2). Como el ancho era de 2,7 m, se optó por una barra de 3m con un ángulo de 27º de inclinación sobre la normal, de manera que siempre se tienen tres medidas en cada barra, para emplear toda la superficie útil del ceptómetro. El sensor se colocó (Figuras 1 y 2) fuera del sistema, en una superficie perfectamente nivelada, con objeto de medir la radiación incidente (R_i) al principio y al final del recorrido en cada periodo de medida. El sensor se colocó debajo de la vegetación y orientado hacia arriba, de tal manera para medir la radiación transmitida por la cepa (R_t), que llega al suelo después de pasar por el conjunto de atmósfera y cubierta vegetal (Figuras 1 y 2).

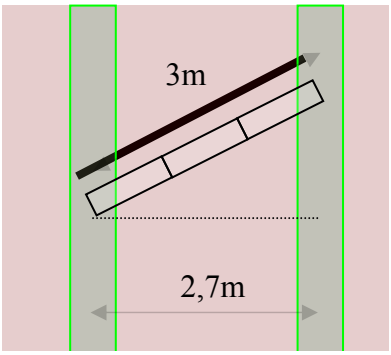


Figura 2. Esquema de la planta del viñedo donde las barras horizontales se colocan en la diagonal y del ángulo de 27º que forman con la distancia real de la calle del viñedo.

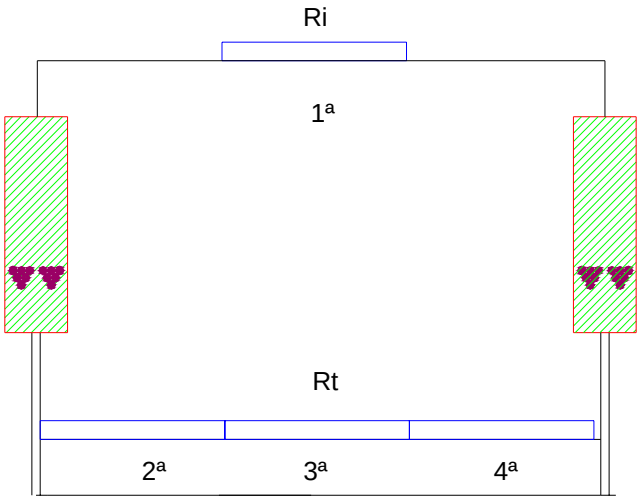


Figura 1. Esquema del dispositivo de medida de PAR.

Se establecieron dos repeticiones por tratamiento y, el tiempo empleado para cada medida horaria fue de unos 20 minutos aproximadamente, en completar el recorrido de los seis puntos de medida.

Se escogió un día completamente despejado, desde el amanecer hasta el anochecer, entre envero-maduración. En total se realizaron 15 balances horarios, desde las 5:00 hasta las 19:00 hora solar.

Los seguimientos evolución de la composición de la baya durante la maduración se realizaron con periodicidad semanal entre envero y vendimia. Se muestrearon 100 bayas por tratamiento y bloque, con las cuales se determinó el peso medio de la baya, y posteriormente se obtuvo el mosto para los análisis. La extracción del mosto se realizó mecánicamente mediante un

pasapurés, se centrifugó para eliminar las sustancias en suspensión, y se recogió el líquido sobrenadante en una probeta para llevar a cabo los análisis.

La medida de la concentración de sólidos solubles totales (SST) se realizó con un refractómetro digital (ATAGO, serie Palette PR 32) con corrección por temperatura. El valor del pH del mosto se realizó con un pHmetro digital (micropH 2001, Crison) directamente en el mosto centrifugado. La acidez total (g ácido tartárico/L) se ha determinado mediante la neutralización volumétrica con sosa 0,1 N hasta pH 8,2 (Ough y Amerine, 1988).

RESULTADOS Y DISCUSIÓN

Superficie foliar expuesta real. Para ver la relación de la superficie foliar (vegetación y porosidad) y la radiación interceptada por la planta, el mejor indicador existente es la superficie real expuesta real (Carbonneau 1995). Es un indicador que engloba la superficie total foliar (L.A.I.; m^2 área foliar / m^2 suelo) y el porcentaje de radiación fotosintéticamente activa interceptada (P.A.R; $\mu mol \cdot m^{-2} \cdot s^{-1}$) por la planta. Los resultados medidos a las 8 h.s.; 12 h.s. y 16 h.s. reflejan (Tabla 3) la mayor exposición a lo largo del día del tratamiento sprawl2 en relación a los otros dos, llegando a alcanzar valores muy superiores comparado con la espaldera (entre 36,4% y 68,4% más; P<0,001). El sprawl1 obtuvo valores intermedios, por lo que se hace patente que el efecto combinado de la carga y el sistema provocó mayores eficiencias de exposición foliar entre las plantas de los tratamientos estudiados.

Tabla 3. Superficie expuesta real (S.F.E.r.) para los tres tratamientos.

Año	Tratamiento	SFEr 8hs	SFEr 12hs	SFEr 16hs
2006	Espaldera	0,47[c]	0,31[c]	0,41[b]
	Sprawl 1	0,83[b]	0,56[b]	1,12[b]
	Sprawl 2	1,15[a]	0,98[a]	1,23[a]
	EEM[1] (n=32)	0,08	0,08	0,08
	Sig[2]	***	***	***
2007	Espaldera	1,08[b]	1,03[b]	1,38
	Sprawl 1	1,01[b]	0,74[b]	1,59
	Sprawl 2	1,78[a]	1,62[a]	1,65
	EEM[1] (n=32)	0,08	0,08	0,08
	Sig[2]	***	***	**ns**

[1] EEM: error estándar de la media para n= 32 muestras por tratamiento.
[2] Sig: diferencias significativas; ns y ***, no existen diferencias significativas y P<0,001 respectivamente. Los valores con la misma letra son iguales.

Asimismo, las mayores tasas de eficiencia en la captación de la radiación por la superficie foliar se obtienen entre las 8:00 y las 12:00 hora solar, cuando la planta registra la mayor actividad fotosintética neta durante el día, hasta el cierre estomático producido por la diferencia entre las pérdidas por transpiración y el descenso de la actividad fotosinética de las hojas.

Balance de radiación. Este hecho se ve reflejado en las tasas de intercepción de radiación acumulada a lo largo del día durante maduración (Ilustración 1e Ilustración 2) según el balance calculado como diferencia entre la radiación incidente sobre la planta (Ri) y la radiación interceptada tras el paso por la vegetación de la misma (Rt).

El tratamiento sprawl2 presenta mayores tasas de radiación interceptada por hora y día en los dos años de estudio y, además, con mayor eficiencia, debido principalmente al efecto del sistema de conducción y, en menor medida, a la carga.

Los datos del balance en el año 2006 muestran cómo el tratamiento que mayor R_t presenta es el sprawl2, desde primeras horas de la mañana hasta el mediodía solar, momento en el cual el tratamiento sprawl1 es el que mayores tasas de radiación interceptada refleja. En cualquier caso el tratamiento espaldera siempre es el que menor radiación intercepta,

igualando estos valores sólo a primera y última hora del balance (5 h.s. y 19 h.s. respectivamente).

Esta conducta también se observa en el año siguiente (2007) con menores diferencias, dado que la R_i es menor en valor absoluto para ese año, por lo que el efecto del sistema parece claro, siendo mejor la intercepción de radiación con la división del *canopy*, al aumentar la superficie foliar expuesta (Carbonneau, 1980; Mabrouk et al, 1997).

Ilustración 1. Balance de radiación acumulado (Ri-Rt) en mol/h para los tres tratamientos durante la campaña de 2006.

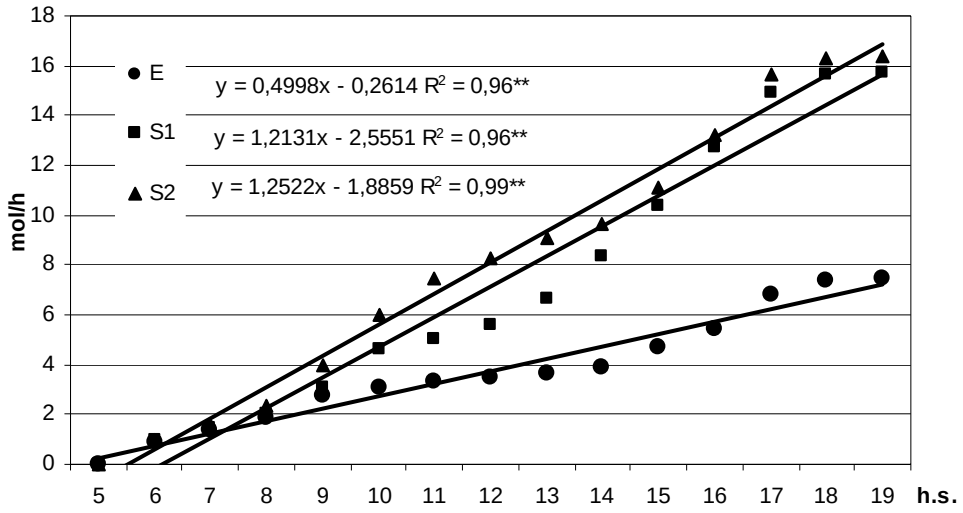
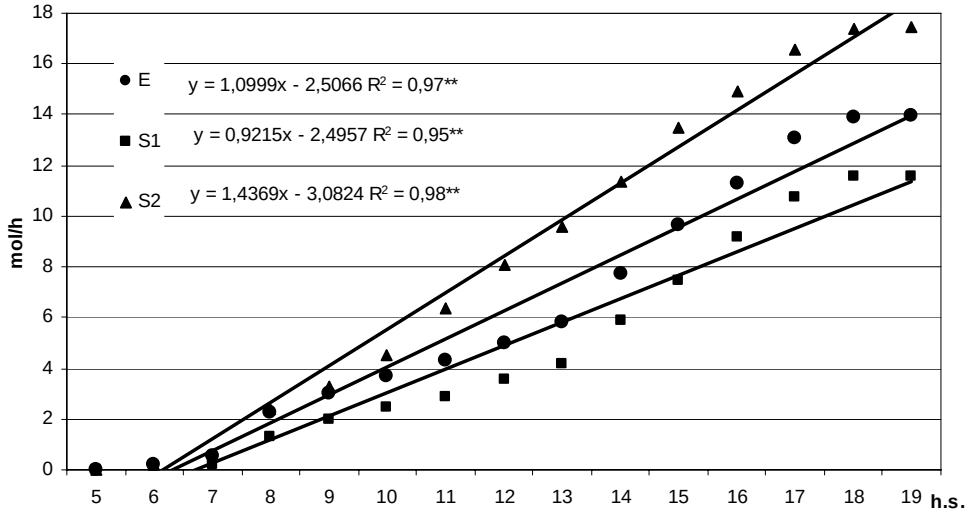


Ilustración 2. Balance de radiación acumulado (Ri-Rt) en mol/h para los tres tratamientos durante la campaña de 2007.



Otro concepto a tener muy en cuenta es la eficiencia de la captación energética (ε_i). En condiciones climáticas calurosas, donde el estrés hídrico es una situación más que frecuente y la temperatura, humedad relativa y demanda atmosférica evapotranspirativa, son factores limitantes para la mayoría de los procesos fisiológicos, es muy importante el óptimo aprovechamiento de la reserva hídrica del suelo y de la eficiencia de la captación de radiación

del medio; es decir, del cociente entre radiación incidente y radiación interceptada (Varlet-Grancher et al. 1989 en Mabrouk et al, 1997):

$$\varepsilon_i = 1 - (R_t/R_i)$$

Dónde R_t s la radiación transmitida por la cubierta al suelo y R_i la incidente en el sistema. Este valor se encuentra entre 0 (suelo desnudo) y 1 (cubierta totalmente opaca).

Según los resultados mostrados en Tabla 4 en relación al balance medio diario, se puede observar que, para los dos años de estudio el sistema que mayor tasa de radiación interceptada presentó fue el tratamiento sprawl2, donde las diferencias al final del día supusieron entre un 54% y un 42% (2006 y 2007 respectivamente; P<0,05) más que el sistema vertical (espaldera). El sprawl1 marcó niveles superiores a la espaldera (48 y 33% para 2006 y 2007 respectivamente; P<0,05) pero inferiores al sprawl2 (12 y 25% para 2006 y 2007 respectivamente; P<0,05).

Tabla 4. Balance diario medio (Ri-Rt.) y eficiencia de captación energética (εi) para los tres tratamientos.

Año	Tratamiento	Ri-Rt (mol/h)	ε_i
2006	Espaldera	3,74 [b]	0,18 [b]
	Sprawl 1	7,15 [a]	0,29 [a]
	Sprawl 2	8,13 [a]	0,31 [a]
	EEM[1] (n=16)	0,92	0,04
	Sig[2]	**	*
2007	Espaldera	4,88 [b]	0,38
	Sprawl 1	6,29 [ab]	0,32
	Sprawl 2	8,41 [a]	0,42
	EEM[1] (n=16)	1,04	0,04
	Sig[2]	*	**ns**

[1] EEM: error estándar de la media para n= 16 medidas por tratamiento.
[2] Sig: diferencias significativas; ns y *,**, no existen diferencias significativas y P<0,05 y P<0,01 respectivamente. Los valores con la misma letra son iguales.

Esto implica unos niveles superiores de eficiencia en la intercepción de la radiación en los tratamientos conducidos en *sprawl* frente al conducido vertical tipo espaldera, que de manera particular se reflejó en la campaña 2006 siendo un 40% superior (P<0,05).

Producción de materia seca y su reparto. Las diferencias en la intercepción de la radiación deberían reflejar un incremento en la producción de la planta debido al mayor uso de los recursos naturales, en este caso de la radiación solar interceptada. Los resultados de la producción quedan expuestos en la materia seca evaluada (Tabla 5 y Tabla 6), para los tres tratamientos en el 2006 reflejan que el peso neto de las hojas tanto de principal como de nietos, es mayor en los tratamientos de *sprawl* que en la espaldera , esto implica que las hojas de estos tratamientos tienen un tamaño mayor y por tanto al ser principales fuentes de fotoasimilados, responsables de tasas mayores de acumulación de materia seca global en la planta. Este comportamiento también se manifiesta en la campaña siguiente (2007), aunque las diferencias no fueron tan patentes. El tratamiento sprawl1 obtiene mayores tasas de materia seca en racimos (8%) y hojas (23%) y, por otro lado, menor en tallos (5%) que el tratamiento vertical en espaldera con distinto sistema de conducción.

Por otro lado, el efecto de la carga también es claro, pues para el tratamiento de mayor carga (sprawl2) se obtiene un menor desarrollo de nietos (17%; P< 0,05) frente al sprawl1 con el mismo sistema de conducción, con lo que el pámpano principal tiene mayor funcionalidad y responsabilidad en el cómputo global productivo.

Tabla 5. Distribución de la materia seca para los tres tratamientos (E, S1 y S2) en vendimia (243 DOY) del 2006.

Año 2006	Principal (g/pámpano)			Nietos (g/pámpano)		
	Tallo	**Hojas**	**Racimos**	**Tallo**	**Hojas**	**Racimas**
Espaldera	46,9	16,2 b	131,3	3,4	7,9 b	0,9
Sprawl 1	44,8	21,1 a	142,7	1,9	15,6 a	1,8
Sprawl 2	25,9	20,8 a	83,8	2,3	5,7 b	0,0
EEM[1] (n=8)	7,02	2,27	30,32	0,61	1,76	0,40
Sig[2]	**ns**	*	**ns**	**ns**	**	**ns**
	Principal (nº)			**Nietos (nº)**		
	Nudos	**Hojas**	**Racimos**	**Nietos**	**Hojas**	**Racimas**
Espaldera	12,5 b	11,0 b	2,4	11,0 b	27,4 b	2,0
Sprawl 1	18,5 a	17,2 a	2,2	13,4 a	37,4 a	1,7
Sprawl 2	20,1 a	19,7 a	2,1	11,2 b	21,6 b	0,0
EEM[1] (n=8)	1,40	1,67	0,22	1,02	3,15	0,54
Sig[2]	*	*	**ns**	*	**	**ns**

[1] EEM: error estándar de la media para n= 16 medidas por tratamiento.
[2] Sig: diferencias significativas; ns y *,**, no existen diferencias significativas y P<0,05 y P<0,01 respectivamente. Los valores con la misma letra son iguales.

En el 2007, las diferencias se reflejan en el número de nudos y de hojas del pámpano principal de la espaldera que es significativamente menor que los tratamientos en *sprawl* (como ya ocurriera en 2006), debido al manejo del cultivo. También se mantiene la tendencia del mayor peso de hojas de principal de los sistemas en *sprawl*, frente a la espaldera, aunque no presente diferencias significativas. La principal diferencia vuelve a ser el menor número de secundarios del tratamiento de mayor carga (S2), efecto positivo desde el punto de vista de la producción de azúcares y acumulación en racimos del principal.

Tabla 6. Distribución de la materia seca para los tres tratamientos (E, S1 y S2) en vendimia (254 DOY) del 2007.

Año 2007	Principal (g/pámpano)			Nietos (g/pámpano)		
	Tallo	**Hojas**	**Racimos**	**Tallo**	**Hojas**	**Racimas**
Espaldera	37,1	17,6	102,7	8,1 a	20,7 a	2,9
Sprawl 1	38,7	19,7	99,6	6,5 a	17,7 a	4,6
Sprawl 2	36,8	19,6	90,8	2,4 b	9,3 b	1,2
EEM[1] (n=8)	2,7	1,29	9,4	1,06	1,93	0,41
Sig[2]	**ns**	**ns**	**ns**	**	**	**ns**
	Principal (nº)			**Nietos (nº)**		
	Nudos	**Hojas**	**Racimos**	**Nietos**	**Hojas**	**Racimas**
Espaldera	14,8 b	13,3	1,8	11,1	48,8 a	1,0 b
Sprawl 1	18,0 ab	15,1	2,0	11,5	38,4 b	2,0 a
Sprawl 2	19,3 a	16,3	2,0	10,3	25,3 c	1,0 b
EEM[1] (n=8)	1,29	1,48	0,65	0,82	3,22	0,05
Sig[2]	*	**ns**	**ns**	**ns**	**	*

[1] EEM: error estándar de la media para n= 16 medidas por tratamiento.
[2] Sig: diferencias significativas; ns y *, **, no existen diferencias significativas y P<0,05 y P<0,01 respectivamente. Los valores con la misma letra son iguales.

Por último, conviene recordar que para todos los cálculos de rendimiento en materia seca por planta, hay que tener en cuenta que el tratamiento sprawl2 tiene 18 pámpanos/ml frente a los 12 de la espaldera y sprawl1. Estas diferencias por planta y metro lineal se ven en la Tabla 7, el rendimiento del sprawl2 es mayor en número de racimos (31% más que sprawl1 y espaldera) y hojas (46% más que sprawl1 y espaldera), así como en peso de tallos y hojas (17% y 31% respectivamente más que sprawl1 y espaldera), independientemente del año estudiado. Por otro lado, el menor peso de tallos (45%) y hojas (25%) de nietos en este

tratamiento en relación a los otros dos, sí es dependiente de la campaña estudiada, así como el número de nietos, que muestra una diferencia del 45% menos de nietos del sistema en espaldera frente a los tratamientos en *sprawl*.

Tabla 7. Distribución de la materia seca por planta y metro lineal para los tres tratamientos (E, S1 y S2) en vendimia de las dos campañas estudiadas.

Rdto (g/ml)	Principal			Nietos		Total	
	Tallo	Hojas	Racimos	Tallo	Hojas	Hojas	Tallo
Espaldera	452,15[b]	200,24[b]	1189,31	69,17[a]	185,23[ab]	385,45[b]	514,89
Sprawl 1	475,01[b]	236,00[b]	1298,71	69,50[a]	194,27[a]	430,26[ab]	534,73
Sprawl 2	558,93[a]	355,70[a]	1545,29	38,32[b]	142,96[b]	468,66[a]	625,70
EEM[1] (n=18)	44,10	17,13	133,70	9,58	19,37	26,54	46,21
Sig[2]							
Trat	*	***	**ns**	***	*	**	**ns**
Año	**ns**	**ns**	**ns**	**	**	*	**ns**
Trat*Año	**ns**	**ns**	**ns**	**ns**	**ns**	**ns**	**ns**
Rdto (nº/ml)	**Principal**			**Nietos**			
	Nudos	**Hojas**	**Racimos**	**Nietos**	**Hojas**		
Espaldera	154,26[c]	145,83[b]	24,31[b]	129,63[b]	487,40[a]		
Sprawl 1	214,12[b]	185,99[b]	24,31[b]	140,00[ab]	435,10[b]		
Sprawl 2	345,14[a]	308,20[a]	35,0[a]	184,72[a]	418,05[b]		
EEM[1] (n=18)	16,19	17,41	1,23	33,41	9,35		
Sig[2]							
Trat	***	***	***	**	**		
Año	**ns**	**ns**	**ns**	**ns**	***		
Trat*Año	**ns**	**ns**	**ns**	**ns**	**ns**		

[1] EEM: error estándar de la media para n= 16 medidas por tratamiento.
[2] Sig: diferencias significativas; ns y *,**, ***, no existen diferencias significativas y P<0,05; P<0,01; P<0,001 respectivamente. Los valores con la misma letra son iguales.

Ahora bien, es evidente que con un incremento del 50% de la carga (sprawl2 vs. sprawl1 y espaldera) se consigue para las condiciones de ensayo, mayor intercepción de radiación y, por ende, mayor producción de materia seca por planta, pero también es importante la calidad de la baya que se puede producir con este incremento de carga. Los resultados del análisis de mosto en maduración (Tabla 8) reflejan que el incremento de carga no merma el peso de baya.

Tabla 8. Analíticas de maduración para los tres tratamientos (E, S1 y S2) en fecha de vendimia (30/08 en 2006 y 05/09 en 2007)

Año 2006	Peso de 100 bayas (g)	ºBrix	pH	Acidez (g ac tartárico/L)
Espaldera	111,9	25,1	3,5	5,9
Sprawl 1	105,4	25,9	3,5	5,2
Sprawl 2	107,0	25,8	3,5	5,2
EEM[1] (n=8)	3,99	0,76	0,02	0,46
Sig[2]	**ns**	**ns**	**ns**	**ns**
Año 2007	**Peso de 100 bayas (g)**	**ºBrix**	**pH**	**Acidez (g ac tartárico/L)**
Espaldera	160,0	25,2	3,06[b]	6,3[a]
Sprawl 1	155,1	25,4	3,13[a]	5,8[b]
Sprawl 2	157,4	24,7	3,20[a]	5,9[b]
EEM[1] (n=8)	3,69	0,27	0,02	0,07
Sig[2]	**ns**	**ns**	**	**

[1] EEM: error estándar de la media para n= 16 medidas por tratamiento.
[2] Sig: diferencias significativas; ns y **, no existen diferencias significativas y P<0,01 respectivamente. Los valores con la misma letra son iguales.

Asimismo, los datos del año 2006 no muestran diferencias en el análisis final de vendimia para ninguno de los parámetros estudiados tan sólo cierta tendencia a una mayor acidez del tratamiento en espaldera respecto a los *sprawl*. Además, los *sprawl*, aunque alcanzan valores finales de acidez más bajos, presentan una velocidad de degradación menor, es decir, la acidez se degrada más lentamente durante toda la maduración, hecho debido probablemente a la menor exposición de sus racimos.

El año 2007 sufrió un periodo de maduración más prolongado y se obtuvieron unos valores finales de acidez cercanos a 6,0 g/L de TH_2, dentro de un rango de pH 3,2-3,3, lo cual es deseable para vinificaciones de tintos en climas cálidos.

El hecho de que el tratamiento S2 tenga una carga mayor para la maduración de la baya refleja una conclusión importante: desde el punto de vista de la acidez (pH y g/L de TH_2) no se tienen diferencias significativas en distintos años y situaciones climáticas, pero sí refleja un ºBrix más bajo debido al mayor número de sumideros disponibles, por lo que retrasa ligeramente su maduración, manteniendo niveles adecuados de acidez para su vinificación; lo que en climas cálidos o semiáridos representa una gran ventaja para lograr los objetivos deseados en vendimia.

CONCLUSIONES

En condiciones de clima semiárido, donde la radiación no es un factor limitante, el incremento de la carga en sistemas de conducción del viñedo no debe suponer una merma ni de la cantidad de cosecha producida, ni de la calidad de la misma, siempre y cuando tengamos disponibilidad de agua y nutrientes en el medio suelo-planta-atmósfera.

El uso de sistemas no posicionados y divididos frente a los sistemas verticales y posicionados puede resultar una alternativa muy eficaz para retrasar la maduración y favorecer la acumulación de solutos en la baya, sin mermar otras sustancias del hollejo (antocianos y polifenoles) por efecto de la sobre exposición de racimos.

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REFERENCIAS BIBLIOGRÁFICAS

Baeza, P, J.R. Conde, J.R. Lissarrague y P. Junquera (2005). "Agronomic and ecophysiological responses of field-grown "Cabernet Sauvignon" grapevines to three irrigation treatments." proc. VIIth IS on Grapevine: 373 - 379.

Bonnisseau, M. y T. Dufourcq. (2004). "Adaptation de la conduite du vignoble: la gestion du rapport feuilles/fruits". MONDIAVITI-Bordeaux. 1º-2º Dic. 139-149.

Carbonneau, A. y P. Costanza. (2004). "Réponse du potentiel hydrique foliaire de la vigne à des variations rapides d'exposition de la végétation. Exemple de la manipulation de l'ouverture de la végétation du Merlot (Vitis vinifera L.)". Journal International des Sciences de la Vigne et du Vin. 38. 27-33.

Carbonneau, A. (1995). "La surface foliare exposée potentielle." Le progrès agricole et viticole 112(9): 204-212.

Carbonneau, A. (1980). "Recherche sur les systemes de conduite de la vigne : essai de maitrise du microclimat et de la plante entiere pour produire economiquement du raisin de qualite". I.N.R.A. I.N.R.A. Bourdeax. 234.

Dokoozlian, N y W.M. Kliewer. (1995). "The light environment within grapevines canopies. I. Description and seasonal changes during fruit development". Am.J.Enol. Vitic. 46. 209-218.

Dufourcq, T, L. Gontier, E. Serrano y N. Ollat. (2005). "Leaf area and crop yield ratio: Effects on vine water status, must quality, wine quality for four varieties trained in south-west France". Progrès Agricole et Viticole. 122. 503-507.

Gonzalez-Padierna, C.M. (2003). "Estudio ecofisiológico y agronómico de cuatro sistemas de conducción de la vid (Vitis vinífera L.): cubiertas vegetales simples versus divididas." Producción vegetal. Fitotecnia. E.T.S.I.A. Politécnica de Madrid. Madrid. 280.

Goma-Fortin, N. (1998). "Incidence du palissage sur la qualité de la rècolte de la vigne." Progrès Agricole et Viticole. 115. 337-345.

Howell, S.G. (2001). "Sustainable grape productivity and the Growth-Yield relationship: a review". American Journal of Enology and Viticulture. 52. 165-174.

Hunter, J.J. (2000). "Plant spacing effects on root growth and dry matter partitioning of Vitis vinifera L. cv Pinot noir/99Richter and implications for soil utilization". 526. 63-74.

Jackson, D.I. y P.B. Lombard. (1993). "Environmental and management practices affecting grape composition and wine quality- a review". Am. J. Enol. vitic. 44. 409-430.

Kliewer, W. M. y N. Dokoozlian. (2005). "Leaf area/crop weight ratios of grapevines: influence on fruit composition and wine quality". Am. J. Enol. Vitic. 56. 170-181

Kliewer, W. (1980). "Vineyard canopy management - A review". 342-352.

Kliewer, W. (1977). "Influence of temperature, solar radiation and nitrogen on coloration and composition of Emperor grapes". American Journal of Enology and Viticulture. 28. 96-103.

Mabrouk, H; A.Carbonneau y H. Sinoquet. (1997). "Canopy structure and radiation regime in grapevine I. Spatial and angular distribution of leaf area in two canopy systems". Vitis. 36. 119-123

Miller, D.P y G.S. Howell. (1998). "Influence of vine capacity and crop load on canopy development, morphology and dry matter partitioning in Concord grapevines". Am.J.Enol. Vitic. 49. 183-190.

Murisier, F. y V. Zufferey. (1997). "Rapport feuille-fruit de la vigne et qualité du raisin". Revue suisse de viticulture, arboriculture and horticulture. 29. 355-362.

Ough, C.S y M.A. Amerine. (1988). "Methods for Analysis of musts and wines". John Wiley & Sons. 377.

Patakas, A; B. Noitsakis y K. Chartzoulakis. (1997). "Changes in W.U.E. in vitis vinifera as affected by leaf age". Acta Horticulturae. 449 Vol. 2. 457-460.

Peláez, H. (1999). "Balance de radiación fotosintéticamente activa, microclima luminoso y evaluación fisiológica y agronómica de distintos sistemas de conducción en diferentes regímenes hídricos en el cultivo de la vid (Vitis vinifera L.)". Producción Vegetal: Fitotécnia. E.T.S.I.A. U.P.M. Madrid. 272.

Peláez, H., J.A Rubio; L.M. Robredo; P. Baeza y J. Yuste. (1995). "Canopy radiation balance and dry matter production in different trainning systems. Relationship with geometry and foliar development in irrigated an non-irrigated vines." Acta Horticulturae.

Petrie, P. R; N.M. Coole y P.R. Clingeleffer. (2004). "The effect of post-veraison water deficit on yield components and maturation of irrigated Shiraz (Vitis vinifera L.) in the current and following season". Australian Journal of Grape and Wine Research. 10. 203-215.

Scheneider, C (1989). "Introduction to the ecophysiology of grapevine. Application to training systems." Bulletin de l'O.I.V. 62((701-702)): 498-515.

Schultz, H.R. (1995). "Grape canopy structure, light microclimate and photosynthesis I. A two dimensional model of the spatial distribution of surface area densities and leaf ages in two canopy systems." Vitis. 34. 211-215.

Smart, R. E.y J. B. Robinson, et al. (1985). "Canopy microclimate modification for the cultivar Shiraz I. Definition of canopy microclimate." Vitis 24: 17-31.

Sommer, K. J y M. Islam. (2000). "Light and temperature effects on shoot fruitfulness in Vitis vinifera L. cv. Sultana: Influence of trellis type and grafting." Aus. J. Grape and Wine Research 6: 99-108.

Spayd, S.E; .J.M. Tarara; D.L. Mee y J.C. Ferguson. (2002). "Separation of sunlight and temperature effects on the composition of Vitis vinifera cv. Merlot berries". American Journal of Enology and Viticulture. 53. 171-182.

Stewart, W. P; B. M. Freeman y J. K. Dick. (1996). "Carbon partitioning in potted post-veraison *Vitis vinifera* L. cv. Riesling vines: Effect of dormant cane length". S. Afr. J. Enol.Vitic. 17. 38-42.

Wolf, T.K; P. R. Dry; P. G. Iland; D. Botting; J. Dick; U. Kennedy y R. Ristic. (2003). "Response of Shiraz grapevines to five different training systems in the Barossa Valley, Australia". Aus. J. Grape and Wine Research. 9. 82-95.

Projet paysager viticole :
Concept et retours d'expériences dans différentes régions françaises

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RESUME

Composante d'une multifonctionnalité de la viticulture, les paysages sont des vecteurs de communication historique, culturelle et environnementale qui enrichissent le potentiel des régions viticoles.

Au-delà de l'étude paysagère, qui définit les particularités des terroirs sous un angle esthétique, il est nécessaire de prolonger cette démarche par un travail d'animation locale au travers d'un projet de territoire associant les organisations viticoles et les collectivités territoriales.

Le projet paysage de territoire peut s'intégrer dans une démarche de reconnaissance nationale ou internationale établie sur la base d'une charte (Fontevraud www.chartedefontevraud.org, UNESCO, etc.).

L'objectif de cette communication vise à établir le retour d'expériences de régions viticoles qui ont initié des projets paysagers pour lesquels l'IFV a été impliqué dans l'accompagnement des démarches.

Au regard de l'enjeu lié à la préservation et à la valorisation des paysages, différente instituts techniques agricoles français ont initié un projet APPORT (Paysages agricoles, des outils pour des projets de développement durable des territoires).

Le projet présenté dans la communication comporte notamment :

- 9 brochures concernant les méthodes, les outils et les orientations liées à la relation agriculture et paysage

Un site Internet ressource sur cette thématique regroupant notamment toutes les productions et informations collectées lors de ce projet www.agriculture-et-paysage.fr

SUMMARY

These landscapes are an element of the multifunctionality of viticulture, and are vectors of historical, cultural and environmental communication, enriching the potential of our wine-producing regions.

In addition to the landscape survey, which defined the particularities of these terroirs from an aesthetic perspective, it is necessary to prolong this measure with local activities as part of a territorial project including viticultural organisations and local authorities.

This project could be incorporated in measures to obtain national or international recognition, based on a charter (Fontevraud, www.chartedefontevraud.org , UNESCO, etc.).

The goal of this communication is to establish feedback from the viticultural regions which initiated the landscaping projects for which the IFV (*Institut Français de laVigne et du Vin*) provided support.

Regarding the issues of preservation and promotion of landscapes, various French technical agricultural institutes have led to the introduction of an APPORT project (agricultural landscapes, tools for sustainable territorial projects).

The project presented in the communication comprises in particular:

- Nine brochures on methods, tools and directions to be taken with regard to agriculture and its relationship with the landscape
- A resource website on this theme, grouping together all the information and documentation produced and collected during this project, www.agriculture-et-paysage.fr

INTRODUCTION

La terre, dont l'évolution nous a transmis des empreintes indélébiles, est à l'origine de la variété de la géologie des sols, des reliefs. Le climat conditionne la conduite de la vigne et la végétation des écosystèmes associés au terroir viticole. A partir de ces contraintes naturelles, l'homme a su valoriser les terroirs les plus favorables et adapter au contexte local les techniques viticoles appropriées. Mais au-delà de la vigne, le vigneron est aussi un acteur environnemental du territoire, créateur d'architecture, gardien des coutumes et traditions locales, autant d'aspects historiques et culturels qui participent à l'identité du paysage local.

Les paysages sont des enjeux de la durabilité des terroirs. Ils participent à l'identité et au sentiment d'appartenance culturelle de chaque région. Les éléments constitutifs des paysages viticoles tels que les vignes, les murets, les talus, les aménagements végétaux et les arbres associés, participent au maintien d'une biodiversité spécifique qui peut parfois permettre de lutter contre les parasites de la vigne.
La structuration du paysage peut participer à la conservation des sols et à la préservation de la qualité de l'eau (notamment vis-à-vis du ruissellement et de l'érosion).
Le lien esthétique entre la viticulture et le grand public doit être envisagé comme une valeur ajoutée au produit. Au-delà du rôle de support de communication pour la filière, les paysages participent à la valorisation économique de toute une région, par le biais de l'activité touristique qu'ils génèrent avec tous les emplois induits.

La France bénéficie d'une remarquable diversité de paysages ruraux qui reflète la capacité des agriculteurs à s'adapter à toute la variété des situations géomorphologiques et climatiques. Les paysages viticoles de par leurs qualités souvent exceptionnelles sont reconnus comme un des fleurons du patrimoine paysager national. Les viticulteurs ont été parmi les premiers à comprendre à quel point le paysage constituait pour eux un atout économique et un atout en termes de reconnaissance sociale.
Les vignobles français ont des caractéristiques communes :

- un aspect linéaire et régulier des vignes,
- la présence de constructions particulières incluses ou non dans le parcellaire (cabanes de vignes, chemins, talus, exutoires),
- la qualité fréquente de l'architecture des villages donnant un caractère particulier aux paysages viticoles.

1 UN PATRIMOINE FRAGILE

Dans une approche globale, le patrimoine recouvre « *un ensemble d'éléments matériels ou immatériels qui témoignent des relations particulières qu'une communauté humaine a instaurées au cours de l'histoire avec un territoire* ».
Les paysages ne cessent d'évoluer, du fait des modifications naturelles des milieux, des interventions humaines, elles-mêmes fonction des avancées techniques. Cette dynamique paysagère est un atout

majeur des paysages, leur donnant vie, leur permettant d'évoluer, sans rester figés tels des objets de musées. Néanmoins la viticulture a traversé des périodes charnières (crise phylloxérique, mécanisation), à l'origine d'un bouleversement des pratiques viticoles, support des paysages.

La modernisation du vignoble s'est parfois traduite par :

- une simplification du paysage par agrandissement des parcelles,
- la modification des aménagements de coteaux,
- l'élimination de nombreuses structures paysagères (arbres isolés ou alignés, haies, talus, bosquets...) ou architecturales.

Les paysages viticoles sont parfois soumis à des arrachages importants, liés à la crise du secteur qui modifie la typicité paysagère locale. Parallèlement certains vignobles subissent des agressions esthétiques en liaison avec l'urbanisation.

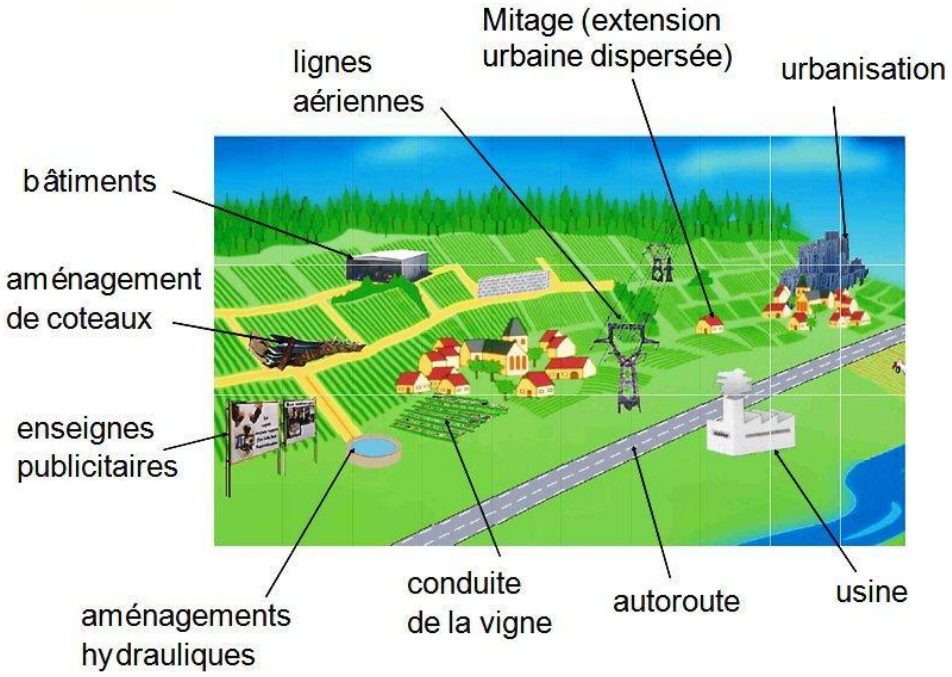

Figure 1 : Sources d'agression des paysages viticoles

Au-delà de son aspect fonctionnel, le terroir illustre cette dimension patrimoniale en donnant un sens à un territoire. Les terrasses, composantes architecturales emblématiques fragiles, illustrent les fonctions multiples de la viticulture. Sans entretien, la nature reprend ses droits. La friche, l'érosion redonnent progressivement à la pente son profil naturel. Pour le vigneron il est plus aisé de se concentrer sur les parcelles de plaine.

Les terrains de pelouses sèches, ou en friche accélèrent le passage du feu. Bien évidemment, parallèlement à son intérêt fonctionnel, la terrasse est souvent favorable à la qualité des vins, mais encore faut-il pouvoir la valoriser pour supporter les surcoûts d'exploitation.

La Suisse, l'Allemagne et le Luxembourg ont su encourager par des aides financières le maintien voire la réimplantation de vignobles dans ces zones sensibles à forte pente. Au delà des aspects financiers, le décloisonnement entre les acteurs du secteur viticole et les autres usagers du territoire (agriculture, collectivités territoriales) s'impose pour initier, formaliser et concrétiser un projet collectif.

2 UNE DEMARCHE DE PROJET:

Le paysage, support sensible des choix d'aménagement locaux, constitue une entrée pertinente pour une réflexion durable car il permet d'identifier et de conforter les supports identitaires, de redonner une vision spatiale à un projet technique viticole et de l'intégrer dans les échelles multiples d'un territoire.

Une démarche de paysage s'appui sur un besoin local, exprimé par une collectivité ou des viticulteurs. Elle se décompose en une première étape de lecture et de diagnostic de paysage permettant d'établir la place, le rôle et l'image du viticulteur dans le territoire.

Elle se base sur un diagnostic de territoire et sur un plan de gestion, rédigé en concertation avec tous les acteurs du projet et qui donne des pistes d'actions pour le territoire concerné. Cette approche vise à développer une démarche paysagère qui, à la fois, améliore le système de production, contribue à insérer l'exploitation dans une dynamique locale et assure une bonne gestion des ressources naturelles et du paysage.
Ensuite sont déclinées les actions associées au plan de gestion, qui répondent aux besoins identifiés tels que par exemple un dysfonctionnement ou besoin viticole local, les aménagements des abords de cave, des sentiers pédagogiques, la réimplantation de haies, la préservation des cabanes de vignes, la restauration des murets, etc.…
Les viticulteurs, principaux gestionnaires d'un terroir viticole, doivent être au cœur des discussions et force de proposition quant à l'avenir de leur territoire et aux priorités du projet. La préexistence d'un mouvement collectif et d'une conscience environnementale au sein de la profession viticole et des acteurs locaux est un atout important pour favoriser les dialogues et les dynamiques d'un projet.

Les éléments-clés de cette démarche sont les suivants :
- Définir et identifier le territoire viticole concerné
- Collecter des informations (cartes, données numériques, photographie, enquête…)
- Lire le paysage (composante, place du vignoble)
- Etablir la typologie du vignoble (principaux éléments identitaires)
- Intégrer les composantes du bâti (village, monuments…)
- Recenser les sources d'agression du paysage
- Préciser et formaliser éventuellement des démarches de protection
- Etablir des scénarii prospectifs vis-à-vis de l'évolution du paysage
- Formaliser un plan de communication

Le diagnostic de paysage apporte la connaissance nécessaire d'un territoire pour le comprendre et le gérer. Le diagnostic de paysage détermine son identité, ses sous-unités caractéristiques, ses spécificités, les éléments caractéristiques, les éléments à valoriser et ceux à améliorer. . Les dynamiques d'évolution et les enjeux pour ce territoire doivent être formulés. Il convient de discuter, de compléter et de valider le diagnostic de paysage en concertation avec les acteurs du projet.
Au final le diagnostic permet de décliner des actions qui seront décidées collectivement et mises en œuvre au cours du temps. Une articulation optimale doit être trouvée entre les acteurs opérationnels (exploitations), les zones viticoles (communales, régionales, aires d'appellation) et les institutions territoriales (commune, groupement de communes, etc.).
L'animation du plan de gestion incombe à une personne ressource, présente au quotidien pour porter l'action, organiser les sorties terrain, suivre les réalisations, relancer la dynamique, répondre aux interrogations, monter, si nécessaire, les dossiers financiers.

Au regard de l'enjeu lié à la préservation et à la valorisation des paysages, différents instituts techniques agricoles français ont initié un projet APPORT (Paysages agricoles, des outils pour des projets de développement durable des territoires).
Le projet présenté dans la communication comporte notamment :

- 9 brochures concernant les méthodes, les outils et les orientations liées à la relation agriculture et paysage (Figure 2).
- Un site Internet ressource sur cette thématique regroupant notamment toutes les productions et informations collectées lors de ce projet www.agriculture-et-paysage.fr

Figure 2 : Plaquettes du projet APPORT (Paysages agricoles, des outils pour des projets de développement durable des territoires). Téléchargeables sur le site : www.agriculture-et-paysage.fr

La formalisation et surtout la finalisation d'une démarche suppose de s'appuyer sur un réseau pluridisciplinaire, d'intégrer le retour d'expérience d'autres régions et dans la mesure du possible d'associer une reconnaissance nationale voire internationale du projet. C'est dans cet esprit qu'a été développé le Réseau International Charte de Fontevraud, animé par l'Institut Français de la Vigne et du Vin qui est présenté ci-dessous.

Réseau international Charte de Fontevraud

La Charte Internationale de Fontevraud est née de l'association de l'interprofession Inter-Loire et de la Mission Val de Loire, à la suite de l'inscription sur la liste du patrimoine mondial par l'UNESCO du Val de Loire en 2000 et du colloque international de Fontevraud « paysages de vignes et de vins » qui s'est tenu en juillet 2003.
L'IFV a été missionnée par les fondateurs pour développer et animer la Charte.
Le Comité Scientifique et Technique International, qui associe des spécialistes viticoles et paysages, est animé par l'IFV qui instruit également les dossiers de candidature des régions.
La Charte de Fontevraud a pour ambition d'inciter tous les acteurs des territoires viticoles, collectivités locales, syndicats viticoles, opérateurs de la culture et du tourisme, universités et laboratoires à s'engager dans des démarches paysagères volontaires et concertées conjuguant, dans une logique de développement durable, l'optimisation de la production viticole et la valorisation culturelle et touristique de ces paysages, dans le cadre d'un réseau international d'excellence.

3 RETOURS D'EXPERIENCES

Une dizaine de régions françaises ont initié des démarches paysagères. A titre d'exemple deux régions signataires de la Charte de Fontevraud ; Costières de Nîmes et Château-Chalon sont présentées ci-dessous.

- COSTIERES DE NIMES

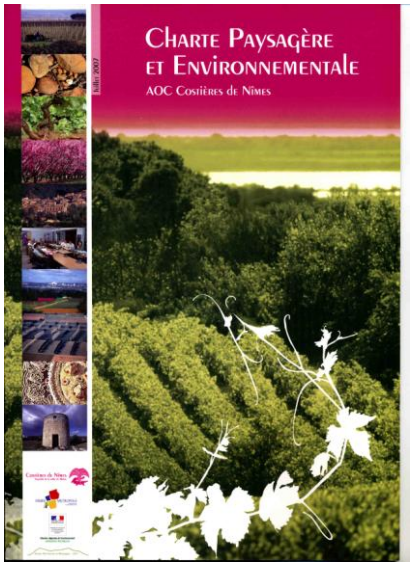

Figure 3 : Charte paysagère des Costières de Nîmes
Source : AOC Costières de Nîmes, Nîmes Métropole, Direction Régionale de l'Environnement Languedoc-Roussillon, Atelier Territoires et Paysages – Juillet 2007

Fin 2005, le Syndicat de défense de l'appellation Costières de Nîmes a engagé une réflexion sur le devenir de son territoire de production, en partenariat avec la Communauté d'Agglomération Nîmes Métropole et la DIREN Languedoc-Roussillon (Direction Régionale de l'Environnement). A partir des valeurs culturelles et économiques que constituent le paysage et l'environnement, le projet a eu pour premier objectif de valoriser et de préserver la valeur ajoutée du territoire liée au vin. La concertation menée avec les acteurs du territoire (acteurs politiques, économiques, institutionnelles et associatifs) a permis d'aller au-delà de ces premiers objectifs a travers la signature d'une Charte paysagère et environnementale de l'appellation.
Trois axes sont visés :
- la préservation des ressources naturelles et la biodiversité
- la gestion de l'identité rurale et agricole du territoire
- la valorisation de l'activité agricole par le tourisme et la communication.

Concernant la valorisation, un inventaire de l'accueil a été réalisé auprès des exploitations afin d'identifier les structures et les communiquer à l'Office du Tourisme de Nîmes. Un « package » Œnotouristique « vin et patrimoine » a été crée en partenariat avec l'Office du Tourisme de Nîmes, qui intègre des visites des monuments de la ville, d'un domaine et dégustation, en liaison avec un hébergement et une restauration dans un bar à vins. Une lettre de liaison trimestrielle présente l'avancée progressive de la mise en œuvre de la charte.

Un poste « animateur charte » a été financé par les différents partenaires institutionnels, qui a pour mission de coordonner, d'informer et de soutenir les vignerons et partenaires dans les actions menées sur l'ensemble du territoire. Il est appuyé par un groupe de travail qui associe les vignerons du cru (caves coopératives, domaines particuliers) avec l'appui d'un comité de suivi composé des partenaires institutionnels et organismes techniques spécialisés (locaux ou extra-régionaux).

Vis-à-vis de la biodiversité, le secteur des Costières de Nîmes est impliqué dans un projet européen en cours de finalisation associé notamment à la plantation de haies.
La charte a dépassé son rôle initial puisqu'elle sert maintenant de référence dans une approche territoriale partagée et défendue (suppression d'un projet de centrale électrique thermique portant atteinte à l'image de la zone).

- **CHATEAU-CHALON**

Figure 4 : Vue du vignoble de Château-Chalon

La démarche est décrite par C. VILLAUME Président de la communauté de communes des Coteaux de la Haute Seille et également par le Maire de Château-Chalon.
Les vignerons ont décidé au cours des années 70 de donner une impulsion nouvelle à l'exploitation du terroir concerné par l'AOC. Ils ont notamment procédé à un remembrement. Les friches ont été ainsi sensiblement réduites, et les efforts consentis se poursuivent encore aujourd'hui.Les pêchers, les amandiers, les noyers, ainsi que les autres arbres fruitiers des vergers sont régulièrement entretenus. Les « avanchers » (les osiers) sont taillés chaque hiver.
Les parcelles sont souvent difficiles à exploiter à Château-Chalon, mais plus encore peut être qu'ailleurs, une relation affective très forte unit les vignerons à leur terre et à leur vigne, ainsi qu'au site remarquable qu'ils contribuent à préserver. Des murs en pierres sèches et des maisonnettes de vigne ont été restaurés, des sentiers ont été balisés.Cette démarche est intégrée dans un programme « LEADER », dont l'un des objectifs est le maintien de l'ouverture des paysages.
Le projet intègre deux équipes de réinsertion qui défrichent, qui entretiennent les sentiers, et qui restaurent des murs et des cabanes en pierres sèches. Ces emplois verts constituent le volet humain et social « troisième pilier », trop souvent négligé, les projets de développement durable.
Parallèlement, ce projet a permis de supprimer une partie du réseau électrique aérien préjudiciable à la qualité esthétique du paysage grâce à un financement du fournisseur de l'électricité.
Quelques jours seulement après la signature du décret de classement, le 16 janvier 2006, un colloque international dédié aux paysages viticoles, a été organisé. A l'issue de la manifestation, le préfet, le directeur de la nature et des paysages du ministère de l'environnement, les élus locaux, les représentants des viticulteurs, ont signé la charte de Fontevraud.
Dans le cadre de la charte, la suppression du réseau électrique aérien moyenne tension qui traverse le site depuis a été pris en charge par le fournisseur d'électricité EDF qui a également remplacé un transformateur de type cabine haute par une cabine basse intégrée au paysage.

CONCLUSION
De la diversité des reliefs et des climats naît une variété de paysage. L'esthétique de la vigne témoigne de cette subtile harmonie que l'homme a su établir avec la nature. Les paysages-vignerons

témoignent d'une diversité géologique unique et d'une histoire culturelle de la vigne et du vin sans égal.
Cette dimension, qui relie la terre à l'esprit, associe en premier lieu le savoir-faire du vigneron, architecte et jardinier, au sens noble du terme, du terroir. Sa maîtrise technique est le fruit d'observations empiriques acquises de génération en génération et d'un esprit d'innovation permanent. Le savoir-faire de l'homme ne s'arrête pas à la vigne. L'architecture des chais, des caves, mais également celle des villages, des églises ou d'autres bâtisses historiques participent pleinement à l'harmonie et à la splendeur des paysages viticoles français. Vitrines des pratiques viticoles, ils sont porteurs d'enjeux écologiques (bonnes pratiques environnementales), économiques (image promotionnelle du vin, tourisme) et sociaux (cadre de vie, identité locale). Ce sont également des liens identitaires, supports de projets collectifs à l'échelle d'un territoire, indispensables au développement de la viticulture durable.
Mais cet écosystème culturel est fragile. La modernité, dans son approche fonctionnelle a souvent occulté ces richesses patrimoniales léguées par les anciens, héritage parfois perçu comme une contrainte face aux évolutions technologiques et aux impératifs économiques. Sachons protéger et transmettre à nos enfants ce patrimoine esthétique, mémoire d'intelligence, de sensibilité, de sueur et parfois de combats. C'est un capital qu'il convient de connaître, de préserver et de valoriser, si l'on veut pérenniser la culture du vin.

BIBLIOGRAPHIE

BRAUDEL F., 1986. L'identité de la France– Espace et histoire, les Hommes et les Choses, tomes 1 et 2.Les éditions Arthaud.

CABANEL J., SAUVAIRE R., 2006. Pays et paysages de France. Editions du Rouergue Rodez,

Direction départementale de l'équipement et de l'agriculture du Jura. 2009. Journal électronique du cadre de vie, de l'environnement, du développement et de l'aménagement durables

ENITA Clermont-Ferrand, Chambre d'agriculture de la Haute-Vienne, CRENA/CNRS UMR 2002. Le paysage dans un projet de territoire – démarche et méthode expérimentées en limousin, Clermont-Ferrand,

FABBRI L., DEMARQUE M., 2003. Les paysages viticoles des Côtes du Rhône gardoises, définition et caractérisation. Rapport d'étude, Laboratoire Mutations des Territoires en Europe CNRS, UMR 5045 – Université Paul Valéry, Montpellier,

FABBRI L., 2008. Mettre en tourisme le paysage viticole. Les Costières de Nîmes et le vignoble champenois. Revue Espaces Tourisme et Loisirs,

LASNIER A., LE ROY H., 2004. Vigne et paysage font bon ménage. In Les 4 saisons du paysage, Solstices, Equinoxes en Champagne-Ardenne. Bulletin d'information de la Fédération Régionale du Paysage,

LASNIER A., 2003. Etude paysagère de Reuil : qualité du paysage et perspectives

MICHELIN Y., Lectures d'un territoire. in La médiation culturelle du territoire. Consultable sur le site :http://enfa.mip.educagri.fr/agri-culture/Ressources/articles/mediation/Michelin-Paysage.htm

PITTE J.R., Histoire du paysage français – De la préhistoire à nos jours

ROCHARD J., 2005.Traité de viticulture et d'œnologie durables, éditions Avenir œnologie

ROCHARD J., HERBIN C., 2006. Les paysages viticoles, éditions Féret

TOUBLANC M., 2004, Paysages en herbe, Editions Educagri,

AN APPROACH FOR EVALUATION OF COMPATIBILITY BETWEEN GRAPE QUALITY AND ENVIRONMENTAL OBJECTIVES IN LOIRE VALLEY PDO WINE PRODUCTION

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ABSTRACT
Social, economic and state increase their demand towards French viticulturists for reduction of their environmental impact, but not at the expense of the quality of the wine. This position paper presents the approach to evaluate the compatibility of grape quality and environmental objectives in Central Loire Valley PDO vineyards. The environmental quality of vineyard management will be assessed using the Life Cycle Assessment method. The relevance of replicating 3 years of measurements is explored.

KEYWORDS: viticulture, Life Cycle Assessment, grape characteristics, multi criteria rating, environment

RESUMÉ
La société, les marchés et l'état imposent à la viticulture française de réduire ses impacts environnementaux tout en produisant des vins de qualité. Ce document expose la démarche prévue pour l'évaluation de la compatibilité des objectifs qualitatifs et environnementaux de la production de raisins de cuve dans les vignobles AOC du centre Val de Loire. La méthode de l'Analyse du Cycle de Vie a été choisie pour l'évaluation de la qualité environnementale des itinéraires techniques. La pertinence du choix de trois années de mesure est discutée.

MOTS CLES : viticulture, Analyse du Cycle de Vie, caractéristiques du raisin, évaluation multicritères, environnement

INTRODUCTION

Social and economic pressure on the wine sector to adopt sustainability is growing. The key points of the French government's policy on ecological and sustainable development were formalized following an Environmental Round Table in 2007 (www.legrenelle-environnement.fr). The policy includes the new requirement for environmental information on mass consumption products from 2012 ("Act Grenelle 2", final version not published yet) and the target of a 50% reduction in the use of pesticides between 2008 and 2018 (ACT No. 2009-967). This is relevant to the wine sector.

French consumers embrace the tradition and natural aspects of wine and their affinity to it might be eroded by their evolving knowledge of production practices (Brugière, 2009). Protected Designation of Origin (PDO), wines embody the localized and traditional technical know-how (Lamine, 2005), but the PDO is only a guarantee of origin, but not environmental

quality (Hirczak, 2007). As the French are very concerned of the risk of agrochemical spraying on food crops (Credoc, 2009), the image of wine could be jeopardised by the use of 20% of pesticides (in mass) on 3.7% of French UAA in viticulture (Aubertot *et etc.*, 2005).

The French PDO wine producers are thus faced with this new societal and institutional demand. Similarly, if they wish to strengthen their position in the world market, they must take into account the environmental requirements of key international markets. It is then necessary to assist the wine industry in addressing this risk through the evolution of its practices towards being more environmentally friendly. The grape growers of the Loire Valley are seeking support for such development in environmental practices without damaging the quality of their wines.

This paper introduces the approach implemented in order to provide, to wines sector agents, inputs useful for choosing viticultural technical paths that meet the objectives of product quality and environmental quality in Loire Valley protected denominations of origin vineyards. This research is implemented in the frame of the scientific programme of UMT Vinitera[*]. The originality of this research is situated in product multi-criteria rating of quality and environment, which corresponds to a new research field emerging internationally, and in the adaptation of Life Cycle Assessment method to the wine grape production.

OBJECTIVES

This project aims to i) measure the levels of compatibility between indicators of grape quality (Qg) and of environmental quality (Qe) of the vineyard management technical paths[†] (TKPv) in these attributes ranging from antagonisitic to synergistic relationships, and ii) to identify, within the TKPv, the techniques responsible for these situations, in order to assist wine industry stakeholders in the choice of TKPv.

The research strategy intends to i) identify the diversity of existing vineyard management practices, ii) establish a typology of TKPv, iii) chose existing vineyard plots representing this diversity as an experimental device, iv) characterize the soil and climate of these plots as co-variables, v) observe the TKPv on the plots for three years on the attributes of Qe and Qg, vi) confront Qe and Qp indicators of TKPv in a matrix structured in degrees of compatibility, vii) identify the parts of the process playing the main role on the TKPv position in the matrix, and viii) adapt the matrix into a tool for wine sector agents.

METHOD

This research focuses on grape production, which represents both a significant part of the environmental impact of wine (Gazulla *et etc.* 2010) and is a crucial aspect of the quality of the product. The two main cultivars of the central Loire Valley: cv. Chenin Blanc and cv. Cabernet Franc will be utilised. Measurements are planned for 3 consecutive vintages (2010-2012) and will be performed at the plot level (a single unit in the vineyard with homogeneous characteristics). The project will be conducted in conjunction with key stakeholders so it has strong application in the wine sector.

[*] UMT Vinitera : Unité Mixte Technologique Vins, INnovations, Itinéraires, TERroirs et Acteurs : research unit including staff from INRA-UEVV Angers, ESA- GRAPPE and LARESS research units, IFV- Pôle Val de Loire-Centre and CTV around a common research programme « Construction of terroir wines quality, from producers to consumers ».

† Logic succession of techniques applied on the vineyard by the producer

LCA has been chosen for the evaluation of the environmental quality of the TKPv because it is the most complete tool in the field of global and multi-criteria assessment of environmental impacts (Boeglin *et etc.*, 2005). It has recently been chosen, in a simplified form, to assess and display the environmental impact of consumer products in France, which directly concerns the wine industry. However, this method only deals with potential impacts (Jolliet and Crettaz, 2001). Appropriate models for impacts on biodiversity and soil quality are still under construction. Currently, estimation of the uncertainty of results remains difficult in agricultural LCA (Payraudeau *et al*, 2005).

The method is currently applied and adapted to agricultural systems (Audsley *et etc.*, 2003, Brentrup *et etc*., 2004) and of particular interest to this research, perennial fruit production as well (Mouron *et etc.*, 2006). Research utilising LCA in viticulture and oenology has been published (Aranda *et etc.* 2005; Petti *et al*, 2006; Pizzigallo *et etc.* 2006; Gazulla *et etc.* 2010), but have not addressed the method in detail for application in vineyard management.

This research is broken down into five stages:

Stage 1: Establishment of the experimental and observational network representing the diversity of TKPv of central Loire Valley PDO vineyards.

The diversity of TKPv existing in the region is identified by:

- A survey of 100 grape growers with diverse socio-economic profiles, different production systems, from different PDO, in order to describe their TKPv on 300 plots.
- A typology of TKPv from this survey and existing databases on 100 variables using the data mining platform CORON (Ducatel *et etc.* 2010), and Factorial Multiple Correspondence Analysis (FMCA).

The sample of plots used for the study will be selected by TKPv types in order to contrast potential Qe and Qg. Two networks will be designed: one comparing TKPv in the same environment (soil, climate) and the other observing TKPv in various environments.

Stage 2: Evaluation of Qg and Qe on the selected plots TKPv

The evaluation of Qg requires the following:

- The choice of grape quality criteria (biochemical, sensory, physical, microbiological, xenobiotics) through a survey with expert winemakers.
- The measure of grape quality on the chosen criteria at harvest.

The evaluation of Qe requires the following:

- Adaptation of the LCA method for wine grape production (functional unit, impacts, completion of Eco-invent data base) following the iterative process of LCA.
- Calculation of environmental impacts using LCA (Simapro software, Ecoinvent database).

An inventory of flux data will be made with grape growers once or twice a year depending on their practices traceability.

Stage 3: Evaluation of the compatibility of Qg and Qp for each TKPv

The environment (soil and vintage climate) will be characterised as co-variables through existing detailed cartography and annual weather data.

Qe and Qg datasets will be crossed using Multiple Factorial Analysis (Escofier and Pagès 1998) and including environmental co-variables.

TKPv will be positioned in a matrix crossing Qg and Qe following the design of:
- A typology of Qg and Qe through a combination of criteria
- A matrix of compatibilities between Qg and Qe using this typology

Stage 4: identification, within TKPv, of vineyard management techniques responsible of TKPv position in QgXQe matrix

The key techniques influencing grape quality will be identified through literature review. The techniques causing the main environmental impacts will be identified both by LCA results on the experimental network and literature.

Stage 5: Development of a tool to assist the wine sector agents in their TKPv choices.

The tool will be developed from the matrix.

METHODOLOGICAL ISSUES

This approach identifies five main methodological issues. LCA adaptation to the grape production process and the treatment of complex data have been developed in previous articles (Renaud *et etc*., 2010 (a) and (b)). The relevance of considering 3 vintages for this study is the focus of this paper. The choice of grape quality indicators to be considered for Qg evaluation and construction of a tool to aid decision making will be developed in a subsequent paper.

In order to validate if a sample of 3 consecutive years will be sufficient to explore the potential variability of grape quality according to the climatic conditions, the CV of two grape quality indicators have been calculated on series of 2 to 30 consecutive vintages (1979-2009). This longitudinal data has been obtained from a sample of Loire Valley Cabernet Franc grapes from the same plot (Data sourced from INRA EU-1117 in the framework of UMT VINITERA).The indicators utilised are sugar (figure 1) and anthocyanins content (figure 2).

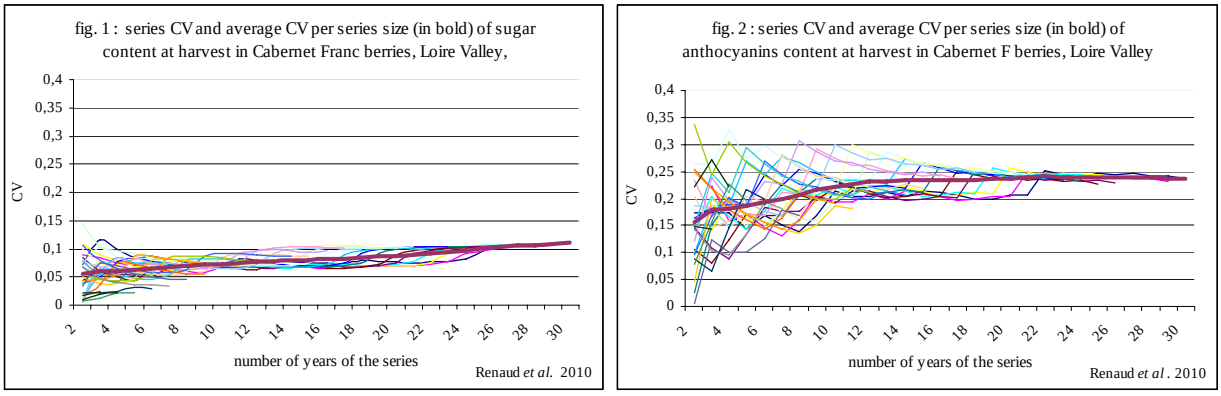

Figure 1 and 2: CV of sugar and anthocyanins contents for 3 to 30 consecutive vintages series, cv. Cabernet Franc, Montreuil Bellay, Loire Valley (INRA EU-1117 in the framework of UMT VINITERA data)

The average CV for both criteria is stable and this increases with the number of years utilised.

Whilst affected by climatic variation, the sugar content's natural variation appears to be limited and most likely regulated by the grower's choice of harvest date. Even if the individual Sugar CV values vary 5 times more for a 3 year series than for an 8 years series, the average CV remains low (under 0.12).

Anthocyanins are subject to uncontrolled variations, with an average CV reaching 0.25, but with lower individual CV values variation than sugar. A consecutive sample of three vintages might not be sufficient to get results consistent with a longer period (i.e. 30 years). Concerning the proposed measurements for this research, the 3 vintages will need to be tested in comparison to present data and charts in order to determine if the sample is representative.

CONCLUSIONS

The expected results are i) the identification of TKPv diversity, ii) a built typology of Loire Valley TKPv for the studied cultivars, iii) an operational method to characterize TKPv by the relationship between Qe and Qg, iv) the positioning of each TKPv type within the QeXQg matrix, structured in increasing degrees of compatibility, v) a list of the vineyard management techniques responsible for this position in the matrix TKPv QeXQg, vi) an advisory tool developed with the actors from this matrix, vii) adapted LCA method for grape production processes in the Loire Valley, viii) results of methodological development on LCA which should benefit viticultural scientists and technicians wanting to use LCA for wine grape production. This work should also contribute to improve multi criteria methods for production processes evaluation.

These results should provide the wine industry the opportunity to increase its awareness of environmental issues and to further increase the environmental quality of grape production processes. The findings could contribute to changes in viticulture towards more environmentally friendly practices. This research could propose new tools for actors in charge of advising the wine sector and enable them to better integrate environmental objectives into the specifications of labelled productions, including PDO, in line with consumers and societal expectations.

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BIBLIOGRAPHY

Audsley, E., Alber, S., Clift, R., Cowell, S., Crettaz, P., Gaillard, G., Hausheer, J., Jolliet, O., Kleijn, R., Mortensen, B., Pearce, D., Roger, E., Teulon, H., Weidema, B., van Zeits, H., (2003): Harmonisation of Environmental Life Cycle Assessment for Agriculture. Final Report Concerted Action AIR3-CT94-2028. European Commission, DG IV version at 20 august 2003, pp. 101.

Aubertot J.N., Barbier J.M., Carpentier, A., Gril J.J., Guichard L., Lucas P., Savary S., Savini I., Voltz M., (2005): Pesticides, agriculture et environnement. Réduire l'utilisation des pesticides et limiter leurs impacts environnementaux. Expertise scientifique collective, synthèse du rapport, INRA et Cemagref (France), 64 p.

Brentrup F. Küsters J., Kuhlmann H., Lammel J., (2004): Environmental impact assessment of agricultural production systems using the life cycle assessment methodology, I. Theoretical concept of a LCA method tailored to crop production *Europ. J. Agronomy* 20 (2004) : 247–264

Boeglin N. Veuillet, D. (2006) : Introduction à l'Analyse de Cycle de Vie (ACV), Note de synthèse externe, mai 2005, éd. ADEME

Brugière F., (2009) : Pratiques culturales sur vignes et pratiques oenologiques : connaissances et opinions des Français, Viniflhor-Infos Vins et cidres n°160, Février 2009, p1-10.

CREDOC, (2009), Baromètre des perceptions alimentaires MAAP/CREDOC 2009 (synthèse), 9p., consulté le 12/04/10 sur : http://agriculture.gouv.fr/sections/thematiques/alimentation/comprendre-informer/

Combris, P., Lecocq S., Visser, M., (1999): Price of Bordeaux and Burgundy wines : is quality important ?, *INRA Sciences Sociales v. 12(1)* p. 1-3, juillet 99.

Ducatel B., Kaytoue M., Marcuola F., Napoli A., Szathmary L. (2010) : Coron : Plate-forme d'Extraction de Connaissances dans les Bases de Données, conférence RFIA (Reconnaissance de Forme et Intelligence Artificielle) - Caen 2010

Escofier B., Pagès J. (1998) Analyses factorielles simples et multiples ; objectifs, méthodes et interprétation. 3rd edition., éd. Dunod, Paris. 284 p

Gazulla C., Raugei M.,Fullana-i-Palmer P. (2010): Taking a life cycle look at crianza wine production in Spain: where are the bottlenecks?, *International Journal of LCA* (2010) 15:330–337

Hirczak M., (2007) : La co-construction de la qualité agro-alimentaire et environnementale dans les stratégies de développement territorial. PhD Thesis, Université Joseph Fourier, Grenoble I, institut de Géographie Alpine, 356p.

Jolliet O., Crettaz P. (2001) : De la critique à la réalisation d'un écobilan, Ed. EPF de Lausanne, Suisse, 121p.

Jolliet O, Saadé M., Crettaz P. (2005) : Analyse du cycle de vie: comprendre et réaliser un écobilan, Ed. Presses polytechniques et universitaires romandes, 235p.

Lamine, C., (2005) : Santé et qualification des produits. Coopération de scientifiques et d'agriculteurs autour de la nutrition méditerranéenne, Natures Sciences Sociétés 13, 403-412 (2005)

LOI no 2009-967 du 3 août 2009 de programmation relative à la mise en œuvre du Grenelle de l'environnement, *Journal Officiel de la République Française* du 5 août 2009

Mouron P., Scholz RW, Nemecek T., Weber O. (2006): Life cycle management on Swiss fruit farms: Relating environmental and income indicators for apple-growing, Ecological Economics 58 (2006) 561– 578.

Payraudeau S., Van der Werf H.M.G. (2005) : Analyse de l'incertitude associée au calcul des émissions azotées sur un groupe d'exploitations agricoles dans le cadre de l'application de l'Analyse du Cycle de Vie. 10ème Colloque international annuel du SIFEE, Evaluation, environnement et développement d'une agriculture durable, 20-24 juin 2005, Angers, France

Petti L., Raggi A., De Camillis C., Matteucci P., Sára B. and Pagliuca G. (2006): Life cycle approach in an organic winemaking firm : an Italian case-study. 5th Australian Conference on LCA, Melbourne, 22- 24 Nov. 2006,

Pizzigallo A.C.I., Granai C., Borsa S. (2006): The joint use of LCA and « emergy » evaluation for Italian wine farms, Journal of Environmental Management 86 (2008) 396–406

Renaud C., Benoît M., Thiollet-Scholtus M., Jourjon F., (2010) a, Evaluation des impacts environnementaux des itinéraires techniques viticoles de production de vins AOP en Val de Loire : démarche d'adaptation de la méthode de l'Analyse du Cycle de Vie (ACV), proceedings VIII International Terroir Congress, June 14th-18th, 2010, SOAVE, ITALY (in press)

Renaud C., Benoît M., Jourjon F., (2010) b, Proposal of approach for Evaluation of compatibility between grape quality and environmental objectives assessed by LCA in PDO wine production, Proceedings of 7th International Conference on Life Cycle Assessment in the Agri-Food Sector, September 22 – 24, 2010, Bari (Italy), (in Press).

DIFFERENTIATION DE PARCELLES DE CHENIN DU VAL DE LOIRE, A L'AIDE DE L'ETUDE DES FLORES FONGIQUES DES RAISINS, EN UTILISANT L'OUTIL DGGE

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RÉSUMÉ

Depuis le millésime 2002, une étude est menée sur la diversité de la flore fongique de parcelles du cépage chenin, situées essentiellement sur les appellations de Vouvray et Montlouis ; deux appellations séparées par le fleuve nommé la Loire. Les parcelles se situent dans des conditions pédoclimatiques différentes, qui se retrouvent au travers des suivis de maturité et l'état sanitaire.

L'objectif est d'utiliser la flore fongique comme facteur de différenciation entre les parcelles, et d'évolution au cours de la maturité. C'est dans ce cadre qu'un outil d'écologie microbienne a été utilisé : Denaturating Gradient Gel Electrophoresis (DGGE). Après une étude spécifique sur les moisissures des raisins, qui ont permis d'établir un référentiel de souches pures, les échantillons complexes constitués de l'eau de lavage des baies de raisins, ont été analysés. Ainsi, nous avons pu analyser et différencier plusieurs parcelles de cépage chenin, situées dans des conditions pédoclimatiques différentes.

MOTS-CLÉ

Flore fongique, état sanitaire, conditions pédoclimatiques, suivi maturité, outil moléculaire, DGGE.

ABSTRACT

Since the vintage wine 2002, a study is led on the variety of the fungal flora of parcels of the Chenin vine, situated essentially on the controlled origin label of Vouvray and Montlouis; two controlled origin label separated by the river named the Loire. The parcels are situated in different conditions of soils and of climate, which meet through the follow-ups of maturity and the sanitary state.

The objective is to use the fungal flora as factor of differentiation between the parcels, and evolution during the maturity. It is in this frame that a tool of microbial ecology was used: Denaturing Gradient Gel Electrophoresis (DGGE). PCR-DGGE is a molecular method which allows the direct analysis of DNA in complex samples without any culture step. This method is based on the separation in a denaturing gradient of double-strand DNA fragments which have the same length but different nucleotide sequences. After a specific study on fungus of grapes, which allowed establishing the reference table, the complex samples constituted by some water of wash of the berries of grapes, were analyzed. This tool will allow us to draw a parallel between the dynamic of fungal populations present in different conditions of soil and of climate.

PCR-DGGE showed its potentialities for a fast characterization of fungi in complex mixes.

KEYWORD
Fungal flora, sanitary state, conditions of soils and of climate, follows-up of maturity, molecular tool, DGGE.

INTRODUCTION

La microflore présente sur la surface de raisins est composée d'espèces diverses de levures, de bactéries et de champignons filamenteux. Certains de ces micro-organismes sont impliqués dans l'élaboration du vin en participant aux fermentations alcoolique et malolactique. Cependant, quelques autres peuvent avoir un impact négatif sur la qualité du vin. Par exemple, les champignons filamenteux peuvent être impliqués dans la production de mycotoxines (Cabanes et *al*. 2002; Battilani et *al*. 2003), dans les maladies de la vigne comme le mildiou et le black rot ou dans des défauts sensoriels comme les arômes de moisi ou terreux (La Guerche et *al*. 2004). L'écologie et la biodiversité des levures présentes à la surface des raisins ont déjà été étudiées en utilisant des méthodes microbiologiques conventionnelles ou des méthodes moléculaires (Versavaud et *al*.1995; Pramateftaki et *al.* 2000; Torija et *al*. 2001; Sabate et *al*. 2002; Fleet 2003; Prakitchaiwattana et *al*. 2004). La présence de champignons filamenteux sur des raisins a été étudiée avec des méthodes conventionnelles (Sage et *al*. 2004; Serra et *al*. 2005), montrant la prédominance d'espèces appartenant aux genres *Botrytis*, *Penicillium* et *Aspergillus*. Cependant, les études basées sur des méthodes moléculaires sont rares (Doaré-Lebrun et *al*. 2006) et la communauté fongique des raisins reste mal connue. En effet, les méthodes de microbiologie conventionnelle peuvent échouer à décrire la diversité totale microbienne à cause de la prédominance de certaines espèces, les inhibitions entre espèces et l'incapacité de certaines d'entre elles à croitre sur des milieux de culture gélosés (Head et *al*. 1998). Les méthodes moléculaires basées sur l'analyse directe de l'ADN environnemental sans étape de culture au préalable ont été développées pour étudier les communautés microbiennes. Parmi ces méthodes, la PCR couplée à un gradient dénaturant (PCR-DGGE) et la PCR couplée à un gradient de température (PCR-TTGE) (Muyzer et *al.* 1993) ont été largement utilisées pour définir des écosystèmes microbiens associés à l'environnement ou associés à l'alimentation. Ces méthodes se sont montrées être plus appropriées que les méthodes microbiologiques conventionnelles pour l'analyse des communautés fongiques (Doaré-Lebrun 2005).

Le but de cette étude est de déterminer l'utilisation de la PCR-DGGE, dans le cadre du suivi d'un réseau de sept parcelles du cépage Chenin, réparties sur les AOC Vouvray et Montlouis, depuis 2002, dont cinq sont définies comme étant sensibles à l'état sanitaire et plus particulièrement à ce qui est défini comme des déviations nommées goûts moisi-terreux (GMT).

MATÉRIELS ET MÉTHODES

Réseau de parcelles :

- Sept parcelles de Chenin sont suivies depuis 2002 et sont nommées : Le Gard (sensible à l'état sanitaire), Fosses rouges (sensible à l'état sanitaire), Brosses 10 (sensible), Brosses 20 (non sensible), Cormier roux (sensible), Marronniers (sensible à l'état sanitaire), et Epinay (non sensible)
- Des analyses de résistivité ont été réalisées courant 2006, ainsi qu'une analyse des sols, par la Cellule Terroir Viticole d'Angers (CTV)
- Suivi climatique : méso et microclimat.

Suivi effectué sur le millésime 2007 :

- Suivi de maturité (sucres, acidité totale, acide malique, degré probable, pH, teneur en géosmine), état sanitaire (par observations visuelles : intensité et fréquence *Botrytis*, et *Penicillium sp*.), étude de la flore fongique par PCR-DGGE.

PCR-DGGE (détail dans l'article de Laforgue et *al*, 2009) :

- Les échantillons proviennent de l'eau de lavage de baies de raisins, ou de moûts. Ces échantillons sont incubés dans un milieu de culture (PDB), pendant 15 heures, avant d'effectuer l'extraction d'ADN. L'ADN est amplifié grâce à des amorces de β-tubuline, permettant l'amplification des ascomycètes filamenteux, et une amorce spécifique à *Botrytis cinerea*. Pour ensuite effectuer l'analyse en gel d'électrophorèse en gradient dénaturant, deux différents gradients de dénaturant ont été utilisés : un premier de 20 à 70 % de solution dénaturante, et un second de 40 à 45 % de solution dénaturante. La solution dénaturante définie à 100% est un mélange de 40% (v/v) de formamide et d'urée à 7 mol/l. Les échantillons sont chargés sur le gel, et la migration a lieu pendant 16 heures à 120V et 60°C. Après migration, le gel est observé sur une table UV, après coloration, à l'aide de bromure d'éthidium.
- Les photos de gels sont ensuite analysées à l'aide du logiciel Bionumerics. Les photos sont normalisées grâce à un marqueur introduit dans le gel. Ensuite, chacune des bandes du profil complexe est comparée aux bandes présentes dans le référentiel constitué d'une cinquantaine de profils de souches pures de moisissures issues de collections et/ou de raisins.

RÉSULTATS ET DISCUSSION

Les parcelles :

La plupart des sols étudiés dans ce réseau sont des sols bruns, sauf un qui est lessivé (bournais).

Quatre des six sols sont représentatifs des terroirs de leur AOC d'appartenance. Dans les deux autres, l'un est de type anthropique, l'autre est une perruche sableuse peu fréquente à Vouvray et davantage à Montlouis.

La majorité des sols présente vers 30 à 60 cm de profondeur un obstacle potentiel soit à la pénétration de l'eau, soit l'inverse à la remontée capillaire, soit à la pénétration des racines. Cet obstacle potentiel est parfois constaté.

Il n'existe pas une ou plusieurs explications pédologiques communes permettant de relier les sols des parcelles dites pourrissantes.

Si le sol est une variable explicative de l'état sanitaire, alors il existe deux types de parcelles :

- celles réagissant sensiblement à l'effet sol comme les Brosses 10, le Gard ou modérément sensible comme les Marronniers. Le sol intervient à travers la circulation de l'eau.
- celles s'affranchissant de l'effet sol par une autre variable explicative dominante comme les Epinays, les Brosses 20.

Si le profil pédologique permet d'expliquer partiellement certaines situations, l'analyse physico-chimique confirme l'état de chaque sol avec peu d'écart entre parcelles.

Les mesures de résistivité complètent la caractérisation des sols des sept parcelles. Ces nouvelles mesures confirment le mauvais état de ressuyage des parcelles sensibles. Les écarts de résistivité entre avril et août semblent en effet plus importants en surface des sols des parcelles avec GMT. Cet écart est en particulier différent au sein de chaque binôme parcellaire. En revanche, ces mesures ne permettent pas de classer les parcelles du Réseau Chenin de Touraine selon leur niveau de risque GMT. Le sol constitue donc un facteur structurel aggravant pour les GMT.

Climat 2007 :

L'été 2007 est pluvieux et frais en général. Les pluies sont en effet très fréquentes de juin à août. Les épisodes pluvieux reprennent ensuite du 19 septembre au 06 octobre. L'hygrométrie minimum est par conséquent très souvent de 45%, c'est-à-dire assez élevée.

Les conditions climatiques sont donc globalement propices à l'installation de moisissures d'altération sauf au cours de la première quinzaine de septembre.

Flore fongique :

Les six observations visuelles de l'état sanitaire, couplées aux analyses effectuées par PCR-DGGE des raisins, sont effectuées entre le 23/08 (fin véraison) et le 02/10 (sur-maturité et fin des vendanges). Lors de la première observation, la pourriture grise est bien présente sur l'ensemble des parcelles sauf les Brosses 10 et 20, avec parfois des moisissures vertes. Ensuite, la fréquence de pourriture se développe fortement entre les deux premières observations pour se stabiliser pendant les trois semaines de temps sec de septembre. Ensuite, le *Botrytis* se développe à nouveau. L'intensité de *Botrytis* croît en revanche de façon quasi-linéaire entre le 23/08 et le 02/10. L'installation des moisissures vertes est décalée d'une semaine : leur présence est générale et marquée de la troisième à la sixième observation, sauf aux Brosses 10 et 20.

L'essentiel de la production de géosmine est mesuré entre le 09 et 25 septembre, soit quatre semaines après le dernier épisode pluvieux, et est marquée préférentiellement sur les parcelles : Marronniers et Epinays. Ce pic intervient pendant la phase stationnaire de développement des moisissures vertes (cf. figure 1), période pendant laquelle l'hygrométrie régresse sauf durant deux journées chaudes et humides les 6 et 17 septembre.

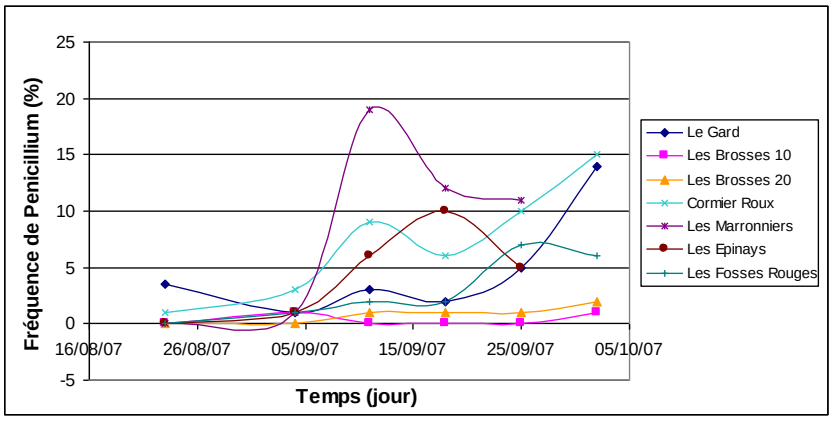


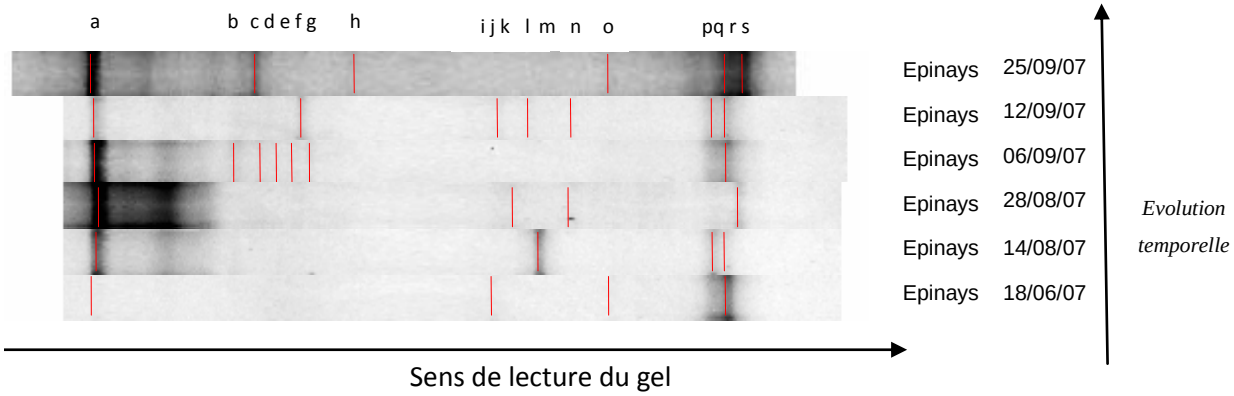
Figure n°1 : Fréquence de ‘moisissures vertes’ observée sur les sept parcelles d’études.

L'évolution qualititive de la flore fongique à partir du 06 septembre est perceptible sur l'image n°1 (gradient 20-70%), à laquelle est associée le détail des espèces détectées (tableau n°1), pour la parcelle 'Epinays'. On peut voir notamment l'apparition d'une plus grande diversité d'espèces, ce qui coïncide avec l'augmentation de la géosmine.De même, si l'on compare l'ensemble des parcelles à une même date (image n°2 (gradient 20-70%), et tableau n°2 associé) du 12 Septembre, on remarque aussi une plus grande diversité d'espèces sur les parcelles Marronniers et Epinays, parcelles où les teneurs en géosmine ont justement fortement augmenté à cette période. C'est ainsi que l'on peut observer cette variation qualitative de la flore fongique à la fois au cours des semaines, mais également entre les parcelles. Penses-tu qu'il faille mettre dans les tableau 1 et 2 les « 100% » concernant les espèces ? Je ne suis pas sûr que cela soit utile sachant que dans le titre on a en plus « identification potentielle ». A toi de voir.

PARCELLE	DATE PRELEVEMENT	ESPECES DETECTEES ET IDENTIFICATION POTENTIELLE
Les Epinays	18/06/2007	*a) B. cinerea; i) P. implicatum; o) Sp. alborubescens; q) inconnue*
Les Epinays	14/08/2007	*a) B. cinerea; m) inconnue ; p) inconnue ; q) inconnue*
Les Epinays	28/08/2007	*a) B. cinerea; k) P. herquei, P. purpurescens; n) Kl. Thermotolerans; r) inconnue*
Les Epinays	06/09/2007	*a) B. cinerea; b) inconnue ; c) P. italicum, P. spinulosum; d) P. viridicatum; e) P. expansum; g) P. purpurescens, P. herquei, S. cerevisiae; q) inconnue*
Les Epinays	12/09/2007	*a) B. cinerea; f) P. herquei; j) inconnue ; l) inconnue ; n) Kl. Thermotolerans; p) inconnue ; q) inconnue*
Les Epinays	25/09/2007	*a) B. cinerea; c) P. italicum; h) P. thomii; o) Sp. alborubescens ; q) inconnue ; s) A. japonicus*

Tableau 1 : Correspondance entre les bandes identifiées sur le gel et les espèces détectées

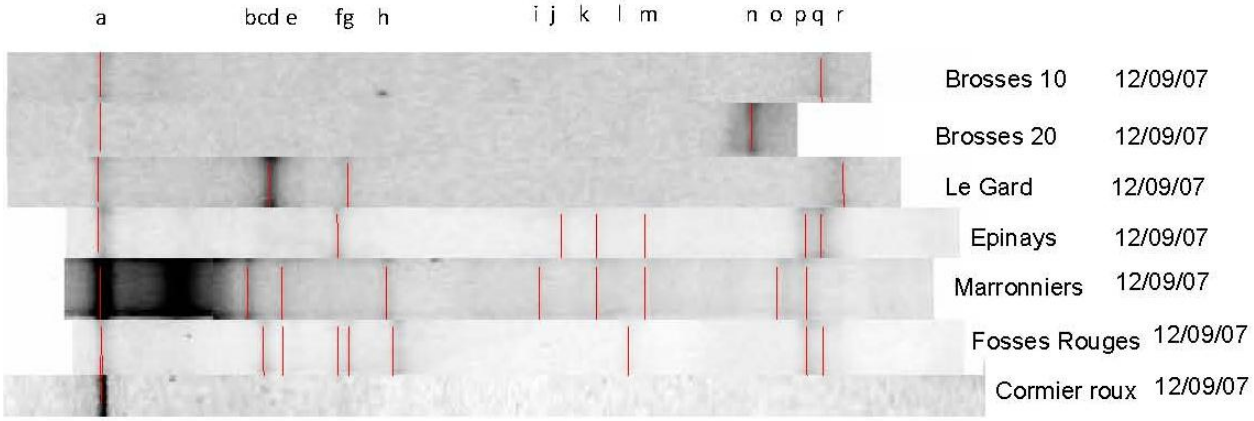
Image 1 : Gel 20-70% de la parcelle Epinays aux différentes dates de prélèvements



PARCELLE	DATE PRELEVEMENT	ESPECES DETECTEES ET IDENTIFICATION POTENTIELLE
Brosses 10	12/09/2007	*a) B. cinerea ; q) inconnue*
Brosses 20	12/09/2007	*a) B. cinerea ; n) inconnue*
Le Gard	12/09/2007	*a) B. cinerea ; d) P. commune ; g) S. cerevisiae, P. purpurescens, P. brevicompactum ; r) inconnue*
Les Epinays	12/09/2007	*a) B. cinerea ; f) P. herquei ; j) inconnue ; k) inconnue ; m) Kl. Thermotolerans ; p) inconnue ; q) inconnue*
Les Marronniers	12/09/2007	*a) B. cinerea ; b) inconnue ; e) P. italicum ; h) P. thomii, M. pulcherima, P. restrictum; i) P. implicatum ; k) inconnue ; m) inconnue ; o) inconnue ; p) inconnue*
Fosses Rouges	12/09/2007	*a) B. cinerea ; c) inconnue ; e) P. italicum, P. spinulosum; g) S. cerevisiae, P. brevicompactum, P.herquei, P. purpurescens; g) P. thomii, M. pulcherima ; j) inconnue ; n) inconnue ; o) inconnue*
Cormier Roux	12/09/2007	*a) P. paxili, B cinerea*

Tableau 2 : Correspondance entre les bandes identifiées sur le gel et les espèces détectées

Image 2 : Gel 20-70% des sept parcelles de Chenin à la même date du 12/09/2007



CONCLUSIONS

Les conditions climatiques de l'été 2007 sont favorables à l'expression des GMT. L'analyse des facteurs confirme en 2007 le rôle principal du climat et de certaines moisissures. En résumé, la progression linéaire de *Botrytis* ne semble pas être corrélée directement à la production de géosmine. En effet, la cinétique de développement des moisissures vertes est croissante et parallèle à celle de géosmine jusqu'au 18 septembre. Au-delà, les moisissures vertes stagnent tandis que la géosmine diminue, peut-être en raison de son caractère volatile, donc éphémère.

Ce pic résulterait peut-être de la conjonction de trois phénomènes : des moisissures installées depuis le 05 septembre, des conditions climatiques très favorables (T° maxi ≈ 20.5°C, hygrométrie minimum ≈ 70%) aux moisissures, des raisins déjà altérés par *Botrytis*. Si l'hypothèse est vraie, cela signifie que la production maximale de géosmine a lieu environ 12 jours après la première journée très favorable aux moisissures.

Ainsi, il est possible de pouvoir déterminer la flore fongique présente et spécifique à chaque parcelle. Cependant, aujourd'hui, la seule détection de souches appartenant au genre *Penicillium sp*. est nécessaire mais pas suffisante pour déterminer un niveau de risque GMT à la parcelle. C'est la raison pour laquelle, il est nécessaire de réaliser les dosages de géosmine. Cette étape de meilleure connaissance de la flore fongique est nécessaire afin de pouvoir déterminer les facteurs favorisant la production de cette molécule.

BIBLIOGRAPHIE

Battilani, P., Pietri, A., Bertuzzi, T., Languasco, L., Giorni, P. and Kozakiewicz, Z. (2003) Occurrence of ochratoxin A – producing fungi in grapes grown in Italy. *J Food Prot* **66**, 633-636.

Cabanes, F.J., Accensi, F., Bragulat, M.R., Abarca, M.L., Castella, G., Minguez, S. and Pons, A. (2002) What is the source of ochratoxin A in wine? *Int J Food Microbiol* **79**, 213-215.

Doaré-Lebrun, E. (2005) Caractérisation de la microflore des raisins par méthodes FISH et PCR-TTGE- Application à la résolution des gouts terreux dans les vins. Ph.D. thesis, Université de technologie de Compiègne.

Doaré-Lebrun, E., El Arbi, A., Charlet, M., Guerin, L., Pernelle J.J., Ogier J.C. and Bouix, M. (2006) Analysis of fungal diversity of grapes by application of temporal temperature gradient gel electrophoresis – potentialities and limits of the method. *J Appl Microbiol* **101**, 1340-1350.

Fleet, G.H. (2003) Yeast interaction and wine flavour. *Int J Food Microbiol* **86**, 11-22.

Head, I.M., Saunders, J.R. and Pickup, R.W. (1998) Microbial evolution, diversity and ecology: a decade of ribosomal RNA analysis of uncultivated microorganisms. *Microb Ecol* **35**, 1-21.

Laforgue R., L. Guérin, J.J. Pernelle, C. Monnet, J. Dupont and M. Bouix. (2009). Evaluation of PCR-DGGE methodology to monitor fungal communities on grapes. *J Appl Microbiol* .107(4):1208-18.

La Guerche, S., Garcia, C., Darriet, P., Dubourdieu, D. and Labarère, J. (2004) Characterization of *Penicillium* species isolated from grape berries by their internal transcribed spacer (ITS1) sequences and by gas chromatography - mass spectrometry analysis of geosmin production. *Curr Microbiol* **48**, 405-411.

Muyzer, G., De Waal, E.C. and Uitterlinden, A.G. (1993) Profiling of complex microbial populations by denaturing gradient gel electrophoresis analysis of polymerase chain reaction-amplified genes coding for 16S rRNA. Appl. *Environ Microbiol* **59**, 695-700.

Prakitchaiwattana, C.J., Fleet, G.H. and Heard, G.M. (2004) Application and evaluation of denaturing gradient gel electrophoresis to analyse the yeast ecology of wine grapes. *FEMS Yeast Res* **4**, 865-877.

Pramateftaki, P.V., Lanaridis, P. and Typas, M.A. (2000) Molecular identification of wine yeasts at species or strain level: a case study strains from two vine-growing areas of Greece. *J Appl Microbiol* **89**, 236-248.

Sabate, J., Cano J., Esteve-Zarzoso, B. and Guillamon, J.M. (2002) Isolation and identification of yeasts associated with vineyard and winery by RFLP analysis of ribosomal genes and mitochondrial DNA. *Microbiol Res* **157**, 267-274.

Sage, L., Garon, D. and Seigle-Murandi, F. (2004) Fungal microflora and ochratoxin A risk in French vineyards. *J Agric Food Chem* **52**, 5764-5768.

Serra, R., Braga, A. and Venancio, A. (2005) Mycotoxin-producing and other fungi isolated from grapes for wine production, with particular emphasis on ochratoxin A. *Res Microbiol* **156**, 515-521.

Torija, M.J., Rozes, N., Poblet, M., Guillamon, J.M. and Mas, A. (2001) Yeast population dynamics in spontaneous fermentations: comparison between two different wine-producing areas over a period of three years. *Antonie van Leeuwenhoek* **79**, 345-352.

Versavaud, A., Courcoux, Ph., Roulland, C., Dulau, L. and Hallet, J.N. (1995) Genetic diversity and geographical distribution of wild *Saccharomyces cerevisiae* strains from the wine-producing area of Charentes, France. *Appl Environ Microbiol* **61**, 3521-3529.

AMPELOGRAPHIC VARIABILITY OF CROATIAN AUTOCHTHONOUS *V. VINIFERA* L. CULTIVARS

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ABSTRACT

The national collection of Croatian autochthonous grapevine cultivars was established in 2001. It contains 120 autochthonous grapevine cultivars from all over Croatia, although most of them come from Dalmatia region. So far 95 varieties have been described by using 39 OIV descriptors determining their ampelographic and basic commercial features. r to estimate ampelografic variability and mutual difference between varieties In orde in collection comparison of OIV descriptor results has been made by using cluster analysis plus a dendrogram. A high degree of similarity has been found between some varieties, such as: Tanetova loza and Vlaška white; Prć & Žumić and Palagružonka while & Stradunska, as well as some others. The varieties which are in the dendrogram shown to have a high degree of similarity will be additionally tested by using genetic methods in order to determine if they are ~~also~~ genetically related or are even synonymous.

Key words: *V. vinifera* L., ampelografy, native cultivars

ZUSAMMENFASSUNG

Die nationale Kollektion kroatischer autochthoner Weinrebensorten wurde im Jahre 2001 gegründet. Dort werden autochthone Weinrebensorten aus ganz Kroatien aufbewahrt, jedoch stammt der Großteil aus dem Gebiet Dalmatiens. Einige Sorten dieser Kollektion sind auch heute noch von wirtschaftlicher Bedeutung, während andere außerhalb der Kollektion kaum existieren. Derzeit befinden sich in der Kollektion 120 Weinrebensorten. Die ampelographischen Charakteristiken dieser Sorten werden durch Verwendung von 39 OIV Deskriptoren festgestellt, um deren ampelographischen und grundlegenden wirtschaftlichen Charakteristiken erkunden zu können. Bisher wurden auf diese Weise 95 Sorten beschrieben. Es wurde ein Vergleich der Resultate der OIV Deskription durch Verwendung von Clusteranalyse sowie ein Dendogramm erstellt, in welchem ersichtlich ist, in welchem Maße sich die Kollektionssorten auf Grund deren ampelographischen Charakteristiken voneinander unterscheiden. Es wurde eine hohe Ähnlichkeit zwischen einigen Sorten festgestellt, wie zum Beispiel von: Tanetova loza und Vlaška bijela, ferner zwischen Prć und Žumić sowie Palagružonka bijela und Stradunska, aber auch zwischen einigen anderen. Diejenigen Sorten, die in dem Dendrogramm eine hohe Ähnlichkeit aufweisen, werden zusätzlich durch genetische Methoden geprüft, um feststellen zu können, ob es sich um genetisch verwandte Sorten oder gar Synonyme handelt.

Schlüsselbegriffe: *V. vinifera,* ampelographie, autochthone Weinrebensorten

INTRODUCTION

Grapevine has been grown in Croatia since ancient times. This is especially through for Dalmatia winegrowing region where it was possible to find several hundred cultivars at the end of 19th century and most of them were considered to be autochthonous (Bulić, 1949). Today more than 80 native cultivars are registered in the official Croatian cultivar list, while an additional 50 rare genotypes remain underutilized, primarily due to the lack of good quality propagation material and the insufficient knowledge about their genetic and oenological potential.

National collection of native grapevine cultivars was founded in year 2001 at the experimental station of the Faculty of Agriculture University of Zagreb, "Jazbina". Most of the cultivars present in this collection ware gathered during the project "Inventarisation and revitalization of native grapevine cultivars" financed by Ministry of science, education and sport from 2001 to 2006.

Nowadays most of these cultivars are not economically important and there is a serious threat of their extinction outside this collection. For all cultivars present in collection it is possible to produce initial planting material in case of interest for their commercial growing. Although for the most cultivars in collection it is not possible to make proper evaluation of their economically important characteristics because they originated from coastal region with different climatic condition, all cultivars are thoroughly described and small breeding program is being started within germplasm present in collection.

Ampelographic and genetic characteristics of some native grapevine varieties ware subject of some earlier researches (Zdunić et. al. 2008, Maletić et. al. 1999, 2004.) but they did not included all of the cultivars present in National collection of native grapevine varieties.

The main goal of this research was to determine level of ampelographic variability between Croatian native grapevine varieties present in the collection.

MATERIALS AND METHODS

In this research 95 Croatian native grapevine cultivars from National collection of native grapevine cultivars have been included. Every cultivar in collection is represented with five vines that where propagated from one mother vine discovered during the inventarisation of Croatian winegrowing regions. Most of the cultivars originated from coastal region of Croatia but some of them have been found in continental region.

Collection is placed in Zagreb (continental region) with moderate climatic conditions. All of the cultivars are grafted on rootstock *V. berlandieri x V. riparia* SO4. Ampelographic description was performed in years 2007 and 2008. 39 OIV descriptors for grapevine qualitative traits (Tab. 1) have been chosen and hierarchical cluster analysis has been performed on dataset from 95 cultivars. Dendrogram was plotted using unweighted pair-group average method (UPGMA) with the Squared Euclidean distance.

Table 1 List of OIV descriptors used int his research

OIV	Descriptor
1	Young shoot: openinig of the shoot tip
3	Young shoot: intensity of anthocyanin coloration of prostrate hairs on the shoot tip
4	Young shoot: debsity of prostrate hair on the soot tip
6	Shoot: atitude (before tying)
7	Shoot: color of the dordsal side of the internodes
8	Shoot: color of the ventral side of the internodes
15-1	Shoot: distribution of anthocyanin coloration on the bud scales
15-2	Shoot: intensity of anthocyanin coloration on the bud scales
16	Shoot. Number of consecutive tendrils
51	Young leaf: color of upper side of blade (4th leaf)
53	Young leaf: density of prostrate hairs between main veins of lover side of blade (4th leaf)
67	Mature leaf: shape of blade
68	Mature leaf:number of lobes
70	Mature leaf: area of antocyanin coloration of main veins on uper side of blade
72	Mature leaf: goffering of blade
74	Mature leaf: profile of blade in cros section
75	Mature leaf: blistering of uper side of blade
76	Mature leaf: shape of teeth
79	Mature leaf: degree of opening/overlaping of petiole sinus
80	Mature leaf: shape of base of the petiol sinus
81-1	Mature leaf: presence of teeth in petiole sinus
81-2	Mature leaf: petiole sinus base limited by vein
83-2	Mature leaf: teeth in upper lateral sinuse
84	Mature leaf: density of prostrate hairs between main veins on lower side of blade
87	Mature leaf: density of erect hairs on main veins of blade
151	Flower: sexual organs
153	Shoot: Number of bunches per fertile shoot
155	Shooot. Fertillity of basal buds(buds 1-3)
202	Bunch: lenght
204	Bunch: density
206	Bunch: lenght of peduncule of primary bunch
208	Bunch: shape
209	Bunch: number of wings on the primary bunch
223	Berry: shape
225	Berry: color of skin
230	Berry: color of flesh
235	Berry: firmnes of flesh
236	Berry: particular flavor
241	Berry: formation of seeds

RESULTS AND DISCUSION

Dendrogram plotted using 39 OIV descriptors result on 95 cultivars is shown in fig. 1. It can be observed that cultivar Dobričić from island of Šolta and Frmentun (from island of Korčula) show highest level of difference from all the other cultivars. Rests of the cultivars are separated in two large clusters. The first cluster shows 6 pairs of cultivars with high level of similarity (98-99%). First three pairs that are showing difference at the level of 1% are Tanetova loza and Vlaška bijela (differs in 6 descriptors) and Palaruša viška and Žlahtina as well as Prč and Žumić (differs in 7 descriptors).

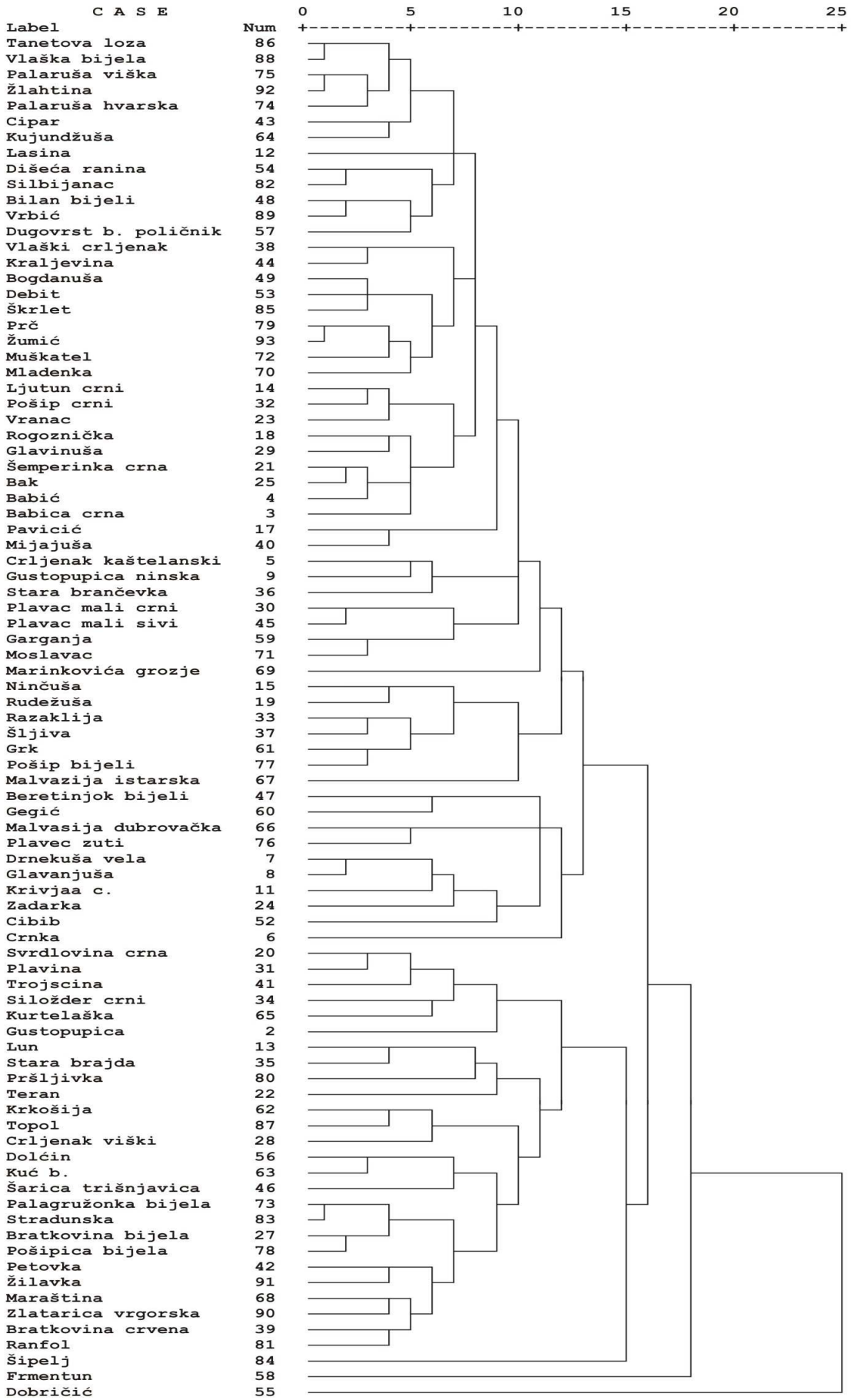

Figure 1 Dendrogram obtained from results of OIV description of grapevine cultivars in National collection of native grapevine cultivars in Jazbina (Zagreb)

In the first cluster cultivar pairs Dišeća ranina and Silbijanac, Bilan bijeli and Vrbić, Drnekuša vela and Glavanjuša (with differences in 10 descriptors), and Plavac mali crni and Plavac mali sivi (with differences in 8 descriptors) differs from each other at the level of 2%. Although Plavac mali sivi is actually a clone of Plavac mali crni with mutation in berry skin color from red to grey, some other differences, mostly regarding the level of anthocyanin coloration of shoot tip, shoot, and buds, can be observed, too. In second cluster only one pair of cultivars is showing difference at the level of 1% and one pair at the level of 2%. The highest similarity is present between cultivars Palagružonka bijela and Stradunska (differs in 6 descriptors) followed by cultivars Bratkovina bijela and Pošipica bijela (differs in 10 descriptors). Rest of the cultivars in both clusters is showing different levels of similarity but in all cases higher then the 4%.

Although high level of similarity between some cultivars has been obtained in this analysis we still cannot claim that they are synonymous without performing additional genetic analyses. Despite of that it is interesting to notice that for cultivar Plavac mali crni and its clone Plavac mali sivi no difference in microsatellite analysis using set of 10 SSR makers and five combination of primers in AFLP analysis has been obtained (Preiner, 2006) but they can be easily differs by simple ampelographic description (berry color).

CONCLUSION

Results of ampelographic description of cultivars in National collection in Zagreb and hierarchical cluster analysis show different level of similarity/difference between them. Morphologically most distinct varieties but also cultivars that are possible synonyms can be determined this way. For cultivar-pairs that show high level of morphological similarity additional genetic analyses will be performed.

REFERENCES

Bulić, S. 1949. Dalmatinska ampelografija. Poljoprivredni nakladni zavod. Zagreb, Croatia

Maletić, E., Sefc, M. K., Steinkellner, H., Karoglan Kontić, J., Pejić, I. 1999. Genetic characterization of Croatian grape cultivars and detection of synonymous cultivars in neighboring regions. Vitis. 38(2): 79-83

Maletić, E., Pejić, I., Karoglan Kontić, J., Piljac, J., Dangl, G.S.; Vokurka, A., Lacombe, T., Mirošević, N., Meredith, C. 2004. Zinfandel, Dobričić, and Plavac Mali: The Genetic Relationship among Three Cultivars of the Dalmatian Coast of Croatia. American journal of enology and viticulture. 55 (3):174-180

Preiner, D. 2006. Ampelographic and genetic evaluation of mutant Plavac mali sivi (V. vinifera L.). Diploma thesis, Faculty of Agriculture University of Zagreb.

Zdunić, G., Pejić, I., Karoglan Kontić, J., Vukičević, D., Vokurka, A., Pezo, I., Maletić, E. 2008. Comparison of genetic and morphological data for inferring similarity among native Dalmatian (Croatia) grapevine cultivars (Vitis vinifera L.). Journal of food agriculture & environment. 6(2): 333-336

EXACT MANAGEMENT OF DISSOLVED GASES OF WINES BY MEMBRANE CONTACTOR

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RÉSUMÉ ENGLISH

Membrane contactor is a new technology which enables implementing liquid-liquid, liquid-gas or liquid-gas-liquid separations. The main component of the system is a hydrophobic porous membrane system. Only gases with low molecular weight like O_2 and CO_2 can pass through the barrier. The system can be installed before the bottling line or in the winery in order to adjust the gas concentration of wine before storage in bulk or bottling.

INRA carried out a first series of tests on an INOXPA WineBrane 2500 pilot unit. The results showed that it is possible to deoxygenize and adjust CO_2 in only one passage on the unit (contrary to a gas injector), thanks to several operating processes which use the vacuum and/or an inert gas flow (Vacuum, Combo and Strip). A deoxygenation, [final dissolved O_2] < 0.5 mg/L, of a white and a red wine (comparison with gas injector) didn't affect the higher alcohols. Only some esters underwent significant quantitative losses. However, a jury of 19 judges could not make the difference between the wines before and after deoxygenation whatever the technique.

RÉSUMÉ FRANÇAIS

Le contacteur membranaire constitue une nouvelle technique qui permet de mettre en oeuvre notamment des séparations liquide-gaz. Le principal composé du système est un assemblage de membranes creuses hydrophobes. Seules, les molécules gazeuses de faible masse moléculaire, comme l'O_2 et le CO_2 peuvent passer la barrière. L'appareil peut être utilisé avant une tireuse ou de cuve à cuve afin de gérer les gaz dissous.

L'INRA a réalisé une première série d'essais sur un WineBrane 2500 manuel. Les résultats ont montré qu'il est possible de désoxygéner et d'ajuster le CO_2 précisément en un seul passage sur l'appareil (contrairement à la technique par injecteur de gaz) grâce à plusieurs modes opératoires utilisant le vide et/ou un écoulement de gaz inerte (vacuum, strip, combo). Une désoxygénation jusqu'à [O_2 dissous] < 0.5 mg/L d'un vin blanc et d'un vin rouge (en comparaison avec l'injecteur de gaz) n'affecte pas les alcools supérieurs. Seuls quelques esters subissent des pertes quantitatives significatives. Cependant, 19 dégustateurs n'ont pas fait de différence entre les vins avant et après désoxygénation quelle que soit la technique.

INTRODUCTION

Oxidation phenomena, depending on the presence of oxygen, affect the evolution of wines. Controlled oxidation contributes to the stabilization of colour and to the reduction of astringency in red wine, as during ageing in barrels or in micro-oxygenated vats. In contrast, protection from oxygen would seem to be necessary for white wines that are to be drunk young (Escudero et etc., 2002; Ferreira et etc., 2002). Finally, it is commonly accepted in oenology that marked oxidation has an adverse effect on wine quality. Several authors

showed also that 2 mg/L of oxygen moreover on white wines involved significant sensory modifications after a few months (Berta et etc., 2000; Boulet, Vidal, 1999). Therefore, various studies undertaken to characterize the appearance of dissolved oxygen during operations performed on wines show that bottling is one of the most critical phases (Vidal et etc., 2004; Castellari et etc., 2004), especially as once the bottles have been sealed, the only remaining means for mastering the evolution of wines are the storage parameters (closure permeability, temperature, relative humidity, light, etc.).

On one side, the presence of O_2 in the wines, following its dissolution, is not a stable state in time. Dissolved oxygen is gradually consumed by various substrates, mainly polyphenols (Singleton et etc., 1979). The disappearance of floral flavours is faster under the effect of oxygen additions even at 15°C and at the organoleptic level, aromatic deteriorations arrive before chromatic deteriorations (Escudero et etc., 2002).

On the other side, CO_2 is produced in a large quantity by yeasts (81 g/L for wine of 10% vol.) and lactic acid bacteria (1.6 g/L for 5 g/L of malic acid) during alcoholic and malolactic fermentations. During storage and various treatments of the wine, the carbon dioxide content tends to decrease because of the tendency to equilibrate the CO_2 partial pressure of the wine with the atmospheric partial pressure. Finally variable quantities remain at the time of the bottling. However the carbonic gas plays a key role in the organoleptic characters of the wines, even if its content is lower than its perception threshold (500 mg/L). Thus adjusting the carbon dioxide content of wines has a great importance from the sensorial point of view. The CO_2 content in bottled wines will have to be adjusted approximately to 300 mg/L for the red wines for ageing and up to 800-1800 mg/L for the white and rosé wines.

Already used in waters, beverages and effluents treatments, membrane contactors present a great interest in oenology because this new technology enables implementing liquid-liquid, liquid-gas or liquid-gas-liquid separations. The main component of the system is a hydrophobic porous membrane system. Only gases with low molecular weight like O_2 and CO_2 can pass through this barrier. It is possible to reduce dissolved gases (eg CO_2, O_2) or to add for example CO_2 in wines before conditioning in bottles or in bag-in box.

After a presentation of this new technology and of the manual membrane contactor used, this study describes the first results obtained by INRA experimental unit of Pech-Rouge (France) for the management of dissolved gases of still wines. The main aim of these trials was to reduce the dissolved oxygen content to a level lower than 0.5 mg/L in order to increase the shelf life of the wines. At the same time, the dissolved carbon dioxide content is adjusted to a level between 300 and 1800 mg/L according to the type and the style of the wine for sensorial reasons.

MATERIALS AND METHODS

WineBrane® membrane contactor:

The WineBrane® skit (INOXPA patent pending) consists of one or several membrane contactors in the way, that when connected with the operation and secondary medium, multiple treating processes are possible. The power spectrum of the plant covers flow rates between 1000 and 20000 litres of wine per hour for the gas management (Schmidt et etc., 2010).

The design and construction of the units is performed in compliance with the requirements of the food-processing industry. The membrane material is polypropylene and it has FDA approval.

The design is CIP cleanable (alkaline solution and disinfectants), units can also be sanitised with hot water.

Operating principle and technical specifications of the used unit:

The Liqui-Cel (MEMBRANA) membrane contactor module consists of a woven assembly of polypropylene hollow membrane fibres of 200 µm interior diameter with an average size of pores of 0.03 µm "Figure 1" which allows only the passage of gases or volatile compounds of low molecular weight.

The hydrophobic character of the membrane confers to the role of interface between the liquid phase which moves outside the hollow fibres (shell side) and the gas phase which moves in opposite direction inside the hollow fibres (lumen side), preventing the mixture of the phases. The balance of concentrations is displaced when a depression and/or a gas flow is introduced. A diffusion force is created due to the differential pressure between gas phase and dissolved gases in the liquid phase, which migrates in the gas through the membrane (or conversely) until a new differential pressure balance is reached. This physical mechanism can be described in a basic way by the principle of osmosis. If there are volatile compounds with a higher concentration on a side of the membrane compared to the other, then these compounds will cross the membrane until the equilibrium of the partial pressures of these compounds on the two sides of the membrane is attained.

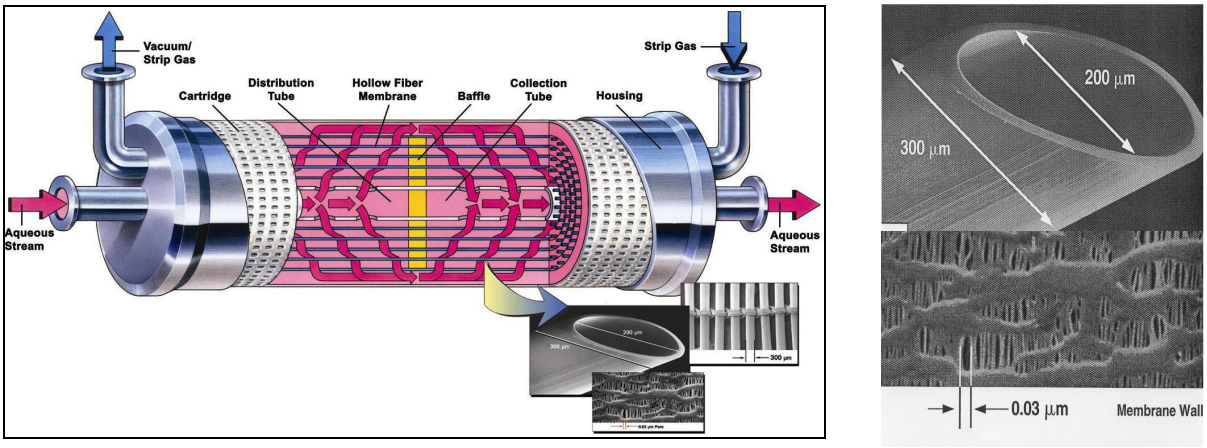

Figure 1: Liqui-cel cartridge and microscopic photos of membranes.

The membrane contactor used was a manual WineBrane® 2500 (25 hL/h), provided with a Liqui-cel 4X13" ExtraFlow X40 cartridge of membrane surface area equal to 8.1 m². A monobloc lobe pump enables wine circulation in shell side of membrane, while the strip gas with or without vacuum (produced by a liquid ring vacuum pump), circulates in opposite direction on lumen side of the membrane "Figure 2".

Figure 2: Manual WineBrane® 2500 used for experiments.

Operating modes of WineBrane 2500:

VACUUM mode: the use of vacuum lower than 100 hPa in lumen side reduces O_2 and CO_2 according to their partial gas pressure out of the wine. The gas molecules, which crossed the membrane wall, are then eliminated by the vacuum pump. Thus, vacuum deoxygenizes and decarbonises the wine at the same time.

CARBONATION mode: to readjust CO_2 content, it is necessary to carry out a second passage without vacuum but by a CO_2 sweep with low overpressure in lumen side (Δ pressure between lumen side and shell side = 200-300 hPa). A back pressure is applied on the outlet of the gas side, to force CO_2 to pass into the wine.

In addition to the two previous modes, INRA carried out two new modes in order to check if it is possible to deoxygenize and adjust the CO_2 content in only one passage in the WineBrane skit.

STRIP mode: an inert gas circulation (N_2, CO_2 or N_2/CO_2 mixture) in lumen side will make it possible to impoverish the oxygen content of wine by keeping the CO_2 concentration. If CO_2 partial pressure of carrier gas is higher than that of wine, the wine will enrich in CO_2. In the opposite case, the wine will be impoverished in CO_2. Roughly, if the carrier gas contains approximately more than 20% of CO_2, a red wine will enrich in CO_2 (50% for the white and rosé wines).

The strip mode is used to increase CO_2 concentration at the same time as to reduce O_2 concentration. If a strong carrier gas flow is introduced, O_2 will be reduced but with a higher carrier gas consumption.

COMBO mode: a partial vacuum is coupled to an inert gas flow in lumen side, in order to deoxygenise the wine and to adjust its CO_2 content.

The combo mode is used when the aim is to keep CO_2 concentration at the same time as to reduce O_2 concentration. This mode is more efficient for deoxygenating at constant CO_2 level because the carrier gas consumption is lower than with strip mode at same deoxygenation level.

Moreover, carrier gas consumption (CO_2) in the combo mode is more important than in strip mode to increase CO_2 content of the wine. And the strip mode is more efficient than combo mode when wine needs a strong carbonation (when initial CO_2 is much lower than target CO_2) at the same time as a deoxygenation.

Methods of Analysis for gaseous molecules:

Oxygen control at inlet and outlet side of the apparatus was carried out by PreSens luminescent probe and PSt3 luminescent spots glued onto inner surface of the sight glass “Figure 3”. A PreSens Fibox 3 trace fiber optic probe was used. The Fibox 3 measures the luminescence decay time of an immobilized luminophore. The luminophore is excited with a sinusoidal intensity-modulated monochromatic light delivered by an optical fibre and its decay time causes a time delay in the light signal emitted by the luminophore. This decay time, or phase angle, Φ, decreases in the presence of oxygen and is correlated to oxygen content.

The PSt3 sensor selected to perform this study can be used for a broad range of oxygen concentration ranging from 0 to 50 %.

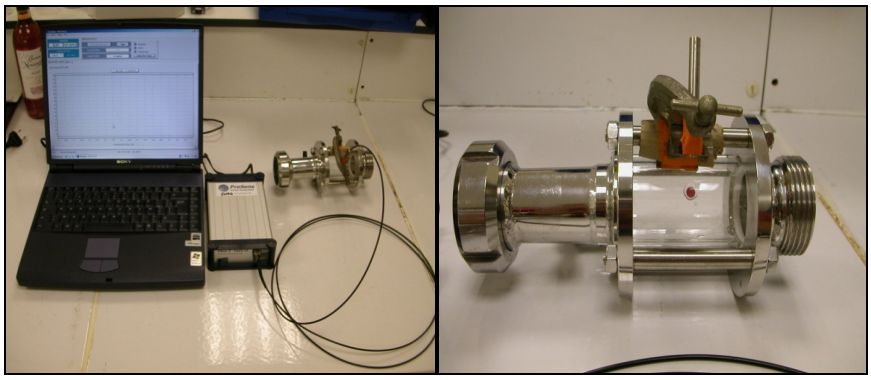

Figure 3: PreSens system with its optical fibre and its PSt3 spot glued in a sight glass.

CO_2 control was carried out by Carbodoseur to the sample taps located at inlet and outlet side of the apparatus.

GC analyses of higher alcohols and esters of wine (headspace sampler coupled to GC Agilent 6850) were performed on samples taken at inlet and outlet side of the apparatus.

RESULTS AND DISCUSSION

The wines used (75% of white or rosé, 25% of reds) were micro filtered before treatment. All treatments were carried out from tank to tank by the racking connection valves. The average temperature of the wines was of 23.9 ± 1.8°C, except for 4 batches (< 7°C).

The combo and strip modes developed by INRA UEPR allowed in only one passage on WineBrane® pilot to deoxygenize the wines as well as by the vacuum mode while controlling the CO_2 content "Tab. 1". The CO_2 content of wines treated by the vacuum strongly decreases. The O_2 content after carbonation is almost stable.

Table 1: Summary of treatments of wines carried out by INRA.

Average ± standard deviation for the 4 last columns.

*For choosing combo, strip or carbonation mode, the final CO_2 content depends on the required CO_2 quantity. The required CO_2 quantity is limited by the temperature and the strip gas CO_2 concentration, according to the abacus of Lonvaud-Fumel, 1976, and also the strip gas mass flow as well as the differential of pressure between lumen and shell side.

Mode	Nb of batches	Total volume hL	Initial [O_2] mg/L	Initial [CO_2] mg/L	Deoxygenation output %	Final [CO_2] mg/L
Vacuum	10	107	4.25 ± 2.82	500 ± 643	- 88.1 ± 3.3	133 ± 140
Carbonation	3	55	0.56 ± 0.09	315 ± 33	+ 4.6 ± 3.2	1425 ± 459
Strip	16	360	0.98 ± 0.32	509 ± 227	- 84.5 ± 4.3	645 ± 297
Combo	19	256	2.28 ± 2.31	446 ± 266	- 87.8 ± 4.5	537 ± 359

For carbonation, strip and combo modes, these first tests showed that consumption of carrier gas is close to 0.5 L CO_2 / L of wine for an addition of 300 mg/L of CO_2 from an initial CO_2 content of 700 mg/L.

Moreover, we observed a diminution of the flow on red wines, perhaps because polyphenols constitute a hydrophobic bio film near the membrane.

Oenological and volatile compounds analyses:

Whatever the used operating process, alcohol percentage, volatile acidity, pH values, free and total SO_2 data and absorbance values at 420, 520 and 620 nm were not modified.

GC analyses of higher alcohols and esters of wine showed that only some esters underwent significant losses being able to go up to 40%, especially ethyl-octanoate, ethyl-hexanoate, isoamyl-acetate and hexyl-acetate, which are the most hydrophobic molecules, which thus have most affinity for the membrane passage according to Diban et etc., 2008.

Comparatively, deoxygenation of the wines by nitrogen sparging with porous diffuser causes important losses for the same molecules because of their volatility.

Sensory analysis by triangular test

A white and a red wine with respective O_2 initial contents of 0.79 and 0.82 mg/L and CO_2 initial content of 620 and 260 mg/L were deoxygenized by sparger and with WineBrane® by strip and combo modes (T° wines = 23 ± 1°C). The final average O_2 contents were respectively of 0.20 (white) and 0.14 mg/L (red) and for CO_2 of 669 ± 68 (white) and 255 ± 36 mg/L (red). 15 days after, a triangular test was carried out by 19 tasters in order to compare the wine before and after the deoxygenation for each wine and each treatment. No significant difference was revealed to the α risk of 5%, in spite of the ester losses quoted previously. Only the wines deoxygenized by sparger (porous diffuser) were considered to be different from the control wines to the α risk = 14.6% (white) and 6.5% (red).

CONCLUSIONS

The dissolved gas management by membrane contactor constitutes a real progress during the phase for conditioning of wines. A full automatic WineBrane® unit integrated in a bottling line (like at the wine coop of Besigheim) or in a bag in box line enables the simultaneous control and adjustment of the two most important dissolved gases in oenology: the oxygen major factor of the wines oxidation and the carbonic gas major factor of the sensory quality of wines. Currently no competitive technology is available which is able to meet these needs accurately and simultaneously in line.

The first tests showed that the main analytical parameters (alcohol, acidities and colour) are not affected by the treatment. Only losses in some esters were observed without impact on the tasting of the wine.

Further studies starting this year will focus on gaseous exchange and on the behaviour of polyphenols. For that, other operating conditions like temperature variation, different gas concentrations, wine types, inert gas consumptions … will be verified.

ACKNOWLEDGEMENT

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BIBLIOGRAPHY

Berta P., Spertino M., Vallini E., 2000. Controllo dell'ossigeno durante l'imbottigliamento di vini frizzanti e spumanti. Industrie delle Bevande, 2000, XXIX dicembre, 618-622.

Boulet J.C., Vidal J.C., 1999. Dissolution d'oxygène à la mise en bouteille. Compte rendu d'expérimentation INRA UE PR Gruissan.

Castellari M., Simonato B., Torinielli G.B., Spinelli P., Ferrarini R, 2004. Effects of different enological treatments on dissolved oxygen in wines. Ital. J. Food Sci., vol16,n°3, 387-396.

Diban N., Athes V., Bes. M., Souchon I., 2008. Ethanol and aroma compounds transfer study for partial dealcoholisation of wine using membrane contactor. Journal of membrane science. 311, 136-146.

Escudero A., Asensio E., Cacho J., Ferreira V., 2002. Sensory and chemical changes of young white wines stored under oxygen. An assessment of the role played by aldehydes and some other important odorants. Food Chem., 77, 325-331.

Ferreira A.C.S., Guesdes De Pinho P., Rodrigues P., Hogg T., 2002. Kinetics of oxidative degradation of white wines and how they are affected by selected technological parameters. J. Agric. Food Chem., 50, 5919-5924.

Lonvaud-Fumel A., 1976. Recherches sur le Gaz Carbonique des Vins. Thèse présentée à l'Université de Bordeaux II le 4 mars 1976.

Schmidt O., Vidal J.C., Waidelich G., 2010. New approach by membrane contactor to manage dissolved gases in wine and to reduce alcohol content of the wine. 60th International IVIF congress. Stuttgart. Communication orale.

Singleton V.L., Trousdale E., Zaya, J., 1979. Oxidation of wines I. Young white wines periodically exposed to air. Am. J. Enol. Vitic. , 30, 49-54.

Vidal J.C., Boulet J.C., Moutounet M., 2004. Les apports d'oxygène au cours des traitements des vins. Bilan des observations sur site. 3ème partie. Rev. Fr Oenol., 205, 25-33.

COMPARISON OF WINES OF GEORGIAN (KAKHETHIAN) AND EUROPEAN TYPES ACCORDING TO QUANTITATIVE CONTENT OF PHENOLIC COMPOUNDS AND ANTIRADICAL EFFICENCY

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ABSTRACT

On the basis of the archeological data, the winemaking in Georgia originates 8000 years ago. According to ancient Georgian traditional technology of preparation of the wine, the squeezed grape is placed in a clay vessel (qvevri) dug in the ground, and alcoholic fermentation is carried out together with components of a cluster. During fermentation, from a stem, skin and seeds of a grape in plenty are extracted phenolic compounds which define the composition and essence of Kakhethian wine. The aim of this study is to estimate total amount of phenols, catechins, proanthocyanidins and anthocyans in wines of the European and Kakhethian type, and to compare their anti-radical efficiency with the help of 2,2-diphenyl-1-picrylhydrazyl (DPPH*) radical. According to the received data, by comparison of European and Kakhethian wines for the content in them of phenolic compounds, Kakhethian wines considerably surpass the European wines. In the Kakhethian white wines prepared from various grapes, the total content of phenolic compounds varies within the limits of 1330-2430 mg/L, and in the European white wines - within the limits of 210-468 mg/L. In the Kakhethian red wines these limits make 2898-4416 mg/L, and in the European red wines – 1630-2340 mg/L. The high content of phenolic compounds in the Kakhethian red wines specifies their medical and preventive properties that proves to be true by comparison of anti-radical efficiency of European and Kakhethian wines.

Se référant aux techniques traditionnelles anciennes géorgiennes de production du vin, le raisin pressé est placé dans un pot en argile enterré (kvevri) pour donner lieu à une fermentation alcoolique avec les constituants de la grappe de raisin. Lors du processus de la fermentation, la râpe, la pelure du raisin et le pépin dégagent en grande quantité des composés polyphénoliques définissant la composition et l'esprit du vin du type kakhétien. Se référant aux données obtenues et en comparant la teneur quantitative des composés phénoliques dans les vins des types kakhétien et européen, il a été mis en évidence que les vins du type kakhétien excèdent considérablement les vins du type européen par leur teneur en phénols. Dans les vins blancs du type kakhétien produits à partir de diverses espèces de raisin le total des phénols varie de 1330 mg à 2430 mg par litre alors que le même indice dans les vins blancs du type européen varie de 210 mg à 468 mg par litre. Dans les vins rouges du type kakhétien la différence se situe entre 2898 mg et 4416 mg par litre contre 1630 mg et 2340 mg par litre pour les vins rouges du type européen. C'est notamment cette teneur en grande quantité des polyphénols que confère aux vins du type kakhétien une qualité curative et préventive ce qui s'affirme d'ailleurs par les résultats d'une comparaison des effets anti-radicaux des vins des types kakhétien et européen.

INTRODUCTION

Adverse ecological conditions observable in the most part of the modern world, unbalanced nutrition and the various illnesses break the counterbalanced free-radical processes proceeding in living cells. The reasons causing this problem are pollution of an environment, the stressful influences, radiation, chronic intoxications, smoking and other conditions, as a result of which the uncontrollable free-radical reactions develop. Under influence of these reactions the toxic effects of xenobiotics are amplified, and carcinogenesis, mutagenesis, atherosclerosis and autoimmune diseases are stimulated. In this respect, the rather important factor is revealing such foodstuff which contains in large quantities compounds having antioxidant activity. In this aspect the Kakhethian technology of making of wine which is since old days used in Georgia is especially interesting (Beridze, 1970) the wines, exclusively rich with flavonoids, are prepared by means of this technology which are characterized by high antioxidant activity and considerably reduce injury of tissues and cells (Middleton,et al. 2000, Robles-Sardin, et al. 2010, Williams, et al. 1997).

According to ancient Kakhethian traditional technology of making of the wine, the crushed grape is placed in a clay vessel (qvevri) dug in the ground, and alcoholic fermentation is carried out together with components of a cluster. During fermentation, from a stem, skin and seeds of a grape in plenty are extracted phenolic compounds which define the composition and essence of Kakhethian wine, and interaction of these compounds with the oxidizing enzymes contained in a skin, pulp and stem of a grape, defines taste and aroma, characteristic for Kakhethian wine. Grape seeds play a main role during formation of a wine of the Kakhethian type as seeds basically increase the content of phenolic compounds in a wine, and give to it characteristic aroma. Stems promote clarification of a wine and enrich it with flavonoids and extractive substances. The grape skin gives to a wine gentle, specific, varietal aroma. Flavonoid compounds define character of a wine, at ageing these compounds are oxidized, therefore the wine becomes soft, velvety and pleasant taste. Quality of the wine made in a clay vessel (qvevri) is much better than quality of the wine made in a wooden vessel. The wine made in qvevri has specific fruit taste and is characterized by integrity. Therefore, qvevri is the best vessel for making of the Kakhethian wine. Besides, its advantage consists still that in the qvevri dug in the ground fermentation occurs in more normal conditions, because of smaller fluctuation of temperature.

The aim of this study is to estimate total amount of phenols, catechins, proanthocyanidins and anthocyans in wines of the European and Kakhethian type, and to compare their anti-radical efficiency in the system forming a radical of 2,2-diphenyl-1-picrylhydrazyl (DPPH*).

MATERIAL AND METHODS

White and red wines of the Kakhethian and European type, made of a grape varieties (*Vitis vinifera L.*) cultivated in Georgia, Italy and Slovenia, were given to us by wineries and physical persons whom we express gratitude.The technology of making of white (W) and red (R) wines, variety of a grape, the country of cultivation, wine factory and year of making are given in Tab. 1.

In samples of analyzed wines the total content of phenols is determined with Folin-Chiocalteu reagent (Singleton, & Rossi, 1965), catechins and proanthocyanidins - by method of Swain & Hillis, 1959), anthocyans – by Durmishidze & Sophromadze (1983). Standard

curves are constructed: for phenols - on a basis of gallic acid ("Sigma", maximum absorption 765 nm), for catechins - on a basis of (+) catechin ("Theodor Schuchard", maximum absorption 500 nm), for proanthocyanidins - on a basis of cyanidin isolated from a grape skin (maximum absorption 548 nm) and for anthocyans - on a basis of malvidin-3-monoglucoside (maximum absorption 536 nm).

For determination of antiradical efficiency of wine the stable radical of 2,2-diphenyl-1-picrylhydrazyl (DPPH*) with maximum absorption 520 nm was used (Sanchez-Moreno, et al. 1998). Alcohol was removed from samples of analyzed wine (50 ml) by evaporation on the vacuum - rotational evaporator at 40^{o}C and after that the volume of samples again supplemented up to initial volume with distilled water. Spectrophotometric measurements were carried out on spectrophotometer СФ-26 (Russia). Each experimental variant was repeated five tames. Experimental data were processed statistically by computer program „MS Excel".

Tab. 1

Wine Samples*

Wine	Tecnology of preparation	Grape Variety	Country and Winery	Vintage
1W	Kakhethian	*Rqatsitheli*	Georgia, Kakhethi, „Okros Kvanchkara"	2005
2W	Kakhethian	*Khikhvi*	Georgia, Kakhethi, Physical Person	2005
3W	Kakhethian	*Ribolla*	Italia, Azenda Agricola, Osalavia Francesco Joŝko Grauner	2003
4W	European	*Rqatsitheli*	Georgia, Kakhethi, „Badagoni"	2005
5W	European	*Kakhuri Mtsvane*	Georgia, Kakhethi, Physical person	2004
6W	European	*Tsulukidzis tetra*	Georgia, Racha, „Okros Khvanchkara"	2004
7W	European	*Rebula*	Slovenia, Vipavska dolina, Vinorodni okolis, deadami Azelen	2006
8R	Kakhethian	*Sapheravi*	Georgia, Kakhethi, „Vazi+Ltd "	2005
9R	Kakhethian	*Cabernet Sauvignon*	Georgia, Kakhethi, „Vazi+Ltd "	2005
10R	Kakhethian	*Ojaleshi*	Georgia, Samegrelo, „Vazi+Ltd "	2005
11R	European	*Sapheravi*	Georgia, Kakhethi, Physical person	2007
12R	European	*Aleksandreuli*	Georgia, Racha, „Okros Khvanchkara "	2003
13R	European	*Merlot*	Georgia, Kakhethi, „Besini "	2008

* W – White wine; R – Red wine

RESULTS AND DISCUSSION

According to the received data (Tab. 2), by comparison of the quantitative content of phenolic compounds in the wines made on the Kakhethian and European technologies it is obviously visible that white and red wines of the Kakhethian type (1W, 2W, 3W, 8R, 9R, 10R), under the content of phenolic compounds considerably surpass to the appropriate wines made on the European technology (4W, 5W, 6W, 7W, 11R, 12R, 13R).

In white wines made on the Kakhethian technology, the total content of phenols varies from 1296 mg (a wine 3W) up to 2290 mg (a wine 1W), and in red wines of the Kakhethian type - from 2848 mg (a wine 10R) up to 4416 mg (a wine 8R) per liter, while in white wines made on the European technology, these parameters varies from 210 mg (a wine 7W) up to 456 mg (a wine 6W), and in red wines of the European type - from 1630 mg (a wine 12R) up to 3130

mg (a wine 11R) per liter. Thus, as a result of making of a wine by the Kakhethian technology, the wine is considerably enriched by phenolic compounds.

Tab. 2

The Content of Total Phenolics, Catechines, Proanthocyanidins, Antocyans in White and Red Wines and Antiradical Efficiency

Wine	Total phenolics, mg/l	Catechins, mg/l	Proanthocyanidins, mg/l	Anthocyans, mg/l	EC_{50} (g antioxidant Kg^{-1} DPPH*)	T_{EC50} (min)	AE ($x10^{-3}$)
1w	2290±38	640±007	690±7.1		510±11.7	4.5	0.44
2w	2000±13	453±01	1097±2.4		515±16	4.5	0.43
3w	1296±46	509±4	392±16		847±35	5	0.23
4w	346±11	39±1	47.8±2		1191±14.1	5	0.16
5w	278±7	27±2	43.2±1.1		1447±22.4	5	0.12
6w	456±26	77±2	165±8.7		1219±18.4	4.5	0.18
7w	210±4	8±2	-		893±13.3	5	0.038
8R	4416±100	1010±23	1203±15	1270±45	516±16.5	3.2	0.62
9R	2848±72	798±2	728±13	317±24	382±14.9	5	0.52
10R	3700±85	862±11	872±18	414±20	342±11.5	5	0.58
11R	3130±76	582±5	610±55	1456±36	519±5.1	4.4	0.43
12R	1630±50	378±15	980±69	53.2±5	595±14.5	5	0.34
13R	2318±73	636±33	826±4	322±18	880±4.3	4.5	0.25
α-Tocopherol					625±22.7	5	0.32

Special interest represents study of phenolic compounds of the wines made on the Kakhethian and European technologies, from the same variety of a grape. We have compared the wines made by the Kakhethian (1W, 3W, 8R) and European (4W, 7W, 11R) technology, from the autochthonous Georgian varieties of grape (*Rqatsitheli*, *Sapheravi*) and from a variety of a grape cultivated in some countries of Europe (*Ribolla*) (Tab. 2).

In the Kakhethian wine (1W), made of a variety of a grape *Rqatsitheli*, total amount of phenolic compounds, catechins and proanthocyanidins, is higher in 6.6 times, 16 times, and 15 times, respectively, than in a wine (4W), made from the same variety of a grape by the European technology. Approximately the same patterns of relationship is observed and in case of the wines made in Italy by the Kakhethian technology from a grape of variety *Ribolla*, and in Slovenia from the same variety of a grape by the European technology. In the wine made by the Kakhethian technology (3W), the total phenols are 1296 mg/l, and in the wine made by the European technology (7W) from the same variety of a grape, this parameter is 210 mg/l. Kakhethian wine (3W) contains catechins and proanthocyanidins in amount of 509 mg/l and 392 mg/l, respectively, and the wine made of the same variety of a grape by the European technology (7W) contains only insignificant amount of catechins, and practically does not contain proanthocyanidins.

Among the white wines made by Kakhethian technology, the high content of proanthocyanidins characterizes a wine (2W), made of the autochthonous Georgian variety of grape *Khikhvi*, in which the content of these compounds makes 1097 mg/l, and the content of catechins - 453 mg/l (the total of phenols in this wine makes 2 g/l). The wine made by Kakhethian technology from a grape variety *Khikhvi* has straw color, contains high amount of extractive substances, is perfect and harmonious (Tabidze, 1954).

Among the white wines made from the autochthonous Georgian varieties of a grape by the European technology, the wine (6W), made of a grape variety *Tsulukidzis Tetra* is distinguished, in which the total content of phenols makes 456 mg/l, catechins - 77 mg/l, and proanthocyanidins - 165 mg/l. Also it is necessary to note a wine (5W) made of a grape variety *Kakhuri Mtsvane* by the European technology, in which the total content of phenols makes 278 mg/l, catechins - 27 mg/l, and proanthocyanidins - 43.2 mg/l. This wine tastes very gentle and aromatic.

In red wine made from grape variety *Sapheravi* by Kakhethian (8R) and European (11R) technologies, the total content of phenols makes 4416 mg/l and 3130 mg/l, respectively. The total content of catechins and proanthocyanidins in wine made by Kakhethian technology is 1.7 times and 2 times higher, than in wine made by European technology.

Partially other interrelation is observed in a case of anthocyans. In red wine made by European technology (11R) the content of anthocyans is 1456 mg/l, but in red wine made by Kakhethian technology (8R) their content makes 1270 mg/l. The reason of it, apparently, consists in long process of maceration in a qvevri that causes sedimentation of anthocyans.

Among autochthonous red varieties of grape of Georgia is distinguished *Ojaleshi*. This wine made by Kakhethian technology is characterized by good color, typical viscosity for red wines, extract content, and harmonicity (Ramishvili, 1948). In this wine (10R), the total content of phenols makes 3700 mg/l, catechins - 862 mg/l, proanthocyanidins - 872 mg/l, and anthocyans - 414 mg/l.

The characteristic of the wine made by Kakhethian technology from of the French variety of grape *Cabernet Sauvignon* cultivated in Georgia is also important. In this wine (9R), the total content of phenols makes 2848 mg/l, catechins - 798 mg/l, proanthocyanidins - 728 mg/l, and anthocyans - 317 mg/l. Comparison of the wine made by European technology from the French variety of grape *Merlot* cultivated in Georgia with the wine made in Argentina from the same variety of a grape and by the same technology is rather interesting. In wine made in Kakhethi (13R) the total content of phenols, catechins, proanthocyanidins and anthocyans makes 2318 mg/l, 636 mg/l, 826 mg/l and 322 mg/l, respectively (Tab. 2), while in red wine made in Argentina the total content of phenols is 1637 mg/l, the total content of catechins and proanthocyanidins is 13.30 mg/l, and content of anthocyans is 52.61 mg/l (Sanches-Moreno et al., 2003). It is necessary to note also the wine (12R) made from the autochthonous Georgian variety of a grape *Aleqsandreuli*, which is characterized by pleasant taste and delicate aroma. In this wine the total content of phenols makes 1630 mg/l, catechins - 378 mg/l, proanthocyanidins - 980 mg/l, and anthocyans - 53.2 mg/l.

Thus, study of total amount of phenolic compounds, catechines, proanthocyanidins, and anthocyans of white and red wines prepared by Kakhethian and European technologies has demonstrated that by content of said compounds Kakhethian wines considerably exceed European wines, that specifies in high medical and prophylactic properties of wines of Kakhethian type. This conclusion has been confirmed by investigation of antiradical efficiency (AE) of wines of Kakhethian and European type (Tab. 2). According to these findings average value of AE of white and red wines of Kakhethian type 2.3 and 1.7 times exceeds average value of AE of white and red wines of European type, respectively. Among wines, the white (1W, 2W) and red (8R, 9R, 10R) wines of Kakhethian type are especially distinguished by their antiradical efficiency.

The data of some authors on the enrichment of white wines of European type by poliphenols should be noted (Williams & Elliot, 1997) have carried out researches whose goal was the development of a seed-enhanced (polyphenol-enriched) white wine with greater positive health potential. This, seed-enhanced white wine is currently being evaluated in human

subjects with regard to its potential effect on the cardiovascular system and inhibition of platelet aggregation. According to Auger et al. (2005), white wine prepared from grape variety *Chardonnay*, enriched by polyphenols possesses a protective effect against early forms of atherosclerosis in hamsters, and according to Fuhrman et al. (2001), the white wine enriched by polyphenols possesses similar to red wine antiradical properties.

CONCLUSIONS

As a result of comparison of white and red wines made by Kakhethian and European technologies it is shown that by content of total amount of phenolic compounds, catechines, proanthocyanidins, and anthocyans, and by antiradical efficiency, the Kakhethian white and red wines considerably exceed European white and red wines, that specifies in high medical and prophylactic properties of wines of Kakhethian type.

BIBLIOGRPHY

Auger C., Rouant J. M., Vanderlinde R., Bornet A., Decorde K., Lequeux N., Cristol J.p., Teissedre P.L. 2005. Polyphenols-Enriched Chardonnay White Wine and Sparkling *Pinot Noir* Red Wine Identically Prevent Early Atherosclerosis in Hamsters. *Journal of Agricultural and Food Chemistry*, 53(25): 9823-9829.

Beridze G., 1970. Kakhuri Gvino. 115 p. (Georgian).

Durmishidze S., Sopromadze A., 1983. Spektrofotometricheskoe Opredelenie Antocuanov v Iagodax Vinograda. In: Metody Biokhimicheskikh Issledovanii. Tbilisi, Mecniereba. 134-138 (Russian).

Furman B., Volkova N., Suraski A., Aviram M., 2001. White Wine with Red Wine-Like Properties: Increased Extraction of Grape Skin Polyphenols Improves the Antioxidant Capacity of the Derived White Wine. *Journal of Agricultural and Food Chemistry*, 49(7):3164-3168.

Middleton E., Kandasvami Ch., Theoharides Th.C., 2000.The Effects of Plant Flavonoids on Mammalian Cells: Implications for Inflammation, Heart Disease, and Cancer. *Pharmacological Reviews*, 52(4): 673-751.

Modebadze K., 1948. Megvineoba. Tbilisi. 560 p. (Georgian).

Ramishvili M., 1948. Gurjis, Samegrelos da Ajaris Vazis Jishebi. Tecnika da Shroma. Tbilisi. 134-139 (Georgian).

Robles-Sardin A. E., Bolanos-Villar A. V., Gonzalez Aguilar G.A.,Laura A.de la Rosa. 2010. Flavonoids and Their Relation to human Health. In: Fruit and Vegetable Phytochemicals: Chemistry Nutritional Value and Stability. Laura A. de la Rosa, Emilio Alvarez-Parrilla, Gustavo A. Gonzalez-Aguilar., Eds. Wiley-Blackwell. 155-175.

Sanchez-Moreno C., Cao G., Ou B., Prior R.L., 2003. Anthocianin and Proanthocyanidin Content in Selected Wite and Red Wines. Oxigen Radical Absorbance Capacity Comparison with Nontraditional Wines Obtained from Higbush Bluberry. *Journal of Agricultural and Food Chemistry*, 51(17): 4889-4896.

Sanchez-Moreno C., Larrauri J. A., Sauro-Calixto F., 1998. A Procedure to Measure the Antiradical Efficiency of Polyphenols. *Journal Science Food Agricultural*, 76: 270-276.

Singleton V. L., Rossi J. A., 1965. Colorimetry of Total Phenolics with Phosphomolybdic-Phosphotungstic Acid Reagents. *American Journal of Enology and Viticulture*, 16(48): 144-158.

Swain T., Hillis W. E., 1959. The Phenolik Constituents of *Prunus domestica*. 1.The.Quantitative Analysis of Phenolic Constituents. *Journal Science Food and Agricuktural,* 10: 63-68.

Tabidze D., 1954. Sakartvelos Vazis Ji.shebi. Teqnika da Shroma. Tbilisi. 116-126 (Georgian).

Williams R.L., Elliot M.S., 1997. Antioxidants in Grape and Wine: Chemistry and Health Effects. In: Natural Antioxidants:Chemistry, Health and Applications. Shahidi F., Eds. AOCS Press. 150-173.

OXYGEN TRANSMISSION RATE OF SCREWCAPS BY CHEMOLUMINESCENCE AND AIR/CAPSULE/HEADSPACE/ACIDIFIED WATER SYSTEM

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RÉSUMÉ ENGLISH

375mL white bordelaises were filled with acidified ultrapure water (pH 1.5) and closed with 2 types of caps (Saran tin, Saranex). The liquid was deoxygenated via bubbling with a blend of 50%CO_2 50%N_2. 3-4 dry ice ministicks were placed on the surface. A drop of liquid nitrogen was deposited in the cap just before placement on the bottle. Dissolved and gaseous O_2 contents were measured with a PreSens probe for 63 days of storage at 20 and 35°C in the vertical position on 8 bottles/procedure mechanically agitated for 30mn from T0 to T0+63 days (17 analysis dates). The average total O_2 concentration at T0 was 6.3±3.7 µg/bottle.

At both temperatures, the total O_2 content remained stable for the 63 days for bottles with Saran tin seal proving that there was neither consumption nor penetration of oxygen. The Oxygen Transmission Rate (OTR) of the seal is thus nearly nil. For the Saranex seal, the content increases with temperature. The slope, thus speed of penetration of the oxygen, of the regression line ($R^2 > 0.992$) was 2.6 and 4 µg/d/bottle respectively at 20 and 35°C.

RÉSUMÉ FRANÇAIS

Une mise en bouteille en bordelaises blanches de 375mL d'eau ultrapure acidifiée à pH 1.5 a été réalisée avec 2 types de capsules métalliques (joints Saran étain, Saranex). Le liquide a été désoxygéné par bullage au mélange 50%CO_2 50%N_2. 3-4 ministicks de carboglace étaient versés en surface. Une goutte d'azote liquide était déposée dans la capsule juste avant la pose sur la bouteille. Les teneurs en O_2 dissous et gazeux ont été mesurées avec une sonde PreSens pendant 63 jours de stockage à 20 et 35°C en position verticale sur 8 bouteilles/modalité après agitation. La concentration moyenne en O_2 total à T0 était de 6.3±3.7µg/bouteille.

Aux 2 températures, la teneur en O_2 total reste stable durant les 63 jours pour les bouteilles avec joint Saran étain, prouvant qu'il n'y a ni consommation, ni pénétration d'oxygène. L'OTR du joint est quasiment nul. Pour le Saranex, la teneur augmente avec la température. La pente, donc la vitesse de pénétration de l'oxygène, de la droite de régression ($R^2>0.992$) est respectivement à 20 et 35°C de 2.6 et 4 µg/j/bouteille.

INTRODUCTION

It is widely accepted that oxygen can deteriorate or improve a wine. Oxygen management is therefore a critical aspect in the winemaking process from harvest to post-bottling. This is quite a challenging task as a good oxygen management strategy requires a good understanding of the specific needs of specific wines at specific time throughout the whole process.

For instance, oxygen exposures during fermentation (oxygen is a key nutrient for fermenting yeasts) are different from those needed during ageing (micro-oxygenation plays a role in stabilizing colour, softening tannins and diminishing herbaceous characters) or those needed after bottling (oxygen transferred through closures keeps on participating to wine evolution). And that will depend on grape varietals, winemaking styles and intended shelf-life.

The various studies undertaken to characterize the appearance of dissolved oxygen during operations performed on wines show that bottling is one of the most critical phases (Castellari et etc., 2004; Vidal et etc., 2004).

In addition, Vidal et Moutounet (2006) reported indeed that the headspace can represent a big reserve of oxygen in the bottle, especially in screw cap bottles (more than 3 mg). They showed also that this oxygen quantity was consumed by the wine during time.

As once the bottles have been sealed, the only remaining means for mastering the evolution of wines are the storage parameters like the temperature, the relative humidity, the light and the closure permeability. But the Oxygen Transfer Rate (simplified version of oxygen permeability) remains the only controllable parameter by the conditioner before the wine leaves towards trade-circuit.

Currently, one of the principal methods for the OTR determination of stoppers and screw caps using experimental air/stopper/gas set-ups (Oxtran, Mocon, Minneapolis, USA) based on the transfer of oxygen from outside to inside a container through a stopper.

Lopes et etc., 2005 adapted a method based on the reduction of an indigo carmine solution in a stopped bottle for the non-destructive determination of the release of oxygen through a cork or a cap. Whatever the type of closure, the rate of oxygen release peaked during the first month after bottling because of the release of the oxygen in the cork, according to the authors. The rate then stabilized at between 0.33 and 28 µg/day during the next 11 months, according to the type of closure. In 2006, they showed that oxygen penetration in the bottle was smallest with caps, followed by technical corks, intermediate with natural corks and high with synthetic corks.

Recently, luminescence-based technologies have been developed. These tools are very easy to use and heretofore an interesting new generation of analyzers has been developed with separate sensors. In the case of measurement of Total Package Oxygen, the sensors can be glued in bottles prior to filling with wine and allow for non-invasive measurement (measurements done through the glass using an optical fiber). These sensors can be used to measure dissolved oxygen as well as headspace oxygen (O'Brien et etc., 2009). Dieval et etc., 2009 showed that this new method is more accurate than polarographic probe and micro GC methods particularly because it does not need gas or liquid sample.

A follow-up of the dissolved and gaseous oxygen by PreSens optode on screw cap bottles filled with a liquid which consumes oxygen as low as possible and as poor in germs as possible was carried out. Then this new technique was compared to the Mocon technique based on gas/stopper/gas set-ups for the evaluation of the weak OTR level of screw caps.

MATERIALS AND METHODS

Experimental design:

375 mL glass bottles were filled with acidified ultrapure water and closed with 2 types of caps (Saran tin, Saranex 38) as shown in the plan of the experiment in "Tab. 1". Measurements of dissolved and gaseous oxygen by PreSens optode were scheduled throughout the 63 days of bottle storage to characterize the OTR of caps.

The bottles were stored upright in one room regulated at 20°C or in another one regulated at 35°C.

The analytical monitoring was performed at 17 dates of analysis after bottling (days T0, 3, 6, 10, 13, 17, 19, 24, 27, 31, 35, 38, 41, 52, 56, 60 and 63).

Table 1: Experimental design for bottling of acidified ultrapure water.

Procedure	Code	Screw cap	Storage T°C	Nb bottles	Analysis	Dates of analysis
1	Saran20	Saranex	20	8	Dissolved and gaseous O_2	days T0, 3, 6, 10, 13, 17, 19, 24, 27, 31, 35, 38, 41, 52, 56, 60 and 63
2	Saran35	Saranex	35	8		
3	Etain20	Saran tin	20	8		
4	Etain35	Saran tin	35	8		

Every day of analysis, oxygen concentrations were measured for 8 bottles per procedure. Before the measurement, every bottle was mechanically agitated for 30mn on a plateau orbital shaker (50 mm of amplitude, 120 rpm) in order to reach the equilibrium of oxygen partial pressure between the headspace and the acidified water, especially at T0, just after bottling.

Choice of conditioned liquid:

The choice of this solution was validated by a prior bottling. The microbiological controls carried out on 8 samples were negative contrary to a hydro alcoholic solution of 20%vol (filtration of 50 mL of solution, filters laid down on PCA standard growth medium, 3-5 days of incubation at 30°C).

Moreover with acidified water, the solution foams less than with ethanol at bubbling. The dissolved oxygen content remains stable close to zero in the acidified water bottles contrary to those with the hydro alcoholic solution. Over 21 days of storage at 21°C, the average dissolved oxygen content in acidified water was equal to 2.8 ± 0.8 ppb instead of 18.7 ± 0.8 ppb with the hydro alcoholic solution (n = 8 dates x 6 bottles per procedure).

Bottling:

Before filling, every bottle was rinsed with pure alcohol then drained off during a few minutes in order to reduce microbial contaminations.

The filling of white screw cap bordelaises bottles with acidified ultrapure water at pH = 1.5 by hydrochloric acid was made by weighing of 363 g corresponding to 363.4 mL and an headspace level of 53 mm, in order to avoid any overflow during bubbling. The density of the acidified aqueous solution was equal to 0.9987 g/mL and determined by density meter.

Each bottle was deoxygenated via bubbling of a gas mixture (N_2 50% / CO_2 50%) and thanks to a inlet filter at a pressure lower than 100 hPa by avoiding the overflows until reaching an oxygen concentration lower or close to 10 ppb (4.5 ± 3.6 ppb), corresponding to an approximate time of 30mn. A multichannel bubbling system was used for bubbling 14 bottles at the same time “Figure 1”. Every time a bottle was picked up from the system, it was replaced by another bottle in order to maintain the flow of the unit constant.

3-4 dry ice ministicks were placed on the surface of liquid in order to saturate the headspace of the bottle with carbon dioxide. Then a snowdrop of liquid nitrogen was deposited in the cap just before placement on the bottle. The cap was crimped thanks to a single head capping machine when no visible “smoke” escaped from the bottle.

Figure 1: Multichannel bubbling system of bottles.

Non invasive method for analysis of dissolved and gaseous oxygen:

Dissolved and gaseous oxygen control of bottles was carried out by PreSens luminescent probe and PSt6 luminescent spots glued onto the inner surface of the bottles. A few days before filling, one spot is glued in the body of the bottles to measure dissolved oxygen and the other in the neck of the bottles for the headspace oxygen measurement. A PreSens Fibox 3 trace fiber optic probe was used. The Fibox 3 measures the luminescence decay time of the immobilized luminophore (spot). The luminophore is excited with a sinusoidal intensity-modulated monochromatic light delivered by an optical fibre and its decay time causes a time delay in the light signal emitted by the luminophore. This decay time, or phase angle, Φ, decreases in the presence of oxygen and is correlated to oxygen content.

The system measures the oxygen partial pressure (P_{O2}) in hecto Pascals (hPa). It can also express the values in mg/L or in ppb/ppm or in %v/v according to the studied phase and to the temperature and to the pressure. Considering that the target oxygen contents are weak, the PSt6 spots were selected because their measurement range goes from 0 to 1.8 ppm or 0 to 41.4 hPa, with a limit of detection equal to 0.02 hPa and an accuracy of 3% of the respective concentration.

A manual calibration was be applied with the two calibration values (phase and temperature values at 0% and 6% air-saturation) obtained from the PreSens inspection of the batch of the used sensor spots.

Determination of Total Oxygen content in bottle:

Dissolved O_2 µg/bt = O_2 ppb x O_2 density (T°C) mg/mL x V_L mL / 1000

With

V_L = 363.47 mL (363 g / 0.9987 g/mL)

O_2 density (T°C) in g/L or mg/mL = 32 g / [22.41 L x (T°C + 273.15) / 273.15]*

**By thermostated room, one bottle without PSt6 spot was used for temperature control of liquid by PreSens temperature probe.*

Headspace O_2 µg/bt = [O_2 % v/v / (100 x 1000 x V_{HS} mL*)] x density O_2 (T°C) mg/mL

With

V_{HS} = average volume measured on bottles closed by Saranex beforehand the experiment according to the storage temperature, either 19.02 ± 1.27 mL (n=12) at 20°C or 16.66 ± 1.00 mL (n=12) at 35°C.

Total O_2 µg/bt = Dissolved O_2 µg/bt + Headspace O_2 µg/bt

Operating mode for the measurement of headspace volume of bottle:

The bottles were filled with 363g of the same acidified water and then closed with screw cap to avoid evaporation. The closed bottles were stored during 24 hours for temperature setting, as well as a surplus of the solution in thermostated rooms at 20 or 35°C. Once the temperature was reached, the bottles were also weighted and then they were filled to the brim with the surplus of solution. Finally, the difference of mass was converted into volume by the aid of the density.

Measure of screw caps OTR by Oxtran Method at 23°C:

At the end of storage and after oxygen analysis by PreSens probe, 4 bottles/screw cap were sent to the LNE Trappes (France) for the measure of OTR at 23°C. The used experiment is conform to ASTM D3985 and ISO 15 105-2 standards: 100% O_2 / cap / N_2 under 80-90% of relative humidity; cut and glued neck, 30-40 days of conditioning before measurement by Mocon Oxtran 2-20).

Materials:

Ultrapure water produced by MilliQ-RG (Millipore SAS, St Quentin en Yvelines, France); conductivity ≤ 18 MΩ/cm.

Smoking hydrochloric acid, purity = 37% (Merck, Fontenay sous bois, France).

375 mL screw cap Bordelaise bottles 38.5 BVS, headspace level of 30 mm at 20°C (Saint Gobain, Chalon sur Saône, France).

Screw caps Stelvin (Alcan Packaging, Paris, France) with Saranex 38 or with Saran tin joint.

Density meter Anton Paar DMA 500 (Courtaboeuf, France).

Plateau orbital shaker 500 Gerhardt (Les essarts le roi, France).

Tubing PTFE (0.062 in. ID x 0.125 in. OD) degassing inlet filter in PE (L x Ø: 1.9 x 1 cm; pores diameter: 19 to 32 µm) for multichannel bubbling system provided by Waters (Guyancourt, France).

Dry ice of CO_2 (ministicks of CO_2, purity 99%) and liquid nitrogen (purity 99.7%) supplied by Cryo express (Bobigny, France).

Gas mixture of 50% N_2 / 50% CO_2: Gourmet N 50 (Messer SAS, Puteaux, France) containing 50% ± 3% vol of CO_2, supplemented until 100% with N_2.

Single head capping machine Zalkin for screw cap TM3 32563 (Rueil-Malmaison, France).

RESULTS AND DISCUSSION

The general average of total O_2 concentration at T0 was 6.3 ± 3.7 µg/bottle. That proves the efficiency of our protocol against the oxygen ingresses at bottling.

At both temperatures, the total O_2 content remained stable for the 63 days for bottles with Saran tin seal proving that there was neither consumption nor penetration of oxygen “Figure 2” and “Tab. 2”. The Oxygen Transmission Rate of this seal is thus nearly nil.

For the Saranex seal, the content increases with time and with temperature “Figure 2”. The slope, thus the rate of the oxygen ingress, of the regression line ($R^2 > 0.992$) was 2.6 and 4 µg/d/bottle respectively at 20 and 35°C “Tab. 2”.

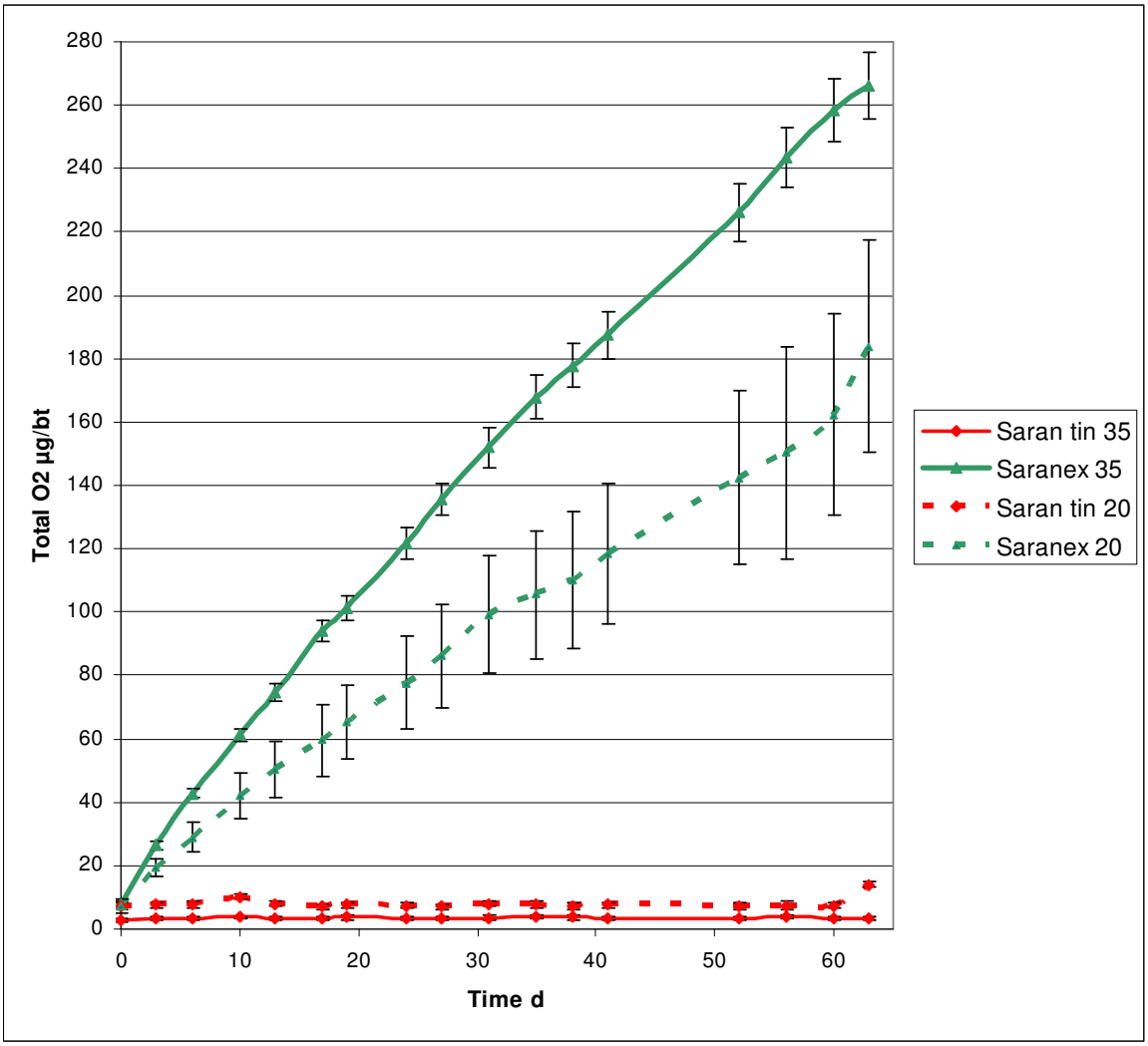

Figure 2: Evolution of Total O_2 at 20°C and 35°C by experimental method.
Unbroken lines: Total O_2 mg/bt at 35°C, dotted lines: Total O_2 mg/bt at 20°C.
Error bars represent the standard deviation of the 8 replicates.

Table 2: Linear regression of Total O_2 curves of experimental method.
Linear regressions were obtained with Microsoft Excel 2002.

Storage T°C	**Screw cap**	**Linear regression Total O_2 µg/bt**	**R^2**	**OTR µg/day/bt**
20	Saran tin	Tot O_2 = 0.025t + 7.3	0.0873	≈ 0
20	Saranex	Tot O_2 = 2.55t + 14.5	0.9929	2.55
35	Saran tin	Tot O_2 = 0.003t + 3.4	0.0702	≈ 0
35	Saranex	Tot O_2 = 4.021t + 20.9	0.9952	4.02

If we compare the OTR values given by this new method with air/cap/headspace/acidified water system at 20°C with those of Oxtran method with 100% O_2/cap/N_2 system at 23°C "Tab. 3", we get an Oxtran method/acidified water method OTR ratio equal to 3.25. This could be partially explained by the difference of operating conditions (higher temperature, atmosphere outside of 100% O_2 instead of 20.95% for the Oxtran method). Moreover, there

are gas flows which sweep the joint on its outside (O_2) and on its interior face (N_2) which don't exist in the ageing of wine bottles.

Table 3: OTR values in mL or µg/day/cap by standard method.
*With d_{O2} at 23°C = 1.317 mg/mL.

Screw cap	Number of bottles	Oxygen Transfer Rate (values at 21% O_2)	
		mL/day/cap	µg/day/cap*
Saran tin	3	0.0015 ± 0.0004	2 ± 0.5
Saranex	3	0.0063 ± 0.0012	8.3 ± 1.6

This air/cap/headspace/acidified water method at 20°C is closer to oenological reality than the Oxtran 100%O_2/cap/N_2 at 23°C type method for which the OTR values obtained were respectively 2 and 8.3 µg/d for Saran tin and Saranex "Tab. 3".

CONCLUSIONS

Following the various trials, this experiment method turns out to be reproducible even with oxygen partial pressures close to the hecto Pascal. Its originality especially comes from the fact that the operating conditions are closer to oenological reality (air/cap/headspace higher than 90% RH/liquid at a given temperature of storage) than the Oxtran method of type 100% O_2/cap/N_2 with 80-90% RH at 23°C.

Furthermore, this work showed the interest to use acidified water to very low pH as solution inducing neither microbial development, nor oxygen consumption, provided to follow those drastic rules against microbial contaminations and atmospheric oxygen.

Finally, this method proves that in conditions of very close to wine bottles storage the OTR values of screw caps are lower than those given by standard tests, even almost zero with the Saran tin caps.

The integration of the follow-up of the atmospheric and headspace pressures in a further study would enable to calculate the oxygen permeability of screw caps.

BIBLIOGRAPHY

Castellari M., Simonato B., Torinielli G.B., Spinelli P., Ferrarini R, 2004. Effects of different enological treatments on disssolved oxygen in wines. Ital. J. Food Sci., vol16,nº3, 387-396.

Dieval J.B., Veyret M., Vidal J.C., Aagard O., Vidal S., 2009. Non-invasive measurement of total package oxygen: validation and application. 32ème Congrès Office International de la Vigne et du Vin, Zagreb. Communication affichée.

Lopes P., Saucier C., Glories Y., 2005. Nondestructive colorimetric method to determine the oxygen diffusion rate through closures used in winemaking. J. Agric. Food Chem., 53, 6967-6973.

O'Brien V. et etc., 2009. Managing oxygen ingress at bottling. Wine Ind. Journal, 24, n°1, 24-29.

Vidal J.C., Boulet J.C., Moutounet M., 2004. Les apports d'oxygène au cours des traitements des vins. Bilan des observations sur site. 3eme partie. Rev. Fr Oenol., 205, 25-33.

Vidal J.C., Moutounet M., 2006. Monitoring of oxygen in the gas and liquid phases of bottles of wine at bottling and during storage. Journal International des Sciences de la Vigne et du Vin, 40 1, 35-45.

Prévention du risque *Brettanomyces* par l'utilisation d'un biopolymère d'origine fongique : le chitosane

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RESUME

En 2009, KitoZyme a initié en collaboration avec l'ICV (Institut Coopératif du Vin), un projet de recherche & développement dans le but d'étudier l'action du chitosane, un nouvel auxiliaire technologique naturel de type polysaccharide, sur l'élimination des *Brettanomyces* afin de répondre aux attentes des professionnels de la filière pour la prévention du risque *Brettanomyces*.

Les résultats de ce projet ont montré que :

- le chitosane est efficace en 5 à 10 jours et son action n'est pas spécifique à une souche de *Brettanomyces bruxellensis.*
- Le chitosane n'a pas d'impact sur les populations de *Saccharomyces cerevisiae* ni sur le déroulement de la fermentation alcoolique.
- Le chitosane n'a pas d'impact sur le déroulement de la fermentation malolactique (soit en mode spontanée, soit en inoculation). Toutefois, il est recommandé d'attendre 8 jours après la fin du traitement avant de réaliser une inoculation en bactéries lactiques.

SUMMARY

Kitozyme has initiated, in collaboration with ICV (Cooperative Wine Institute) ,an R&D project with the aim at studying the effect of chitosan, a new natural oenological to prevent the growth of *Brettanomyces* during winemaking processes.

The results of this project are as follows:

- The antimicrobial activity of chitosan is effective from 5 to 10 days on different strains of *Brettanomyces bruxellensis*
- *Saccharomyces cerevisiae* and alcohol fermentation were not affected by the use of chitosan
- Malolactic fermentation (either spontaneous or inoculated) is note affected by treatement with chitosan. It's recommended to except 8 days post- treatment to realized inoculation with lactic bacteria.

INTRODUCTION

La découverte des levures du genre *Brettanomyces* remonte à 1904, lorsque Clausen les isole dans un moût de brasserie. Depuis, les *Brettanomyces* sont décrits comme un agent de contamination de nombreux produits de fermentation incluant le vin, le cidre, la bière, le

kombucha, le kefyr, la téquila, etc [Greenwalt C.J. *et al.*, 2000 ; Martens H. *et al* 1997 ; Morrissey W.F. *et al.* 2004 ; Silva P. *et al.* 2004 ; Teoh A.L. *et al.* 2004 ; Wyder M.T. *et al.* 1997].
Dans le milieu viticole, la détection de ces levures de contamination au vignoble est rendue délicate par le fait qu'elles sont, à ce stade, minoritaires. Toutefois la fermentation (processus de transformation des sucres en alcool) va entraîner une véritable « sélection » de ces microorganismes. En effet, les *Brettanomyces* sont particulièrement résistantes à l'éthanol et au SO_2 et sont capables de subsister dans le milieu malgré son appauvrissement en sucres. De plus, certaines techniques ou certains protocoles de vinification peuvent favoriser le développement des *Brettanomyces* dans le vin, comme l'élevage sur lies. Pour que les *Brettanomyces* puissent se développer, moins de 500 mg/l de sucre suffisent.

L'impact des levures de contamination *Brettanomyces* a été prouvé par de nombreux auteurs sur des vins dans différents pays [Di Stefano R. 1985 ; Ciolfi G. 1991 ; Tucknott J. 1977 ; Heresztyn T. 1986 ; Henschke P. 1996 ; Fridriere I. *et al.* 1988], des vins mousseux allemands [Barret *et al.* 1955] des vins jaunes d'Arbois [Galzy P. *et al.* 1955] ou des vins du midi de la France. En 1965, Domercq a rapporté l'isolement des *Brettanomyces* dans des moûts des appellations Saint Emilion et Premières Côtes de Bordeaux et dans des vins rouges ou blancs en cours de conservation des appellations Médoc, Graves et Saint-Emilion.
Quant aux cépages, le pinot semble être le plus touché. En Bourgogne, par exemple, pour le millésime 2000, 50% des vins en fermentation issus de ce cépage et 25% après embouteillage avaient été affectés [Gerbaux V. *et al.* 2000].
En définitive, la maîtrise de cette altération reste difficile, même avec des pratiques œnologiques réfléchies. Les moyens de lutte sont essentiellement curatifs, ils permettent d'agir sur les populations de *Brettanomyces* (flash pasteurisation, filtration). Toutefois ces traitements modifient les qualités organoleptiques des vins et ne préservent pas les vins traités des contaminations ultérieures [Ruiz-Hernandez M . 2003 ; Calderon F. *et al.* 2004 ; Delfini C. *et al.* 2002 ; Puig M. *et al.* 2003 ; Comitini F. *et al.* 2004 ; Blateyron L. *et al.* 2008].

Les propriétés antibactériennes et antifongiques du chitosane ont été largement étudiées et documentées et sont aujourd'hui bien reconnues. Récemment KitoZyme a initié en collaboration avec l'ICV (Institut Coopératif du Vin), un projet de recherche & développement dans le but de proposer un auxiliaire technologique naturel de type polysaccharide, le chitosane, qui puisse répondre aux attentes des professionnels de la filière pour la prévention du risque *Brettanomyces*.

MATERIEL & METHODE

1- Le produit de traitement : le chitosane (Cs)

Le chitosane est un polysaccharide linéaire composé de la distribution le long de la chaîne polymère des unités de répétition D-glucosamine (unité désacétylée) et N-acétyl-D-glucosamine (unité acétylée). Le chitosane est obtenu par désacétylation (hydrolyse des groupement N-acétyl) de la chitine. Il est produit à partir d'une source fongique, non-OGM, le mycélium d'*Aspergillus niger*, un champignon microscopique utilisé pour la production d'acide citrique à destination des industries alimentaires et pharmaceutiques.
Le chitosane se présente sous forme d'une poudre fine insoluble dans le vin, de couleur blanche à légèrement brune, inodore et sans saveur. La structure chimique du chitosane est illustrée sur la Figure 1.

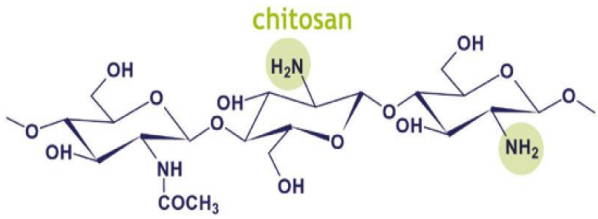

Figure 1- Structure chimique du chitosane

2- Les souches de levure

Les souches de *Brettanomyces bruxellensis* utilisées pour l'inoculation artificielle du vin sont des souches identifiées comme majoritaires sur le vignoble méditerranéen (Blateyron *et al.* 2008) et ont des génotypes 100% A et 100% B. Elles proviennent de la collection des souches ICV-ADN[id.] Elles sont conservées à 4°C sur milieu gélosé incliné (milieu YAC).

3- Le milieu et les conditions de culture du levain

Le levain est un milieu d'acclimatation dans lequel les levures peuvent croître et atteindre une population de l'ordre de 10^7UFC/ml, tout en s'adaptant à un environnement peu favorable (pH acide, taux en éthanol élevé)
La composition du levain utilisé pour les expérimentations est décrite dans le Tab. 1 suivant :

Tableau 1- Composition du milieu levain pour *Brettanomyces bruxellensis*

Composés	Concentrations/conditions
Yeast Nitrogen Base	6,7g/l
Glucose	20g/l
Ethanol	10% (v/v)
Eau déminéralisée	Compléter au volume souhaité
pH	Ajuster à 3,5 avec des cristaux d'acide tartrique
Stérilisation	20 min à 121°C

4- Techniques analytiques de dénombrement

Pour le suivi microbiologique, des échantillons de vins ont été prélevés à T0, T3 (T0+3 jours), T7 (T0+7 jours), T10 (T0+10 jours), T25 (T0+25 jours). Le dénombrement des *Brettanomyces* viables s'est fait par mise en culture sur milieu gélosé spécifique YAC (YEPD + Actidione + Chloramphénicol) selon le protocole suivant : les échantillons de vins sont prélevés et dilués en cascade sous environnement stérile, dans des tubes contenant 9ml ou 9,9ml d'eau physiologique stérile. 100µl sont mis en culture par étalement sur milieu gélosé YAC, à partir de la dilution appropriée pour avoir entre 30 et 300 colonies sur la gélose. Les boîtes sont incubées à 28°C pendant 10 jours. Les mises en culture sont doublées.

RESULTATS & DISCUSSION

1- Effet du chitosane sur la croissance des *Brettanomyces* de profils génétiques différents

Le vin choisi pour cette étude est un vin rouge du Languedoc Roussillon, millésime 2008 dont les paramètres sont décrits dans le Tab. 2. Ce vin a été artificiellement contaminé par des *Brettanomyces* de souche A ou de souche B. Le vin a ensuite subi un traitement suivant les modalités 1, 2, et 3 décrites dans la Figure 2.

Tableau 2- Caractéristique analytique du vin

TAV %	Sucres résiduels g/l	Acidité totale g/l H_2SO_4	pH	SO_2 actif mg/l
13,78	1,7	3,18	3,45	< 0,02

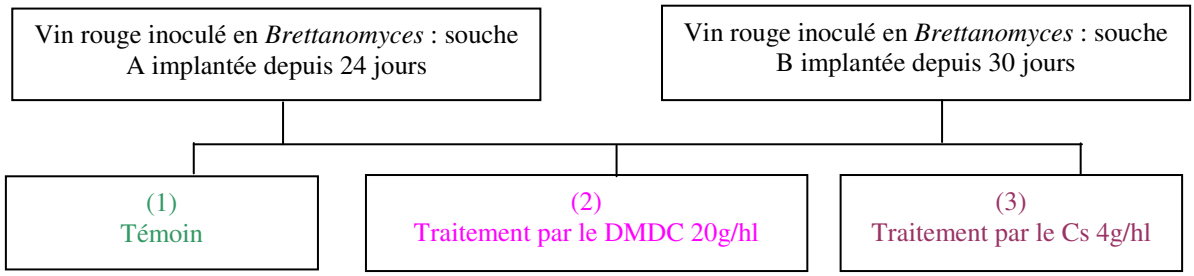

Figure 2- Plan expérimental pour l'étude de l'effet du chitosane sur la croissance des *Brettanomyces* de profils génétiques différents

Nous avons mis en évidence que les effets du DMDC et du chitosane sont similaires quelle que soit la souche de *Brettanomyces*. Les résultats (Figures 3 et 4) montrent une réduction significative des populations en *Brettanomyces* viables sous le seuil de détection (<10UFC/ml) en 5 à 10 jours avec les deux types de traitement.

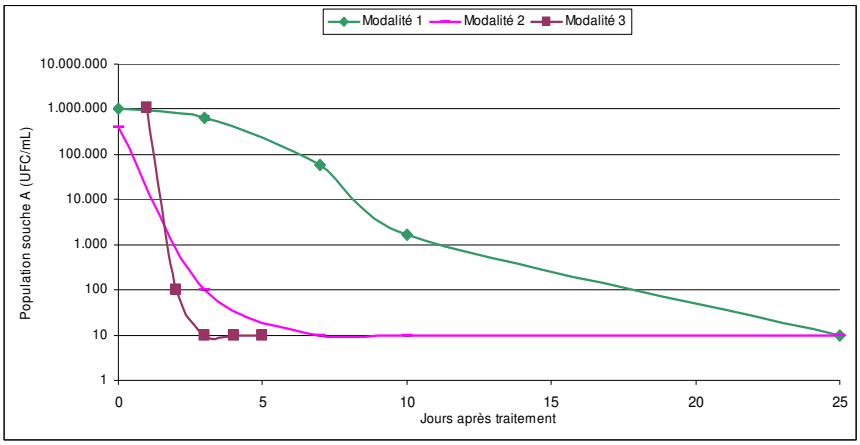

Figure 3- Suivi de la population de *Brettanomyces bruxellensis* souche A dans le vin après le traitement

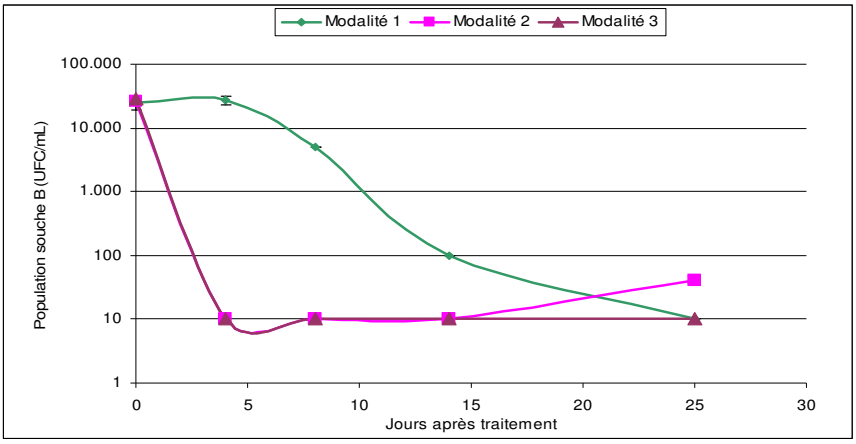

Figure 4- Suivi de la population de *Brettanomyces bruxellensis* souche B dans le vin après le traitement

2- Impact du chitosane sur les autres populations microbiologiques du vin importantes dans le processus de vinification (*Saccharomyces cerevisiae, Oenococcus oeni*)

2.1- Impact sur *Saccharomyces cerevisiae*

Pour cette étude le moût choisi est un jus de raisin blanc de la marque Casino qui a été supplémenté en sucre pour atteindre la teneur de 200g/l et en azote pour atteindre une teneur supérieure 200mg/l. Nous avons par la suite inoculé en *Saccharomyces cerevisiae* ICV D47® à la dose de 30g/hl.

Comme il est décrit dans la Figure 5, l'ajout du chitosane a été réalisé en cours de fermentation alcoolique à deux moments différents :

- densité de 1035 : 3 jours après le levurage
- densité de 1015 : 6 jours après le levurage

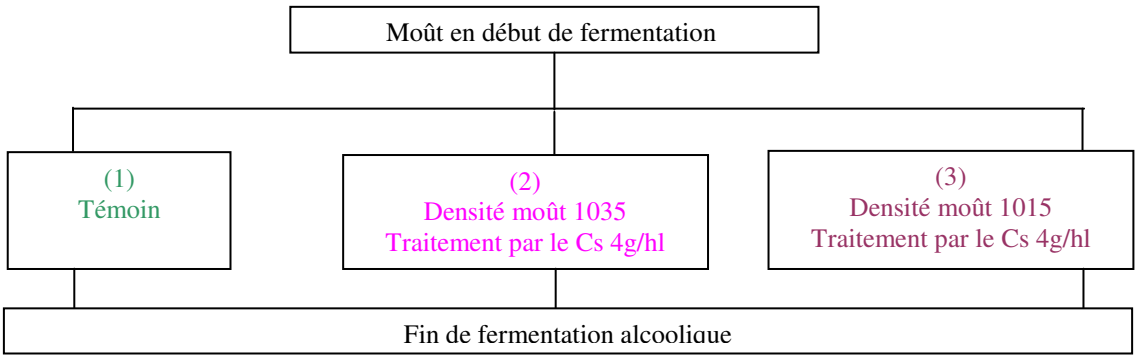

Figure 5- Plan expérimental pour l'étude de l'impact du chitosane sur la croissance des *Saccharomyces cerevisiae*

La Figure 6 permet de démontrer que le chitosane n'a pas d'impact sur le déroulement de la fermentation alcoolique. La période de 13 jours de la réalisation de la fermentation alcoolique est identique quelle que soit la modalité.

La cinétique de croissance de la population de *Saccharomyces cerevisiae* (Figure 8) atteint sa valeur maximale de l'ordre de 6.10^7UFC/ml après 3 jours de fermentation, puis le nombre de

levures se maintient à 5.10^7UFC/ml pendant 10 jours pour finalement amorcer sa phase de décroissance après 13 jours de fermentation.
Cette cinétique est similaire pour toutes les modalités. Par conséquent nous pouvons conclure que le chitosane n'a pas d'impact négatif sur les levures *Saccharomyces cerevisiae.*
Ces résultats sont en accord avec les travaux de Gomez-Rivas *et al.* en 2004 qui ont conclu que le chitosane avait un effet inhibiteur sur *Brettanomyces bruxellensis* et un effet neutre voire positif selon les doses employées sur *Saccharomyces cerevisiae*.

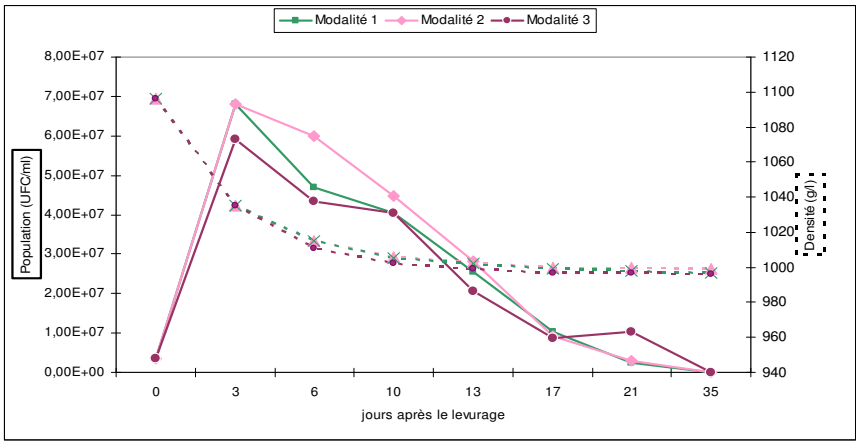

Figure 6- Evolution de la population en *Saccharomyces cerevisiae* dans le moût avant et après le traitement par du chitosane et suivi de la densité

2.2- Impact sur *Oenococcus oeni*
Le vin choisi pour cette étude est un vin rouge du Languedoc Roussillon, millésime 2008 dont les paramètres sont décrits dans le Tab. 3. Nous avons inoculé ce vin en *Oenococcus oeni* Elios 1® suivant les modalités décrites dans la Figure 7.

Tableau 3- Caractéristique analytique du vin

TAV %	Sucres résiduels g/l	Acidité totale g/l H_2SO_4	pH	Acide malique g/l	Acide lactique g/l	SO_2 actif mg/l
12,95	1,4	3,09	3,58	1,26	0,18	0,03

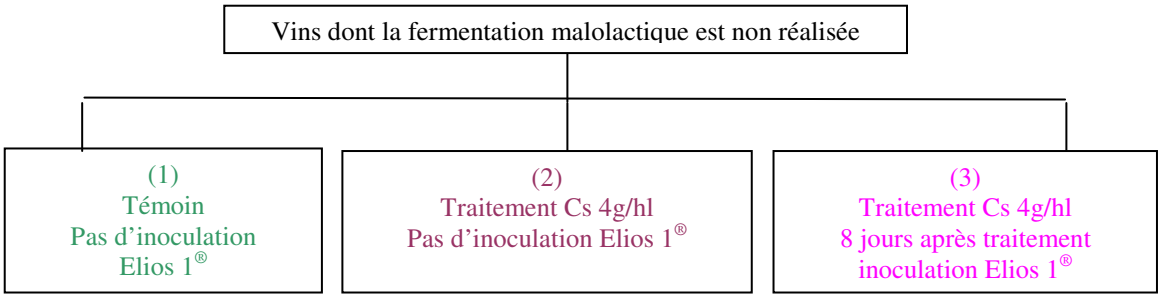

Figure 9- Plan expérimental pour l'étude de l'impact du chitosane sur la croissance des *Oenococcus oeni*

Lors d'un pécédent essai non présenté ici, nous avons démontré grâce au suivi microbiologique des bactéries lactiques et au suivi de la dégradation de l'acide malique qu'un traitement au chitosane à la dose de 4g/hl bloque le développement et l'activité des bactéries lactiques si ces dernières sont inoculées dans les 3 jours suivant le traitement par le chitosane.

Lors de ce second essai, nous avons pu démontrer qu'un traitement au chitosane réalisé 8 jours avant l'inoculation en bactéries lactiques n'a pas d'impact sur la réalisation de la fermentation malolactique (modalité 3).
Dans le cas d'une fermentation malolactique spontanée (modalité 2), le traitement par le chitosane a pour effet de rallonger la phase de latence de la fermentation malolactique de 3 jours.
En conclusion, lors d'un traitement au chitosane, il est recommandé d'attendre 8 jours après la fin du traitement avant de réaliser une inoculation en bactéries lactiques.

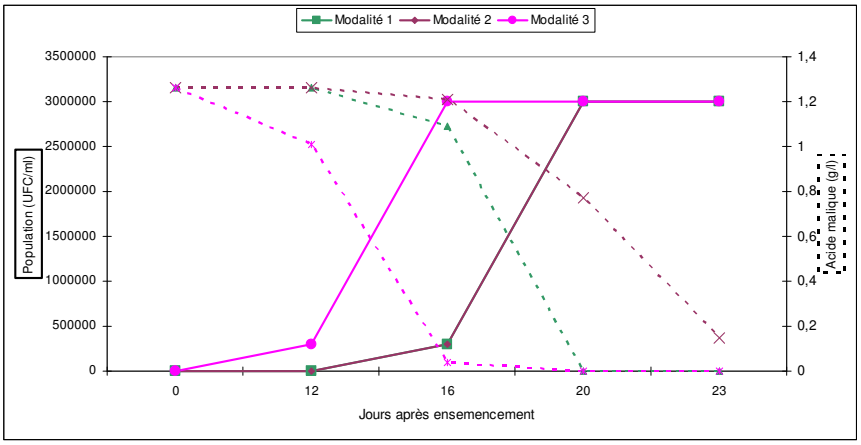

Figure 10- Evolution de la population en *Oenococcus oeni* dans le vin avant et après le traitement par du chitosane et suivi de la FML

CONCLUSIONS

Ce projet de recherche & développement initié en collaboration avec ICV avait pour but de proposer un auxiliaire technologique alternatif au DMDC (limité aux vins avec sucres résiduels, nécessitant un dispositif d'injection spécifique et de ce fait souvent limité à une application au moment du conditionnement) qui puisse répondre aux besoins des professionnels de l'œnologie et du conditionnement tout en tenant compte des exigences de sécurité alimentaire pour le consommateur.
Ce projet a permis de montrer que :

- l'action du chitosane n'est pas spécifique à une souche de *Brettanomyces bruxellensis* : les souches A et B testées ont toutes deux été éliminées par le chitosane en 5 à 10 jours.
- le chitosane n'a pas d'impact sur les populations de *Saccharomyces cerevisiae* ni sur le déroulement de la fermentation alcoolique.
- Le chitosane n'a pas d'impact sur le déroulement de la fermentation malolactique (soit en mode spontanée, soit en inoculation). Toutefois, il est recommandé d'attendre 8 jours après la fin du traitement avant de réaliser une inoculation en bactéries lactiques.

REFERENCES

Barret A. et al. (1955). Sur quelques accidents de vinification dus à des levures à voile. *Communications and Research of the Academy of Agriculture*, Paris, 41, 496.

Blateyron L., Moreau F., Canivenc G.Aliot R. (2008) Caractérisation génotypique des souches de Brettanomyces présentes dans des vins méditerranéens et de la vallée du

Rhône et mis en évidence de l'intérêt du DMDC pour leur élimination dans les vins rouges revue française d'œnologie 220, 8-14

Calderon F. et al. (2004). Aplicaciones de la ultrafiltración en la industria enológica. Últimos avances tecnológicos. *Tecnología del vino.* 16, 49-54.

Ciolfi G. (1991). Ecologia dei lieviti vinari. *Vini d'Italia*. 33-6, 41-46.

Comitini F. et al. (2004). Pichia anomala and Kluveroymyces wickerhammi killer toxins as new tools against Dekkera/Brettanomyces spoilage yeasts. *FEMS Microbiology Letters.* 238, 235-240.

Delfini C. et al. (2002). Fermentability of grape must after inhibition with dimethyl dicarbonate (DMDC). *Journal of Agricultural and Food Chemistry*. 50, 5605-5611.

Di Stefano R. (1985). Gli etil fenoli nei vini. *Vegnevini*. 5, 35-38.

Froudriere I. ; Larue F. (1988). Conditions de survie de *Brettanomyces* (*Dekkera*) dans le moût de raisin et le vin. *Connaissance de la Vigne et du Vin*. 2, 296-303.

Galzy P.; Rioux J. A. (1955). Observations ser quelques vins atteints par la maladie de "la fleur" dans le midi. *Progrès Agric. et Vitic.* 144, 365-370.

Gerbaux V. et al. (2000). Les phénols volatils dans les vins de Pinot noir de Bourgogne. *Bulletin de l'O. I. V.* 73, 581-599.

Gomez-rivas L. and al. (2004) Selective antimicrobial action of chitosan against spoilage yeast in mixed culture fermentations J. Int. Microbiol. Biotehnol. 31, 16-22

Greenwalt, C. J. et al. (2000). Kombucha, the fermented tea: microbiology, composition, and claimed health effects. Journal of Food Protection, 63, 976–981.

Henschke P. (1996). Technical notes: *Brettanomyces* in Porto wine. *Technical Review of the Australian Wine Research Institute*. 105, 12-13.

Heresztyn T. (1986). Metabolism of volatile phenolic compounds from hydroxynnamic acids by *Brettanomyces* yeast. *Archives of Microbiology*. 46, 96-98.

Martens, H. et al. (1997). Microbiological aspects of a mixed yeast-bacterial fermentation in the production of a special Belgian acidic ale. Journal of the Institute of Brewing, 103, 85–91.

Morrissey, W. F. et al. (2004). The role of indigenous yeasts in traditional Irish cider fermentations. Journal of Applied Microbiology, 97, 647–655.

Puig M. et al. (2003). Microbiological and biochemical stabilization of wines by application of high pressure processing. *Bulletin de l'O.I.V*. 76, 596-617.

Ruiz-Hernadez M. (2003). Casein for correction of defects caused by *Brettanomyces* and *Dekkera*. *Semana Vitivinícola*. 58, 1462-1463

Silva, P. et al. (2004). Studies on the wine spoilage capacity of Brettanomyces/Dekkera spp. American Journal of Enology and Viticulture, 55, 65–72.

Teoh A. L. et al. (2004). Yeast ecology of kombucha fermentation. International Journal of Food Microbiology, 95, 119–126.

Tucknott J. (1977). The mousy taint in fermented beverages: its nature and origin. *Thèse de doctorat de l'Université de Bristol* (Royaume Uni de Grande Bretagne).

Wyder, M. T. et al. (1997). Investigation of the yeast flora in dairy products: a case study of kefyr. Food Technology and Biotechnology, 35, 299–304

UTILIZACIÓN DE TÉCNICAS DE BIOLOGÍA MOLECULAR EN EL CONTROL DE FERMENTACIONES VÍNICAS INOCULADAS CON LEVADURAS SELECCIONADAS

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RESUMEN

Mediante la técnica de electroforesis en campo pulsante (PFGE) se realizó un estudio de caracterización analizando la diversidad genética de las levaduras indígenas de las fermentaciones espontáneas de un vino blanco producido en una Bodega del suroeste de España. Se observó una población heterogénea de cepas de levaduras durante dos vendimias consecutivas. Cuatro de las cepas más representativas se eligieron para analizar diversos parámetros de interés enológico. Después se utilizaron como iniciadoras de las fermentaciones industriales desde el año 2001 hasta el 2009. En la mayoría de vendimias, se obtuvo una población homogénea de levaduras durante las fermentaciones, en las que el cariotipo de una de las cepas inoculadas fue el mayoritario. El análisis de los fragmentos de restricción para el ADN mitocondrial (RFLP-ADNmt) fue aplicado como test rápido para controlar las fermentaciones inoculadas sin previo aislamiento de levaduras, a las 11 horas tras el muestreo.

ABSTRACT

By applying electrophoretic karyotype we analyse the diversity of wild yeast in spontaneous fermentations of a white wine in a winery of the southwestern Spain. A heterogeneous population of yeast was observed during two consecutive vintages. Four of the most abundant yeast were selected and tested for properties of enological interest. Autochthonous strains were used as starter for the 2001-2009 years. In the majority of the vintages we observed homogeneous yeast population formed by one of the inoculated strain, that we identified by electrophoretic karyotype. The analysis of restriction fragments length polymorphism of mitochondrial DNA (RFLP-mtDNA) was applied for testing the dominance of the inoculated strain in the vintages 2005-2009, and controlled industrial fermentations without previous isolation of yeast colonies. The results were obtained 11 hours after sampling.

INTRODUCCIÓN

Actualmente, en la industria enológica existe una alta competencia que está llevando a los productores a innovar introduciendo en el mercado nuevos tipos de vinos. Los consumidores demandan cada vez mas vinos elaborados con estilos más personales y específicos (Bisson *et al*., 2002; Bisson, 2004).

Las fermentaciones alcohólicas vínicas son procesos complejos en los que están involucradas distintas especies de levaduras. Durante la fermentación espontánea de los mostos se van sucediendo distintas poblaciones de levaduras las cuales aportan determinadas características al vino, describiéndose tres etapas en el proceso. En la primera, cuando el grado alcohólico es bajo predominan géneros de levaduras como *Candida*, *Hanseniaspora*, *Pichia*, *Kluyveromyces* etc. En la segunda y tercera fase, a medida que va aumentando el grado alcohólico estas levaduras son sustituidas por cepas pertenecientes, en general, a la especie *Saccharomyces cerevisiae*.

Las alta diversidad de levaduras encontrada en las fermentaciones espontáneas puede aportar alta calidad a los vinos con un carácter típico de la región donde se producen, proporcionándoles diferenciación y un valor comercial añadido dentro de un mercado tan competitivo (Fleet, 2008). Sin embargo, el inconveniente de estos procesos naturales es que son impredecibles, pudiéndose producir paradas o fermentaciones lentas, afectando a la calidad de los vinos, aunque la combinación de conocimientos artesanales y tecnológicos pueden asegurar el éxito de esas fermentaciones, las cuales se siguen realizando en numerosas ciudades Europeas (Rainieri, Pretorius, 2000). Una alternativa a las fermentaciones espontáneas son las inoculadas con cultivos iniciadores preparados con levaduras seleccionadas. Estas levaduras están disponibles en el mercado en concentrados secos activos que pueden ser reconstituidos fácilmente para su inoculación en los mostos de uva (Manzano *et al*., 2006). Sin embargo, se está reconociendo que la utilización de estos cultivos puede llevar a una pérdida en la complejidad de los sabores de los vinos haciéndolos más homogéneos (Rainieri, Pretorius, 2000; Mannazzu *et al*., 2000). Todos estos aspectos están proporcionando nuevos retos para mejorar las cualidades y valor de los vinos obtenidos mediante fermentaciones inoculadas. Algunos de estos retos son, por un lado, la utilización de levaduras autóctonas seleccionadas en la zona productora, y por otro lado el seguimiento de la cepa inoculada durante las fermentaciones, lo que permitiría al bodeguero tener un mayor control sobre la fermentación.

Las técnicas moleculares que permiten la caracterización de levaduras industriales y discriminan entre las distintas cepas son habitualmente la Electroforesis en Campo Pulsante (PFGE) y el análisis del polimorfismo para la longitud de los fragmentos de restricción (RFLP) del ADN mitocondrial (Mesa *et al*., 1999, 2000; Cappello *et al*., 2004; Demuyter *et al*., 2004; Schuller *et al*., 2004; Blanco *et al*., 2006; González *et al*., 2007; Rodríguez *et al*., 2010). La primera técnica analiza el número y tamaño de los cromosomas de las levaduras separados mediante electroforesis en campo pulsante (PFGE), y la segunda mide variaciones en la secuencia del ADN mitocondrial afectado por los sitios de corte de determinadas endonucleasas de restricción. En algunos casos, estas técnicas también se han utilizado para determinar si los cultivos de cepas de levaduras utilizados como iniciadores de las fermentaciones llegan a ser los dominantes entre la población de levaduras durante el proceso de fermentación (Esteve-Zarzoso *et al*., 2000; Raspor *et al*., 2002; Lopes *et al*., 2007; Rodríguez *et al*., 2010).

En nuestro trabajo, mediante la utilización de PFGE se analizó la diversidad genética de las levaduras involucradas en las fermentaciones espontáneas de los mostos de un vino blanco producido en una Bodega del suroeste de España. Tras elegir cuatro de las cepas más representativas se analizaron diversos parámetros de interés enológico (Rodríguez *et al*., 2010) y se seleccionaron tres levaduras para utilizar como iniciadoras de las fermentaciones industriales desde el año 2001. Desde el año 2005, aplicando RFLP-ADNmt pudimos comprobar si la cepa de levadura inoculada fue la dominante en las fermentaciones, sin previo aislamiento de colonias.

MATERIALES Y MÉTODOS

Inoculación y características de las fermentaciones industriales

Las fermentaciones se llevaron a cabo en depósitos de acero inoxidable de 400.000 l que se llenaron con mosto procedente de la variedad de uva Palomino. Para inocular las fermentaciones con las cepas de levaduras autóctonas seleccionadas, se elaboró un pie de cuba con dos fases de preparación. La primera etapa se llevó a cabo en condiciones de laboratorio, de manera que a partir de una colonia crecida en medio YPD (1% extracto de levadura, 2% peptona, 2% glucosa, 2% agar) se inocularon 500 ml de mosto esterilizado (126ºC, 20 min). Después de 2-3 días de fermentación, el cultivo se adicionó a 1500 ml de mosto también esterilizado. A los 3-4 días cuando el azúcar disminuyó hasta 1 ºBé, los 2 l de cultivo se adicionaron a 18 l de mosto descongelado pero no esterilizado. Los 20 l de cultivo se transportaron a la bodega donde comenzó la segunda etapa de elaboración del pie de cuba. Allí los cultivos puros de cada hechos con cada levadura se mezclaron y se fueron añadiendo a volúmenes más grandes de mosto fresco hasta alcanzar el volumen final (Fig. 1). Cada escalamiento fue realizado cuando el ºBé de las fermentaciones alcanzó un valor de 1-2. Después, el pie de cuba se propagó a otros depósitos de 400.000 l que fueron recibiendo determinados volúmenes de mosto fresco hasta que se completó el volumen final. La frecuencia de las recargas de mosto dependió del rendimiento de la producción de mosto en cada vendimia.

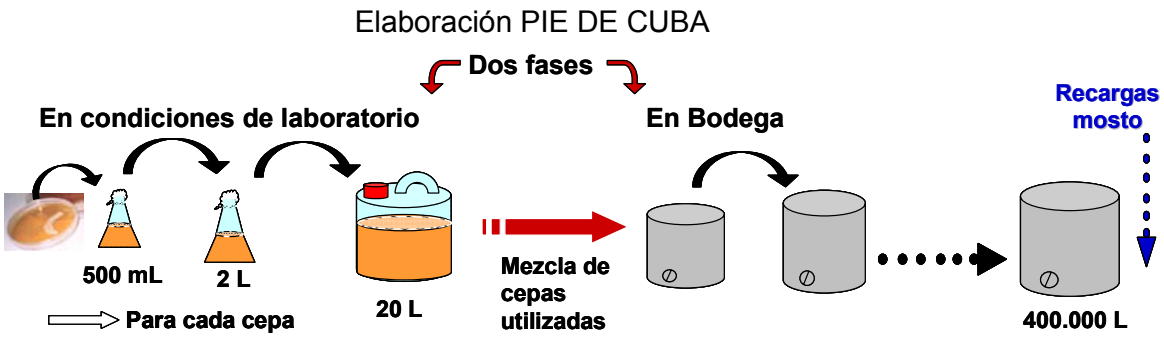

Figura 1. Esquema de la elaboración del pie de cuba en el cual se muestran los sucesivos escalamientos realizados desde el laboratorio, manteniendo los cultivos en condiciones de pureza, hasta la bodega de vinificación.

Muestreos y aislamiento de colonias de levaduras

En la zona de vinificación de nuestro estudio, cada año, las uvas son cosechadas durante los meses de Agosto y Septiembre para obtener el mosto. El seguimiento de las fermentaciones se llevó a cabo midiendo el ºBé. Los muestreos se realizaron diariamente, tomando 25 ml de mosto del centro de los depósitos desde que comenzaron a ser llenados con el pie de cuba

hasta la finalización de las fermentaciones. Las muestras se transportaron en hielo al laboratorio donde fueron centrifugadas y resuspendidas en 600 µl de mosto. A continuación se mezclaron con 400 µl de glicerol al 50% y se conservaron a -80 ºC hasta su posterior procesamiento. Los aislamientos de levaduras se realizaron a partir de diluciones y siembra en placas de YPD. Después las placas se incubaron a 28 ºC durante 3 días. Pasado este tiempo se eligieron al azar 20-30 colonias para caracterizar mediante cariotipo electroforético.

Electroforesis en campo pulsante

Para obtener el cariotipo electroforético de los aislamientos de levaduras, las células fueron embebidas en bloquecitos de agarosa y tratadas siguiendo un procedimiento basado en el protocolo de Carle y Olson (1985). Cada colonia se inoculó en 15 ml de YPD y se incubó toda la noche en agitación a 28 ºC. Después cada cultivo se centrifugó a 3800 rpm y las células fueron resuspendidas en 600 µl de EDTA 0.5 mol/l, pH 8. La suspensión se mezcló 1:1 con agarosa low-melting (Bio-Rad) y se adicionó en moldes donde los bloques o plugs se enfriaron y gelificaron. A continuación los plugs fueron procesados, incubándose primero en una solución reductora, Solución 1 (0.01 mol/l Tris-HCl pH 9, 7.5% (v/v) β-mercaptoetanol, 0.5 mol/l EDTA pH 9) a 37 ºC durante 24 h. Después se lavaron los plugs dos veces con EDTA 0.05 mol/l pH 8 y se incubaron con una slución de lisis o Solución 2 (1% N-lauroilsarcosina, 1mg/ml proteinasa K, 0.5mol/l EDTA pH 9) a 50 ºC durante 24 h. A continuación se volvieron a lavar dos veces con EDTA 0.05 mol/l pH 8. Finalmente, los plugs se almacenaron en la misma solución de los lavados a 4 ºC hasta ser cargados en el gel de electroforesis.

Los cromosomas de las levaduras fueron separados por PFGE en un aparato CHEF-DR II (Bio-Rad). Los geles se hicieron al 1% con agarosa de campo pulsante (Bio-Rad) en tampón de electroforesis TBE 0.5x. Las condiciones de electroforesis fueron las siguientes: pulsos de tiempo inicial y final 60 s y 120 s respectivamente, 24 h, 14 ºC, 6 V/cm y 120º de ángulo. Tras la electroforesis los geles fueron teñidos con una solución de bromuro de etidio (0.5 µl/ml, 0.5x TBE), y la imagen se capturó con una lámpara de luz UV en un aparato Gel-Doc 1000 (Bio-Rad). El análisis de las imágenes se realizó utilizando el programa Quantity One 1-D (Bio-Rad).

Análisis del polimorfismo para la longitud de los fragmentos del ADN mitocondrial

Para el control microbiológico de las fermentaciones se tomaron 250 ml de mosto fermentando de los depósitos objeto de estudio, se centrifugaron a 3800 rpm y el precipitado celular fue lavado al menos tres veces con agua estéril para eliminar los restos de mosto. A continuación se purificó el ADN del conjunto celular siguiendo el procedimiento descrito por Querol et al. (1992). Cinco µl de ADN se trataron con 10 unidades de la enzima de restricción *Hinf*I (Fermentas) y se incubaron a 37 ºC durante 2 h. Los fragmentos de restricción fueron separados por electroforesis en un gel de agarosa al 1% preparado con tampón 1x TBE y 0.5 µl/mg de bromuro de etitidio. Las imágenes fueron digitalizadas utilizando el mismo equipo descrito en el apartado anterior.

RESULTADOS Y DISCUSIÓN

Esquema de selección de cepas de levaduras autóctonas

Para seleccionar las levaduras más adecuadas al proceso, primero realizamos una caracterización molecular exhaustiva de las cepas de levaduras involucradas en la

fermentación espontánea de uno de los depósitos de la Bodega en la vendimia de 1999. Se analizaron muestras desde el inicio de la fermentación hasta su finalización (0 h, 8 h, 19 h, 56 h, 129 h, 153 h, 221 h, 313 h y 361 h). Aplicando la técnica de PFGE como herramienta adecuada para la diferenciación entre cepas de levaduras (Schuller *et al*., 2004), obtuvimos el cariotipo electroforético de un total de 158 aislamientos.

Los resultados mostraron la existencia de un elevado polimorfismo cromosómico, característico de las levaduras vínicas (Granchi *et al*., 2003; Santamaría *et al*., 2005; Blanco *et al*., 2006), detectándose 17 patrones distintos pertenecientes a cepas S*accharomyces cerevisiae.* Se observó que las cepas con cariotipos I, II, III y V fueron mayoritarias durante toda la fermentación y las principales responsables del proceso. También se detectaron cepas no-*Saccharomyces* durante las primeras horas del proceso, identificadas por no presentar en sus cariotipos bandas con un tamaño molecular inferior a 500 kb. Resultados similares fueron encontrados cuando se analizaron las fermentaciones de varios depósitos en la vendimia del año 2000 (Rodríguez et al., 2010).

Siguiendo nuestro esquema de selección de levaduras, para reducir el número de cepas a estudiar se eligieron al azar cuatro pertenecientes a la última fase de la fermentación de entre aquellas que mostraron los cariotipos más representativos en el depósito analizado, éstas fueron P1, P2, P3 y P5 con cariotipos I, II, III, y V respectivamente.

Tras aplicar distintos criterios de selección, se seleccionaron tres cepas para inocular las fermentaciones industriales en vendimias posteriores (Rodríguez et al., 2010).

Fermentaciones inoculadas

La inoculación de las fermentaciones se llevó a cabo preparando un pie de cuba como se describe en el apartado de Materiales Métodos.

Las cepas con cariotipos II, III y V se utilizaron como iniciadoras de las fermentaciones industriales en el año 2001. Se analizaron un total de 423 aislamientos y mediante cariotipo electroforético se comprobó la presencia mayoritaria de la cepa con patrón V en un 83%, y una presencia de 7.6% y 8.5% para las de cariotipos II y III respectivamente. En las vendimias de los años 2002, 2003 y 2004 se utilizaron las cepas con cariotipos II y V para preparar el pie de cuba, aunque solamente en el año 2004 la presencia de la cepa con patón V fue mayoritaria, detectándose en un 99.6%. A partir del año 2005 las fermentaciones industriales sólo fueron inoculadas con la cepa de patrón V, ya que cuando esta cepa se encontró de forma mayoritaria en las fermentaciones el vino resultó mejor calificado por personal especializado en catas (Fig.2). En las vendimias 2005, 2007 y 2008 la levadura inoculada se encontró en un 79.8%, 50% y 100% respectivamente. La presencia de esta levadura en alta proporción durante las fermentaciones y la mejora en la calidad del producto sugiere que esta cepa contribuyó a las propiedades organolépticas de este vino local. La técnica de PFGE nos permitió tomar decisiones para mejorar el proceso a lo largo de los años de estudio.

Control de la levadura inoculada durante las fermentaciones

Para que la levadura inoculada se desarrolle bien durante el curso de las fermentaciones es necesario un buen control de los primeros pasos de inoculación o preparación del pie de cuba. En los años en los que la levadura con cariotipo V fue predominante en las fermentaciones, la preparación del pie de cuba fue similar y se siguieron los pasos que se indican a continuación. Primero, antes de cada escalamiento se esperó a que el azúcar fuera consumido (< 1 ºBé). Segundo, el inóculo siempre supuso el 10% del total del volumen a fermentar. Tercero, la temperatura se mantuvo en torno a 17 ºC. Pensamos que estos criterios favorecieron la

adaptación del inóculo a las condiciones del mosto obtenido en cada vendimia y a las condiciones finales de fermentación en los depósitos de 400.000 l.

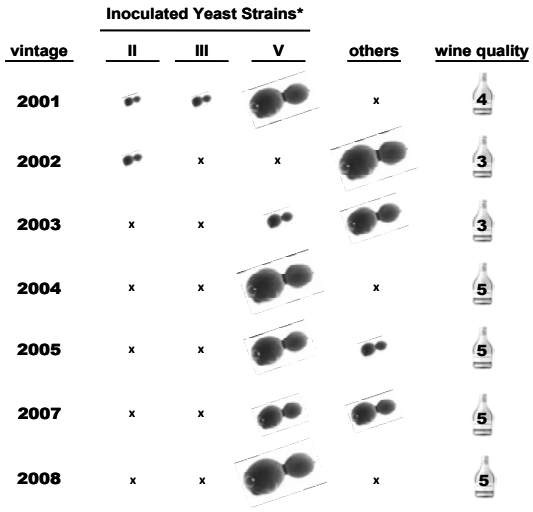

Figura 2. Composición de la población de levaduras vínicas en cada vendimia y su relación con la calidad del producto final. Para cada año el tamañote la célula de levadura representada es proporcional a la contribución de cada cepa dentro de la población de levaduras analizadas. El símbolo X indica que la proporción de la población de la cepa o cepas inoculadas estuvo por debajo del 5%. La calidad del vino, evaluada por personal de la bodega especializado en catas, fue graduada en una escala de 1 a 5 basándose en las propiedades organolépticas deseadas por los productores. Se encontró una correspondencia entre la predominancia de la cepa con cariotipo electroforético V y una alta calidad del vino.
* La cepa con patrón II fue inoculada en los años 2001 al 2004, la de patrón III solamente en el 2003, y la de patrón V se inoculó en todas las vendimias.

Por otro lado una de las necesidades del enólogo es poder realizar el seguimiento de la levadura inoculada durante las fermentaciones. Aunque se han aplicado otros métodos rápidos de diagnóstico para controlar la implantación de cepas de levaduras inoculadas (López *et al*., 2003; Ambrona *et al*., 2006), nosotros aplicamos la técnica de RFLP-ADNmt a muestras tomadas directamente del mosto fermentando, sin previo aislamiento de colonias de levaduras, como método rutinario para detectar la presencia mayoritaria de la levadura inoculada. Los resultados se obtuvieron 11 horas tras la toma de muestras. El estudio comenzó en la vendimia de 2005 a partir de la cual se empezó a utilizar solamente la cepa seleccionada con cariotipo V para inocular las fermentaciones. Cuando el patrón de restricción obtenido con la enzima *Hinf*I de las células totales presentes en la muestra coincidió con el patrón de la cepa inoculada, consideramos que la levadura inoculada fue dominante en el momento de la fermentación analizado. De esta forma testamos y controlamos la dominancia de la cepa inoculada, sobre todo durante la elaboración del pie de cuba, y durante las fermentaciones en los depósitos de 400.000 l. En total se analizaron 16 muestras en la vendimia 2005, 37 muestras en 2006 y 2008, 50 muestras en 2007 y 52 muestras en 2009.

En la Fig. 3 se muestran ejemplos de resultados en los que la cepa inoculada fue dominante durante todo el proceso de elaboración del pie de cuba. Estos resultados fueron contrastados con los obtenidos de PFGE tras analizar el cariotipo de 20 aislamientos por muestra. De manera que todos los aislamientos tuvieron el mismo cariotipo electroforético que la cepa inoculada (datos no mostrados). Las muestras analizadas del pie de cuba en cada vendimia dependieron de los escalamientos realizados, los cuales se realizaron en función del rendimiento de la entrada de mosto en los depósitos.

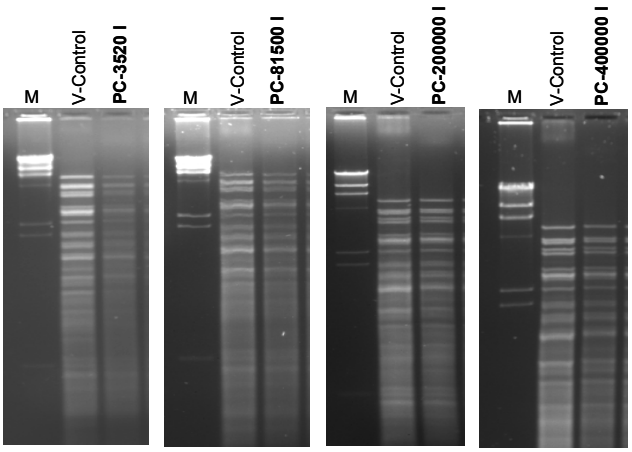

Figura 3. RFLP-ADNmt de células totales de muestras tomadas directamente de distintos volúmenes del pie de cuba (PC) en la vendimia 2007. V-Control es el perfil de restricción de la cepa inoculada. M es el marcador de pesos moleculares λ-*Hind*III.

CONCLUSIONES

Nuestro trabajo muestra que la utilización de las técnicas de biología molecular pueden ser aplicadas en la industria del vino para mejorar y controlar las fermentaciones industriales, cubriendo así dos de las principales necesidades de los enólogos. La técnica de PFGE fue una herramienta muy útil a la hora de seleccionar levaduras autóctonas y nos ayudó a tomar decisiones para mejorar el proceso de las fermentaciones inoculadas, ya que se fueron descartando las levaduras menos competitivas en las fermentaciones industriales, mejorando así la calidad del producto final obtenido en cada vendimia. La aplicación de la técnica de RFLP-ADNmt a muestras tomadas directamente de la fermentación sin previo aislamiento de colonias ha supuesto una ventaja para el enólogo, ya que puede controlar de forma rápida (11 horas) si la levadura inoculada es la que está llevando a cabo las fermentaciones dentro en un proceso industrial complejo como se describe en este trabajo.

AGRADECIMIENTOS

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REFERENCIAS

Ambrona J., Vinagre A., Maqueda M., Álvarez M.L., Ramírez M., 2006. Rhodamine-pink as a genetic marker for yeast populations in wine fermentation. *Journal of Agricultural and Food Chemistry*, 54: 2977-2984.

Bisson L.E., Waterhouse L.A., Ebeler S.F., Walker M.A., Lapsley J.T., 2002. The present and future of the international wine industry. *Nature*, 418: 696-699.

Bisson L.E., 2004. The biotechnology of wine yeast. *Food Biotechnology*, 18: 63-96.

Blanco P., Ramilo A., Cerdeira M., Orriols I., 2006. Genetic diversity of wine *Saccharomyces* strains in an experimental winery from Galicia (NW Spain). *Antonie van Leeuwenhoek*, 89: 351-357.

Cappello M.S., Bleve G., Grieco F., Dellaglio F., Zacheo G., 2004. Characterization of *Saccharomyces cerevisiae* isolated from must of grape grown in an experimental vineyard. *Journal of Applied Microbiology*, 97: 1274-1280.

Carle G.F., Olson M.V., 1985. An electrophoretic karyotype for yeast. *Proceeding of the National Academy of Sciences of the United States of America*, 82: 3756-3760.

Demuyter C., Lollier M., Legras J.L., Le Jeune C., 2004. Predominance of *Saccharomyces uvarum* during spontaneous alcoholic fermentation, for three consecutive years, in an Alsatian winery. *Journal of Applied Microbiology*, 97: 1140-1148.

Esteve-Zarzoso B., Gostincar A., Bobet R., Uruburu F., Querol A. 2000. Selection and molecular characterization of wine yeast isolated from the "El Penedes" area (Spain). *Food Microbiology*, 17: 553-562.

Fleet G.H., 2008. Wine yeasts for the future. *FEMS Yeast Research*, 8: 979-995.

González S.S., Barrio E., Querol A., 2007. Molecular identification and characterization of wine yeast isolated from Tenerife (Canary Island, Spain). *Journal of Applied Microbiology*, 102: 1018-1025.

Granchi L., Ganucci D., Viti C., Giovannetti L., Vincenzini M., 2003. *Saccharomyces cerevisiae* biodiversity in spontaneous commercial fermentations of grape musts with 'adequate' and 'inadequate' assimilable-nitrogen content. *Letters in Applied Microbiology*, 36: 54-58.

Lopes C.A., Rodríguez M.E., Sangorrín,M., Querol A., Caballero A.C., 2007. Patagonian wines: implantation o fan indigenous strain of *Saccharomyces cerevisiae* in fermentations conducted in traditional and modern cellars. *Journal of Industry Microbiology and Biotechnology*, 34: 139-149.

López V., Fernández-Espinar M.T., Barrio E., Ramón D., Querol, A., 2003. A new PCR-based method for monitoring inoculated wine fermentations. *International Journal of Food Microbiology* 81: 63-71.

Mannazzu I., Clementi F., Ciani M., 2002. Strategies and criteria for the isolation and selection of autochthonous starters. *Biodiversity and Biotechnology of Wine Yeast.* Ciani M., ed. Research Signpost, Trivandrum, India. 19-34.

Manzano M., Medrala D., Giusto C., Bartolmeoli I., Urso R., Comi G., 2006. Classical and molecular analyses to characterize commercial dry yeast used in wine fermentations. *Journal of Applied Microbiology*, 100: 599-607.

Mesa J.J., Infante J.J., Rebordinos L., Cantoral J.M., 1999. Characterization of yeasts involved in the aging of sherry wines. *Food Science and Technology*, 32: 114-120.

Mesa J.J., Infante J.J., Rebordinos L., Sánchez J.A., Cantoral J.M., 2000. Influence of the yeast genotypes on enological characteristics of sherry wines. *American Journal of Enology and Viticulture*, 51: 15-21.

Querol A., Barrio E., Ramón D., 1992. A comparative study of different methods of yeast strain characterization. *Systematic and Applied Microbiology*, 15: 439-446

Raspor P., Cus F., Povhe Jemec K., Zagorc T., Cadez N., Nemanic J., 2002. Yeast population dynamics in spontaneous and inoculated alcoholic fermentations of *Zametovka* must. *Food Technology and Biotechnology*, 40: 95-102.

Rainieri S., Pretorius I.S., 2000. Selection and improvement of wine yeasts. *Annals of Microbiology* 50: 15-31.

Rodríguez M.E., Infante J.J., Molina M., Domínguez M., Reboordinos L., Cantoral J.M., 2010. Genomic characterization and selection of wine yeast to conduct industrial fermentations of a white wine produced in a SW Spain winery. *Journal of Applied Microbiology*, 108: 1292-1302.

Santamaría P., Garijo P., López R., Tenorio C., Gutiérrez A.R., 2005. Analyisis of yeast population during spontaneous alcoholic fermentation: Effect of the age of the cellar and the practice of inoculation. *International Journal of Food Microbiology*, 103: 49-56.

Schuller D., Valero E., Dequin S., Casal M., 2004. Survey of molecular methods for the typing of wine yeast starins. *FEMS Microbiology Letteers*, 231: 19-26.

IMPORTANCE OF AEROBIC YEASTS ON THE VARIETAL WINE SENSORY

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ABSTRACT - INTRODUCTION

Several factors influence the aroma profile during the alcoholic fermentation of the grape must. One of the widely discussed topics is the use of apiculate microflora in starting of winemaking. Several authors have studied[1,2] the behaviour of various yeast genera - *Kloeckera, Pichia* - and their contribution to the wine aroma, considering the apiculate microflora as spoilage. To avoid spoilage of the wine and its unpredictable changes of flavour starter *Saccharomyces* cultures are used, however they may also cause a loss of characteristic aroma of wine. The diversity and the composition of the yeast micropopulation significantly contribute to the wine sensory characteristic. The growth of each wine yeast species is characterized by a specific metabolic activity, which determines concentration of flavour compounds in the final wine. The grape variety is characterized by typical aroma according to content and type of compounds forming it. The apiculate yeasts such as *Hanseniaspora uvarum* and its anamorphic form *Kloeckera apiculata*, and oxidative non-*Saccharomyces* yeasts, for example, *Candida*, *Pichia*, *Rhodotorula* and *Kluyveromyces*, are the predominant species as the grape[5]. They are frequently found in fermented drinks and foods where it plays a role in the spontaneous fermentation[5].The non-*Saccharomyces* yeasts grow well during early stages of fermentation.

Our previous results confirmed the role of apiculate microflora in the wine aroma production under semiaerobic conditions[4] and support of the original character of wine by apiculate microflora[3,5]

The aim of this work is study typical yeast strains of some variety of grape for their aroma compounds production. We compared apiculate microflora under semiaerobic conditions. Typical aroma compounds for each genus were recognized by using the GC MS that was used for their identification.

KEY WORDS: grape must, non-*Saccharomyces* yeasts, *Pichia anomala*, GC MS

GERMAN : BEDEUTUNG DER AEROBIC HEFEN AUF DIE SORTENANERKENNUNG WEINSENSORIK

Verschiedene Faktoren beeinflussen die Aromaprofil während der alkoholischen Gärung von Traubenmost. Eines der viel diskutierten Themen ist der Einsatz von apiculate Mikroflora in der Weinbereitung ab. Zur Vermeidung von Verderb des Weines und seiner unvorhersehbaren Änderungen des Geschmacks Starter *Saccharomyces* Kulturen verwendet werden, sie können jedoch auch zu einem Verlust der charakteristischen Aroma des Weines. Die Vielfalt und die Zusammensetzung der Hefe micropopulation erheblich dazu beitragen, den Wein sensorischen charakteristisch. Das Wachstum der einzelnen Arten Weinhefe wird durch eine spezifische metabolische Aktivität, die Konzentration von Aromastoffen bestimmt in den letzten Wein

charakterisiert. Die Rebsorte ist durch typische Aroma nach Inhalt und Art der Verbindungen bilden sie aus. Die apiculate Hefen wie *Hanseniaspora uvarum* und seine anamorphen Form *Kloeckera apiculata* und oxidative non-*Saccharomyces* Hefen wie *Candida, Pichia, Rhodotorula* und *Kluyveromyces*, sind die vorherrschenden Arten, wie der Traube [1] und wachsen auch während der frühen Stadien der Gärung. Sie sind häufig in fermentierten Getränken und Lebensmitteln, wo es spielt eine Rolle bei der spontanen Gärung gefunden [1].

Unsere bisherigen Ergebnisse bestätigten die Rolle der apiculate Mikroflora in dem Wein Aroma Produktion unter Bedingungen semiaerobic [3] und die Unterstützung der ursprüngliche Charakter des Weines durch apiculate Mikroflora [2,4].

Das Ziel dieser Arbeit ist typisch Studie Hefestämme einiger Rebsorte für ihre Aromastoffe Produktion. Wir apiculate Mikroflora unter semiaerobic Bedingungen. Typische Aromastoffe für jede Gattung wurden mit Hilfe der GC MS, die zu ihrer Identifizierung verwendet wurde anerkannt.

MATERIALS AND METHODS

We have isolated and identified various yeasts species from defined grape varieties in different stages of fermentation procedure – from grapes-health and damaged berries, from pedicel, in the stage of active fermentation, and in the stage of maturation.

Typical yeasts species of grape variety, in this presentation oxidative non-*Saccharomyces* yeasts, we have fermented for 24, 48, 72 hours at 10ºC by semiaerobic conditions. We compared also inoculation of *Pichia anomala* isolated from must of Riesling blanc (aromatic variety) and Grüner Veltliner (less aromatic grape variety) to must of Riesling blanc.

After fermentation we used SPME method for preparation the samples before GC MS: volatiles produced were sampled by means of headspace solid-phase microextraction (SPME - 100µm polydimethylsiloxane).

The samples were analysed by gas chromatography using a mass detector. Gas chromatograph with mass spectrometer were used from Shimadzu GCMS-QP2010.

Injection:

10g of sample and 4g NaCl

column GC:

- name: VF-mS
- length: 30,0 m
- thickness: 0,25µm
- diameter (id): 0,25mm
- temperature programe: 40ºC hold for 1min., from 40 to 220 ºC by 5ºC/min, 5 min hold by 220 ºC,
  total programe time 61 min

- carrier gas: helium
- injektion mode: splitless
- flow control mode: pressure 60kPa

detection:

- MS quadrupole
- mass range: m/z 29-390

RESULTS

Inoculating grape must by various microorganisms and fermentation occured, the aroma compounds increased significantly, in all cases by more than 60 compounds. Most of them were recognised as typical fermentation products – ethanol, izoamylacohol, propanol, etylester of caproic, caprylic acid, caprinic acid, ethylacetate, isovaleric acid, pentylacetate, 2,3-butandiol, furfural, 3-hydroxybutyrát, methionon, 1,4-buthandiol. 2-metyl buthan acid, 3-methylbutan acid, 2-fenyletylacetate, izoamylacetate, cis 3- hexenylacetate, etylbenzoate, α-terpineol, ethyl isobutyrate, ethyl butyrate, ethyl 2-metylbutyrate, ethyl isovalerate, isoamyl acetate, ethyl hexanoate, cis-3-hexenol, ethyl octanoate, furfural, linalool, ethyl furoate, ethyl decanoate, ethyl benzoate, α-terpineol, fenylethyl acetate, geraniol , a-ionone, guaiacol , b-Ionone , g-nonalactone, ethyl cinnamoate, g-decalactone, eugenol, 4-vinylguaiacol, d-decalactone etc. These compounds were confirmed also by other authors [7] as a product of grape must fermentation by various yeast strains.

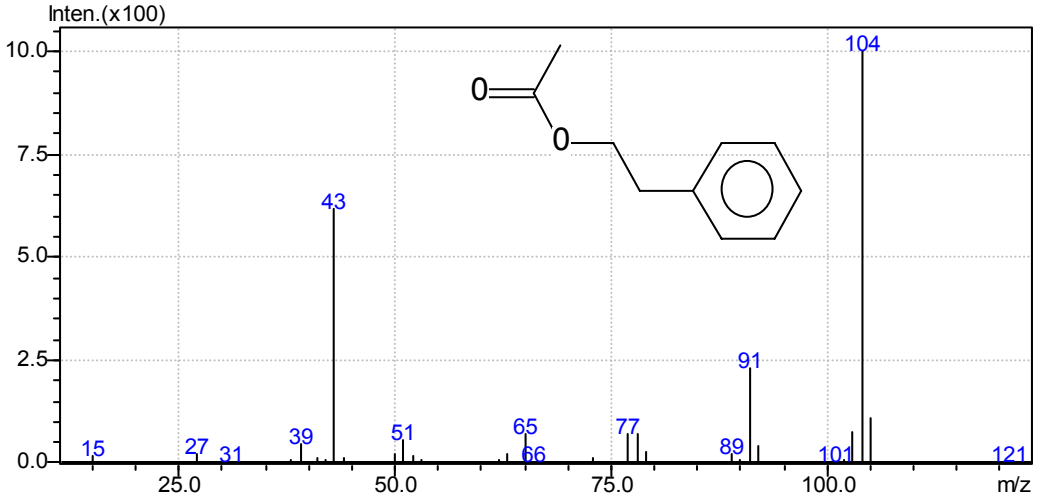

Fig.1 Mass spectrum of 2-fenyletyl acetate. Detected in must fermented by *Pichia anomala*, retention time 27,805 min.

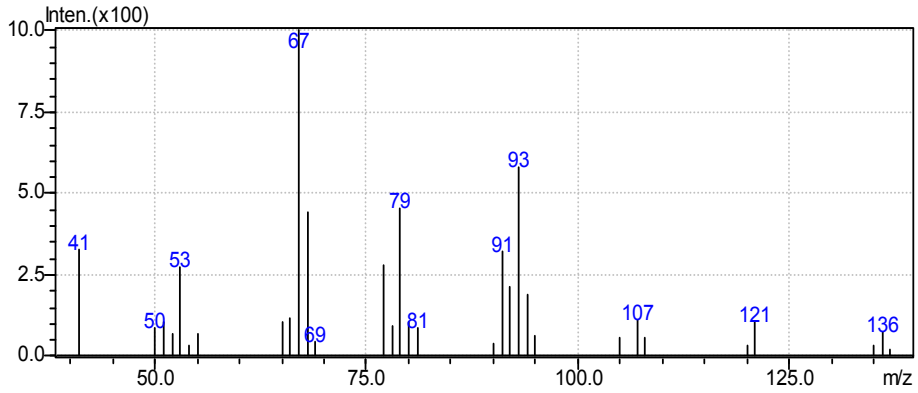

Fig.2 Mass spectrum of limonene. Detected in must fermented by *Pichia anomala*, retention time 19,44 min.

The volatile organic compounds produced were found to be alcohols (amyl alcohol and isoamyl alcohol), aldehydes (2-methyl-2-hexenal and 2-isopropyl-5-methyl-2-hexenal) and esters (ethyl isobutyrate, isobutyl acetate, isoamyl acetate, 2-methylbutyl acetate, ethyl isovalerate, isoamyl propionate and phenylmethyl acetate).

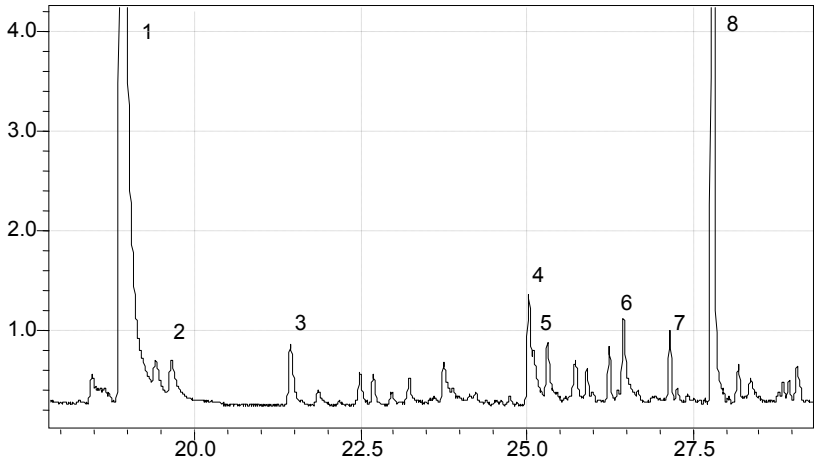

Fig.3 Compounds detected in must Riesling blanc fermented by *Pichia anomala*, 1.day by inoculation:

1. hexylacetate, 2. limonene, 3. dihydromyrcenol, 4. octane acid, 5. 4-izopropyl-1-metylcyklohexanol, 6. 2-metylundecanol, 7. hexyl-2-metylbutyrate, 8. 2-phenyletylacetate

In our work we compared also the influence of yeasts origin on the volatile organic compounds production. The same apiculate strain *Pichia anomala* was isolated from Grüner Veltliner and Riesling blanc and they were subsequently used for the same must fermentation.

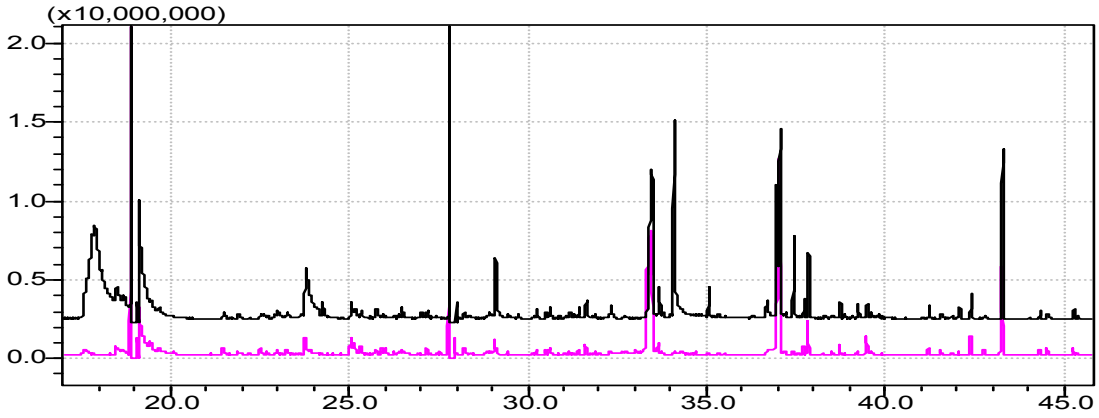

Fig.4 Comparison of volatile organic compounds detected in must Riesling blanc fermented by *Pichia anomala* isolated either from must of Riesling blanc (black) or Grüner Veltliner (lila), 1.day after inoculation

CONCLUSION

Aerobic yeasts strains contributes positively to the wine aroma by the production of volatile compounds.

We determined 2-fenyletyl acetate produced by *Pichia anomala*, recognised sensorialy by fruity aroma. On the other hand, too much production of this compound, in concentrations above 200mg/l, can also act as wine spoilage[6] .Therefore, the positive impact of that non-*Saccharomyces* yeasts could be achieved well during early stages of fermentation, subsequently replaced during the following stages by *Saccharomyces* yeasts.

Our results have confirmed one, max. two day fermentation under semiaerobic conditions. The origin of yeast *(Pichia anomala* isolated from Grüner Veltliner, and Riesling blanc) had no influence on their behaviour and VOC profile.

There were produced in highest concentration 2-fenyletyl acetate and hexylacetate for *Pichia anomala*. The sensorial evaluation confirmed analytical results (apple and pear aroma involved by 2-fenyletyl acetate).

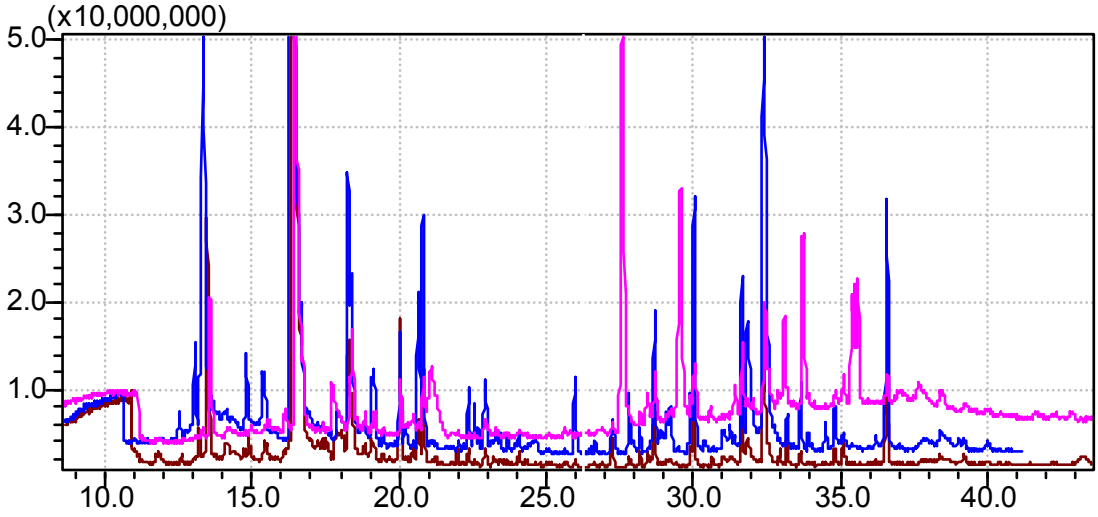

Fig.5 Comparison of production of aromatic compounds by various non-*Saccharomyces* yeasts*: Pichia anomala, Rhodotorula mucilaginosa, Kloeckera apiculata.*

REFERENCIES

[1] Fleet, G.(2003 Sep 1),Yeast interactions and wine flavour. Food Microbiologica, 86 (1-2):11-22

[2] Romano P., Fiore C., Paraggio M., Caruso M., Capece A.,(2003) Function of yeast species and strains in wine flavour. Int. J. Food Microbiol., 86, 169-180.

[3] Breierová E., Kaňuchová-Pátková J., Vajcziková I. (2008) Nonsaccharomyces yeast in grape must-advantage or spoilage? Chem.listy, 102, 537-539.

[4] Breierová E., Nemcová K., Kaňuchová-Pátková J., Vajcziková I. (2008) The effect of fermantation conditions on the yeast aroma profile. Chem.listy, 102, 599-600.

[5] Breierová E., Nemcová K., Kaňuchová-Pátková J., (2009) Expression of indigenous grape microflora on the sensory of varietal wine. 37th annual conference on yeasts, 103.

[6] Passoth, V. et.al.(2006), Biotechnology, physiology and genetics of the yeast Pichia anomala, Yeast Research 6: 3-13

[7] Ferreira, V., Hernandez-Orte, P., Escudero, A., Lopez, R., (1999) Semipreparative reversed-phase liquid chromatographic fractionation of aroma extracts from wine and other alcoholic beverages. Journal of Chromatography A, 864, 77–88.

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METHOD OF ANALYSIS OF AROMATIC COMPOUNDS IN WINE

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ABSTRACT

A simple and inexpensive headspace method for analysis of broad spectrum wine aroma compounds (from acetaldehyde up to 2-phenylethanol) using microcolumn filled with 2,5 mg of Tenax TA is presented. The microcolumn was thermally desorbed in the inlet of gas chromatograph.

Introduction

Sample preparation is one of the most critical steps in chromatographic analysis. Solvent-free extraction methods based on the partitioning of analytes between a gaseous and a stationary phase has become increasingly important and widely applied in research during the last decade. Solid-phase microextraction (SPME) is a solvent-free extraction method introduced by Pawliszyn and co-workers (Arthur, Pawliszyn, 1990). However, conventional SPME has some drawbacks such as fibre fragility, and low sorption capacity (Bigham *et al*., 2002). Some alternative extraction techniques derived from SPME, such as microextraction in a packed syringe (Lou *et al.*, 2008) and stir bar sorptive extraction (Baltussene *et al*., 1999) address these limitations.

The main objective of presented work was to develop a rapid, simple and inexpensive method for analysis a broad spectrum of wine aroma compounds (from acetaldehyde up to 2-phenylethanol) in one sample run.

Material and Methods

Analysis were carried out on a GC 8000 Top Series, CE Instruments (Rodano-Milan, Italy) equipped with a modified inlet (Hrivňák *et al*., 2009a, b). The microcolumn of 1mm I.D. was packed with 60-80 mesh Tenax TA (Alltech, Deerfield, IL, USA). VF-WAXsm silica capillary column of 30 m length × 0.25 mm I.D. and 0.25 μm film thickness (Varian, Lake Forest, CA, USA) was used. The chromatographic elution was temperature programmed as follows: isothermal at 30 ˚C (5 min), then increased to 220 ˚C at a rate of 5 ˚C/min and hold 5 min. The inlet chamber temperature was 230 ˚C and the temperature of FID detector 250 ˚C. Helium was used as a carrier gas.

A 100 ml of the wine sample containing 20 % NaCl was transferred into a 500 ml Erlenmeyer flask and vigorously shaken for 1 min at the temperature of 22±1˚C. After equilibration the microcolumn was inserted into the flask and from the distance of 1 - 2 cm from the level the headspace of 10 ml was aspirated through the microcolumn at a flow rate of 2-3 ml/min using an all-glass syringe (Poulten & Graf, Wertheim, Germany). The loaded microcolumn was transferred into the modified GC inlet and the trapped analytes desorbed for 1 min at 230 ˚C and carrier gas pressure of 10 kPa. After desorption, the pressure was increased to 60 kPa and the chromatographic program was started.

Results and Discussion

A chromatogram of wine aroma compounds (´GrüneVeltliner´, Slovak origin) analysed under the above conditions, it is shown in the fig. 1. The identification of peaks was performed by analysing model mixtures containing pure compounds.

The static headspace was preferred, because in a relatively short time the highest possible concentration of analytes in the gas phase can be obtained. The limiting factor of the amount of adsorbent in the microcolumn is the breakthrough volume of analytes and the aspirated volume of headspace. On Tenax TA at 20 ˚C, among the compounds listed in fig.1, ethanol exhibit the lowest breakthrough volume of 1,80 L/g (Scientific Instrument Services, 2004). On this basis, all the compounds in fig.1 are selectively adsorbed on 10 - 15 mg of Tenax TA, when 10 ml of the headspace is used. Due to low water affinity and low desorption temperature, Tenax TA was selected to trap the volatiles.

During the adsorption studies we have found that full adsorption of all the sample volatiles is not preferable, because the broad peak of ethanol on chromatograms interferes with a near eluting peaks. Therefore we have decided to use only 2,5 mg of Tenax TA in the microcolumn and work beyond the breakthrough volume of ethanol. In such condition, the compounds obtaining up to 3 – 4 carbon atoms are beyond their breakthrough volumes, therefore their peaks are lower and the ethanol peak narrower, but still can be used for analysis.

Advantage of the method is the low dead volume of the microcolumn. The distance between the adsorbent and the head of a column in the inlet is only 10 mm (×0.25mm I.D.). This means that the microcolumn is practically a part of the capillary column, which results in obtaining of non-dispersed peaks even at the beginning of a chromatogram (without cryofocusation or subambient temperatures). The method is useful for analysis of both very volatile and high boiling aroma compounds in one sample run and can be used to compare volatile profile from different types of wines (Kružlicová *et al*., 2008).

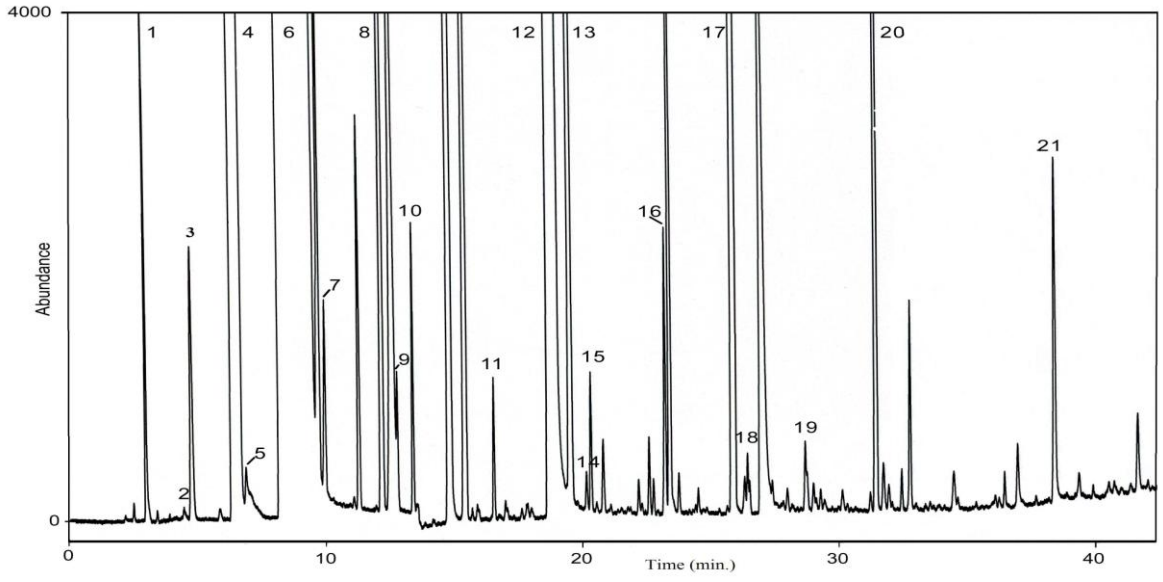

Fig. 1: Chromatogram of wine aroma compounds (´GrünerVeltliner´, Slovak origin).

Peaks: (1): acetaldehyde; (2) aceton; (3) methylacetate; (4) ethylacetate; (5) 2-butanon; (6) ethanol; (7) propylacetate; (8) i-butylacetate; (9) propanol; (10) butylacetate; (11) butanol; (12) i-amylalkohol; (13) ethylhexanoate; (14) pentanol; (15) hexanol; (16) hexylacetate; (17) ethyloktanoate; (18) heptanol; (19) linalool; (20) ethyloktanoate; (21) 2-phenylethanol

Conclusions

A simple and inexpensive headspace method for analysis of broad spectrum wine aroma compounds (from acetaldehyde up to 2-phenylethanol) using microcolumn filled with 2,5 mg of Tenax TA is presented. The microcolumn was thermally desorbed in the inlet of gas chromatograph.

Acknowledgements

This work was supported by the Slovak Research and Development Agency APVV - 0550-07.

Bibliography

Arthur C. L., Pawliszyn J., 1990. Solid phase microextraction with thermal desorption using fused silica optical fiber. *Anal. Chem, 62*: 2145.

Baltussen E., Sandra P. J. F., David F., Cramers C. A., 1999. Stir bar sportive extraction (SBSE), a novel extraction technique for aqueus samples:theory and principles. *J. Microcol. Sep., 11*: 737.

Bigham J., Medlar J., Kabir A., Shende C., Alli A., Malik A., 2002. Sol – gel capillary microextraction. *Anal. Chem, 74*: 752.

Hrivňák J., Kráľovičová E., Tölgyessy P., Ilavský J., 2009a. Analysis of unmetabolized VOCs in urine by headspace solid – phase microcolumn extraction. *J. Occup. Health, 51*: 173.

Hrivňák J., Tölgyessy P., Figedyová S., Katuščák S., 2009b. Solid phase microcolumn extraction and gas chromatography-mass spectrometry identification of volatile organic compounds emitted by paper. *Talanta*, 80: 400.

Kružlicová D., Mocák J., Hrivňák J., 2008. Classification of wine varietes using multivariate analysis of data obtained by gas chromatography with microcolumn extraction. *J. Food Nutr. Res.*, 47: 37.

Lou D-W., Lee X., Pawliszyn J., 2008. Extraction of formic and acetic acids from aqueous solution by dynamic headspace – needle trap extraction temperature and pH optimization. *J. Chromatogr*. A, *1201*: 228.

Scientific Instrument Services: Tenax TA Breakthrough Volume Data, Ringoes, New York, USA 1996-2004 http://www.sisweb.com/index/referenc/tenaxta.htm, stored data 10. 5. 2004.

AROMA OF SLOVAK WINES VS. BULGARIAN WINES

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ABSTRACT

Aromatic compounds in wine are one of basic potentials quality of aroma. In wine are present more than 800 aromatic compounds, which are influenced by synergistic effect in their odour threshold concentration and quality.

On research Institute of Viticulture and Enology new method has been develop, which enables to determine 100 compounds by one injection. 20 compounds were identified as of important aroma aspect in wine. Esters were identified with characteristic fruit odour. The highest concentration of isoamylalcohol was observed in Slovak wines, usual by acid, malt flavour. The aromagrams of Slovak wines were compared to foreign wines, for example Bulgarian. Aromatic esters showed lower concentration in Bulgarian wines, compared to Slovakian. The Bulgarian wines were higher in alcohol content.

Introduction

There has been created no complex study by now, that would deal with aromatic wine profile regarding terroir and processing technology (Plutowska, Wardencki, 2007). Moreover, aromatic substances in wine are considered to be one of elementary qualitative potentials of wine aroma. In wine you can find more than 800 aromatic substances influencing each other in threshold value of perception and quality alone (Kaňuchová Pátková, Hrivňák, 2009).

The Institute of Viticulture and Enology has developed an unique method, which can distinguish more than 100 substances by one measuring. Many significant substances have been identified, which are typical for each wine variety. Established database was compared to Bulgarian wines. The results demonstrate huge differences, which are interesting from statistical and oenological point of view.

Material and Methods

Analyses were carried out on a GC 8000 Top Series, CE Instruments (Rodano-Milan, Italy) equipped with a modified split-splitless inlet and flame ionization detector. The inlet was modified so that it was possible to insert a glass microcolumn. The microcolumn was packed with 5.0 mg of 60 - 80 mesh Tenax TA (Alltech, Deerfield, Illinois, USA). The outlet of the microcolumn afforded a tight connection with the capillary column. The fused silica capillary column VF-Wax, 30 m * 0,25 mm * 0,25 μm film thickness (Supelco, Bellefonte, Pennsylvania, USA) was used. The GC inlet and the detector temperatures were 230 °C and the initial column temperature was maintained at 30 °C. Thermal desorption was performed at a pressure of 10 kPa for 1 min, then the pressure was increased to 60 kPa and the column temperature was programmed at a rate of 5 °C.min^{-1} up to 210 °C and maintained at 210 °C for 5 min. Helium was used as the carrier gas.

A volume of 100 ml of the wine sample plus 20 g NaCl was transferred into a 500 ml volumetric flask and the flask was vigorously shaken for 2 min at ambient temperature. Immediately after shaking, an appropriate volume of headspace was taken through the microcolumn using a glass syringe with a glass plunger lauer (Poulten and Graf, Wertheim, Germany). The distance between the microcolumn and the surface of the liquid was about 1 cm. The loaded microcolumn, with the volatile compounds sorbed, was transferred into the GC inlet at 10 kPa carrier gas pressure and the compounds desorbed were analysed as described earlier. Analysis of each wine sample was repeated twice. A computer program Class-VP 7.2 SP1 (Shimadzu, Columbia, Maryland, USA) was used for data acquisition.

Eight wine samples were qualitative analysed by gas chromatography. Fourth wine varieties were Slovak wines, namely Chardonnay, Cabernet sauvignon, Blaufrankisch and Sauvignon produced in 2008. Next fourth wine varieties were Bulgarian wines, namely Chardonnay, Cabernet sauvignon, Dimyat and Gamza produced in 2008.

Results and Discussion

All the samples were analysed by gas chromatography, preextracted with microcolumn filled with Tenax. More than 100 compounds were identified in aromagram, of which 20 were determined as significantly important sensorial active compounds. From alcohols – propanol, butanol, characteristic by alcoholic perception, hexanol evoking windrow grass, important esters recognised especially by fruity aroma. The quantity of these compounds is varietal specific, what is shown in fig. 1. The aromatic profile of four Slovak wines – Sauvignon, Blaufrankisch, Chardonnay and Cabernet Sauvignon was analysed. The highest concentration of isoamylalcohol was noted in all the samples, characteristic by acidic – malt aroma. Even though the wines were fruity, as the ester concentration has reached the threshold concentration (fig. 2-6).

The results were compared with Bulgarian wines. There is a comparison of Dimyat, Gamza, Cabernet Sauvignon and Chardonnay, in fig. 2-6. In all Bulgarian wines the esters were in lower concentration compared to Slovakian wines, however were richer of alcohol. These facts resulted in different aromas, whereby the Bulgarian wines were less aromatic, more spirituous; it is shown in the fig. 7-8.

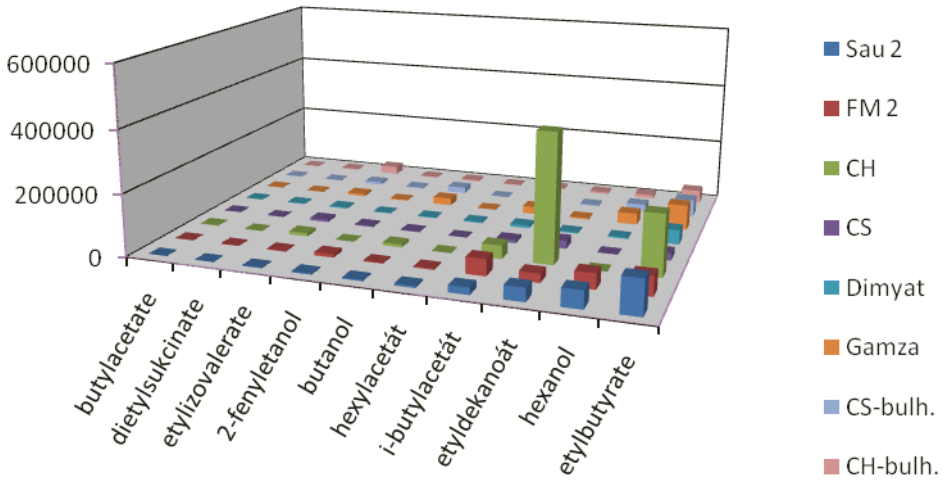

Fig. 1: Aromatic profile analyses samples.

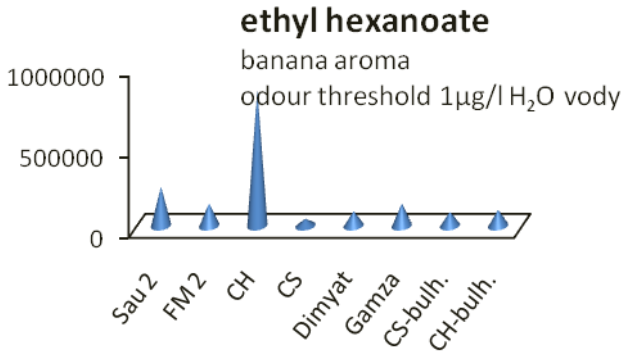

Fig. 2: The concentration of ethyl hexanoate expressed by area.

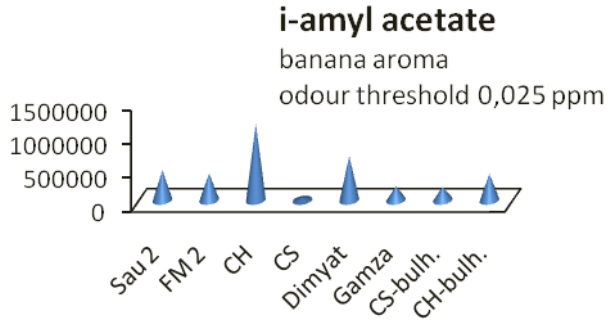

Fig. 3: The concentration of izoamyl acetate expressed by area.

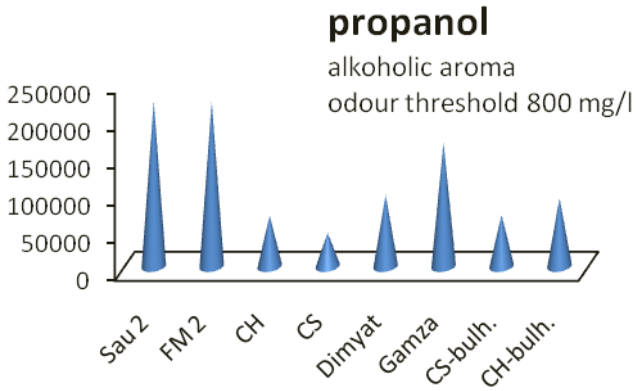

Fig. 4: The concentration of propanol expressed by area.

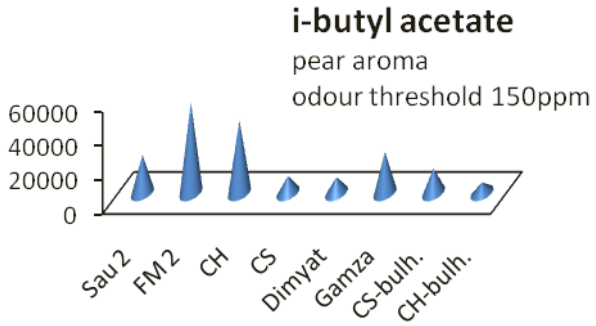

Fig. 5: The concentration of isobutyl acetate expressed by area.

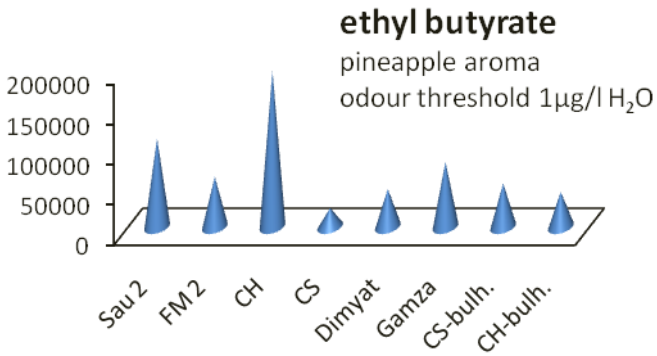

Fig. 6: The concentration of ethyl butyrate expressed by area.

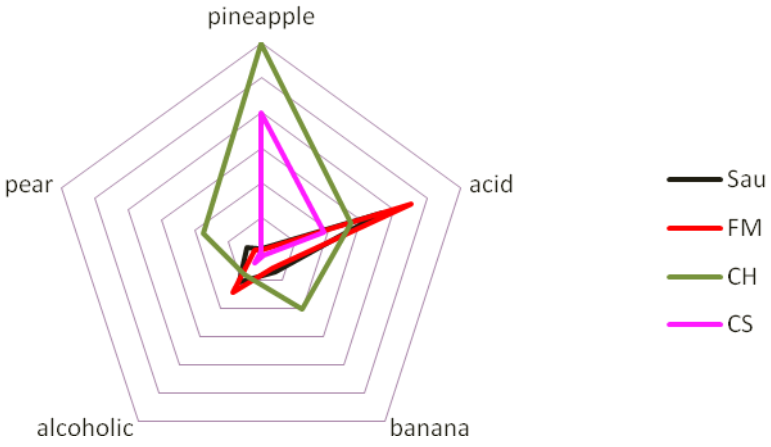

Fig. 7: Aromatic profile of Slovak wine.

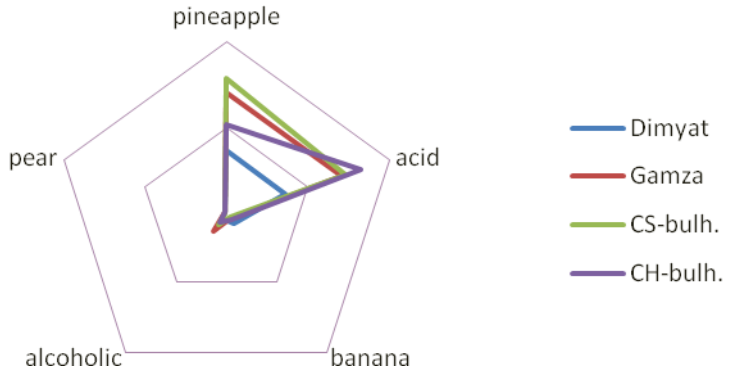

Fig. 8: Aromatic profile of Bulgarian wine.

Conclusions

Aromagrams are characteristic odour imprints, usually acquired using chromatographic methods. Authentication of particular aromatic substances in grape and wines typical for Slovak Republic and its saving in newly-founded database can help to prevent wine falsification. Using this method can significantly reduce negative economic impact on honest wine producers.

Acknowledgements

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Bibliography

Kaňuchová Pátková J., Hrivňák J., 2009. Aká je aróma slovenských vín. *Vinič a víno*, 6 (IX.): 195-196.

Plutowska B., Wardencki W., 2007. Aromagrams–Aromatic profiles in the appreciation of food quality. *Food Chemistry*, 101 (2): 845-872.

Scientific the bases of obtaining the Georgian types of the wines

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Abstract

White Kakhetian and Imeretian wines rightfully can be named one of the achievements of world wine-making culture. The uniqueness of the Georgian technologies of white faults lies in the fact that grape pulp undergoes fermentation in the buried in the earth clay jugs (Kvevri), obtained in this case the fermented must then is maintained on the same pulp during 3- of 4 months. This uncommon technology gives fault special taste - astringent and a little audacious, amazing color of white amber and the aroma of the field colors.

Are presented below the results of the long-term investigations of the process of the alcoholic fermentation of must using "red method" in the conditions for industrial experiment, is shown the influence of technological factors on the accumulation of organic and mineral matter in winemaking material and wine, and also to their quality.

Were set the experiments in the following microzones of Kakheti for the purpose of the study the process of the alcoholic fermentation of must on the pulp of the grapes red types of: Shroma, Teliani, Kurdgelauri and Kistauri. For the experiences there were processed technically ripe, healthy grapes of the types of Saperavi and the Cabernet-Sauvignon, assembled from one and the same sections of the vineyards. Pulp without the crests was distributed in in parallel confronting vats, one of which was without the partition. For the purpose of the aeration of the straying pulp the fermentation was here conducted with mixing of the pulp of 3-4 times in a 24 hour period by the wooden mixer (experience). In the different version the fermentation was conducted in the vat, supplied with partition. Fermentation in this case was carried out by method with "submerged cap". In this case in the course of the process of fermentation was conducted the circulation of the straying must "to itself" (control).

Below is given data of studies of the influence of the duration of the fermentation of must on the pulp and mixing the straying pulp to the chemical composition and the organoleptic indices of winemakings material and wines of the red types of the grapes.

Organic acids. Wine and malic acids compose 90% from about 30 designations of acids, which are contained in the grapes and the wine. From them the tartaric acid is specific. The analysis of obtained data makes it possible to conclude the following:

1) The duration of the persistence of the straying must on the pulp practically does not have a noticeable effect on the content of flying acids in obtained winemakings material. In winemakings material, obtained both from the intermixed, and unmixed pulp flying acids practically remain at one and the same level.

2) A insignificant increase in the tartaric acid is noted in winemakings material, fermented from the intermixed pulp in the comparison with winemakings material from the unmixed pulp. The same picture is observed also for the titrate acids, i.e., it is contained more in winemakings material, obtained from that intermixed in the fermentation process of pulp, during mixing process of the extraction of organic acids from the vacuoles of the cells of the grapes berries pulp, where in essence the organic acids are contained, is improved.

3) Independent of the duration of the fermentation of must on the pulp and the mechanical agitation of the straying mixture, pH of obtained winemaking material it remains at one and the same level. Depending on the place of growth and type of grapes (Saperavi, Caberne -Sauvignon) this index varies in limits of 2,85-3,45.

Nitrous substances are located in all parts of the grapes. Shown in Fig.1 curves describe the dynamics of the content of the nitruos substances in winemaking material and wine of grapes of the type of Saperavi. As can be seen from represented given, in the wines the content of nitrogen changes with the same regularity, which is characteristic for winemaking material, from which is obtained this wine. In contrast to winemaking material, in the wine is contained somewhat less nitrogen than in winemaking material, which is caused by the precipitation in the sediment in the course of endurance of winemakings material of the complex substances, which contain nitrogen.

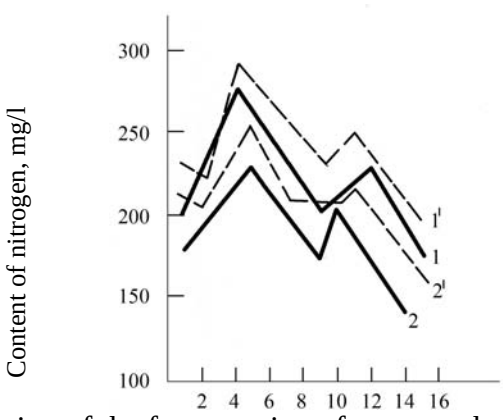

Fig. 1. **Change in the content of nitrogen in winemaking material (1, 1^1) and wine (2, 2^1) of Saperavi, obtained during mixing of the straying medium (-) and without its mixing (- -)**

Tannins (tanning substances) in essence are contained in the skin, the seeds and the crests of grapes.

Data analysis of curves ($1,1^1$) on fig.2 show that a maximum quantity of tannin in winemakings material is accumulated on 7-10 days of the fermentation of must on the pulp. Then, as a result indicated above processes of condensation and polymerization of phenols, and also reduction in the solvent power of the saturated by organic matter must, occurs precipitation of the generatrix of complex compounds into the sediment. As the result of this, the content of tannin in winemakings material begins to descend.

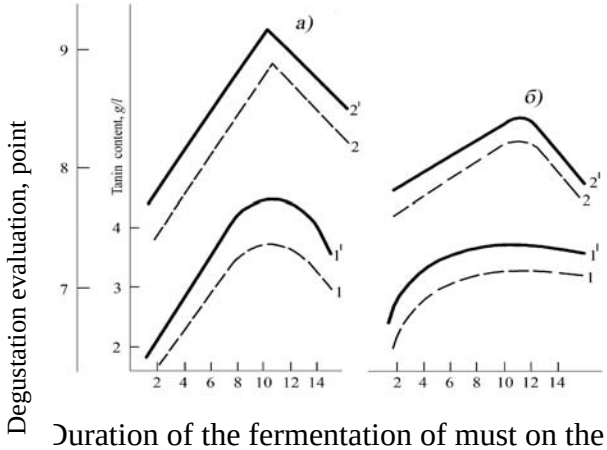

Fig. 2. **Dynamics of the content of tannin (1, 1[1]) and indices of degustation evaluation (2, 2[1]) of winemakings material, obtained with the fermentation of must on that intermixed (-) and that not mixed (---) to pulp in different viticulture microzones of Kakheti**:

а*) Kurdgelauri; б) Shroma*

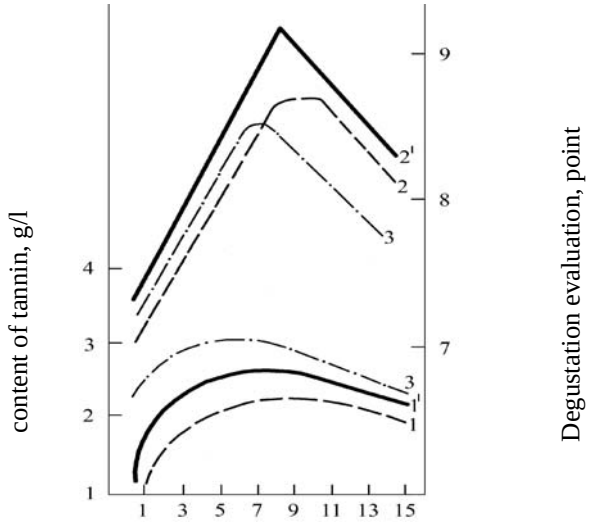

Fig.3. **Dynamics of the content of tannin and indices of degustation evaluation of winematerials and their corresponding wines, obtained from mixing (-) and nonmixing (---) in the fermentation process of pulp;**

1,1[1] – the content of tannin in the wines;
2,2[1] – wine degustation evaluation ;
3 – consistence of tannin in winematerials;
3[1]- winematerial degustation evaluation

We observe in view of this, that in parallel with an increase in the content of tannin during the first ten days of fermentation on the pulp rises the organoleptic estimation of winemakings material (curved 2, 2[1] on fig.2). The analysis of the curve

dependence of the degustation evaluation of winemakings material on the duration of the alcoholic fermentation of must on the pulp shows that the quality of winemakings material begins to deteriorate immediately after a reduction in them of the total content of tannin as one of the indices of quality of the end product

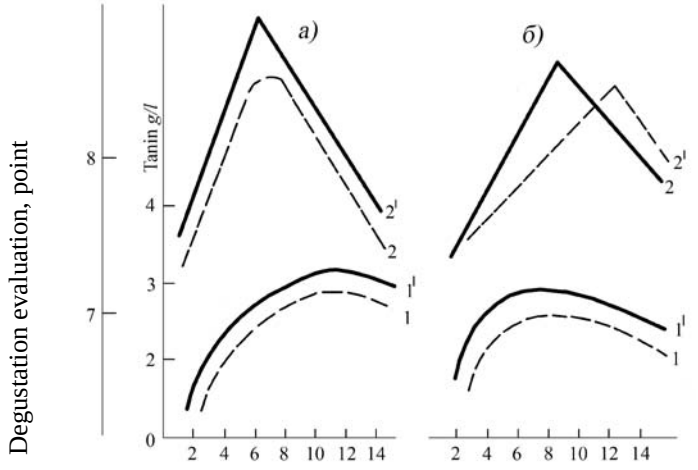

Fig. 4. **Dynamics of the content of tannin (1,1[1]) in winemakings material of Caberne - Sauvignon (a) and Saperavi (b) and their degustation evaluation (2,2[1]) depending on the duration of the process of the alcoholic fermentation of must on that intermixed (-) and that not mixed (---) to the pulp**

The chemical reactions of the transformation of tannins, as it was noted above, in essence occur in the process of endurance of winemakings material. Tannins, as strong antioxidants, are oxidized first of all. Then oxidizing reactions continue also in them other substances are implicated. Chemical reactions in the comparison with the enzymatic occur considerably slower.

The comparison of curves 1 and 1^1 on fig.3 shows that in the self-possessed wine is contained less than the tannin in the comparison with winemakings material, which is caused by the continuous reactions of condensation and polymerization of tannin in the young wines. In this case the degustation evaluation of wine Saperavi, which is been the summary index of the transformations of all substances of organic guilt complex, is considerably above in the comparison with winemaking material (young wine). Wines, obtained from the intermixed pulp, are analogous with winemakings material, as is evident of the comparison of curved 2 and 2^1, have the higher degustation evaluation than wine, obtained from the unmixed pulp, since mixing contributes to obtaining the more extractive wine.

Summarizing that outline aboved it is possible to conclude that is outlined the clearly expressed regularity in the passage of tanning substances (tannin) from the solid parts of the pulp into the must straying on them, which consists in the fact that first of all, winemakings material are saturated by tannin still before the completion of the process of transforming the fermented sugar into ethyl alcohol, or immediately after this process and, secondly, mixing the straying medium contributes to an increase in the content of tannin both in winemaking material and in the wine, obtained with endurance of the same winemaking material.

Anthocyan in effect in all types of grapes is located in the skin of berries. Their greatest quantity is contained in the cells adjacent to the pulp; therefore during the

extrusion of the heated grapes juice comparatively easily washes out anthocyan from the destroyed cells and it becomes painted.

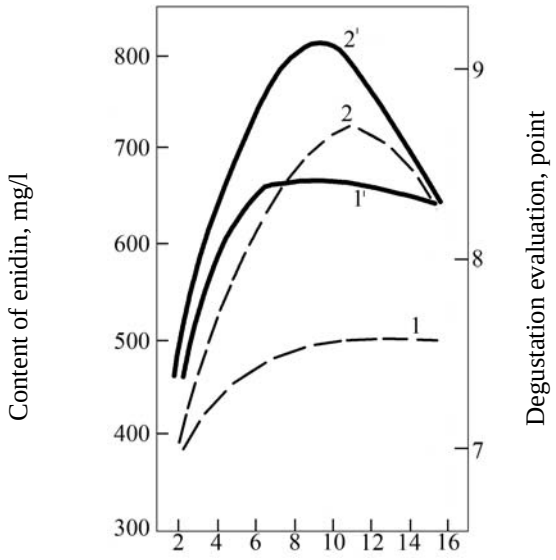

Fig.5. **Content of the anthocyan (1, 1^1) and degustation evaluations (2, 2^1) of wines, obtained from that intermixed (-) and that not mixed (- -) the pulp of grapes of Saperavi**

The basic task of wine making using “red method” is the extraction of the maximally possible quantity the coloring substances from the skin of grapes in the process of the alcoholic fermentation of must on the pulp and their subsequent retention in the wine.

Data analysis of fig. 12 show that mixing the straying pulp has an essential effect on an increase in the quantitative content of anthocyan in the wine.

The extract (without sugar) is the sum of all dissolved in the wine non-volatile substances minus the fermented sugar. In the white table wines into the composition of the given extract the wine and malic acids, nitrous and other enter, the substance of must, and also resultant in it in the course of the alcoholic fermentation non-volatile substances. The extract of red wines additionally contains the substances extracted from the solid parts of the pulp (skin, seeds) non-volatile.

The dynamics of the accumulation of extractive substances in red winemakings material as this is shown in Fig.6, are described by one-vertex curve with the maximum on the 7th or 10th day of the fermentation of must on the pulp. By analogous curve is described the dynamics of a quality change of obtained winemakings material, with the difference that maximums in these two curves do not coincide one with another. i.e. the enrichment of winemakings material by extractive substances positively affects their quality to a definite limit, which on the average is 25-27 g/l. Further enrichment of winemakings material with extractive substances makes them rough.

During the continuation of the fermentation process of must on the pulp are more than 10 days, as a result changes in the solvent power of the straying must, caused by an increase in its alcohol content, and also in view of the occurring polymerization reactions and condensation of the substances of the organic complex of the straying

medium, the part of the substances falls out into the sediment, decreasing thus extract content of obtained winemakings material.

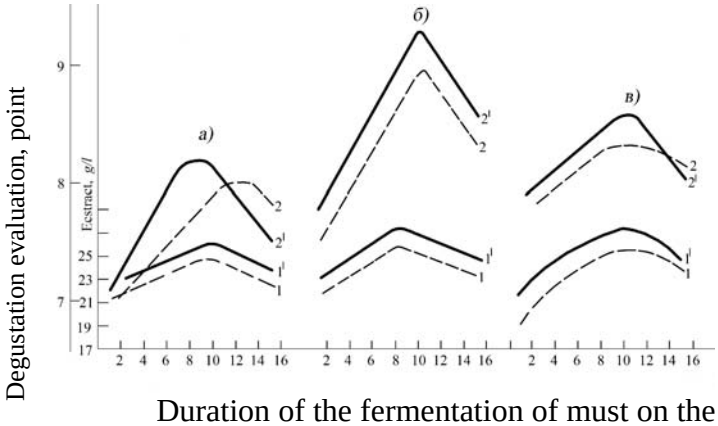

Fig. 6. Content of extractive substances (1,1[1]) and the degustatoin evaluation of winemakings material (2,2[1]), obtained in different microzones of Kakheti from the grapes of the type of Saperavi during mixing of pulp (-) and without the mixing (---):

a) Kistauri; b) Kurdgelauri; c) Shroma

Data analysis of fig.6 also show that mixing pulp in the course of the process of the alcoholic fermentation has an essential effect both on exstract content of obtained winemaking material and on its quality. In particular, in the represented figures are visible clearly expressed laws governing the increase in exstract content of winemakings material during mixing of the straying medium, which is caused by the intensification of the process of the extraction of extractive substances out of the solid parts of the pulp. As a result the best aeration pulps during its mixing are intensified the process of multiplication and metabolism of yeast(s), which positively affects the quality of the end products.

Comparison data of a), b), c) in fig.6 make it possible to conclude that the geographical factor has an essential effect on the course of the process of the alcoholic fermentation, which is evinced by a change in the forms of the curves of the accumulation of extractive substances and curves of the degustation evaluation of winemakings material obtained in different city blocks and wines. The influence of geographical factor on the motion of the alcoholic fermentation of must on the pulp is, apparently, caused by both the different chemical composition of the grapes, assembled in different microzones, and by different composition of microflora in the environment of the zones of the viticulture indicated.

From the moment of the beginning of the process of the alcoholic fermentation an increase in exstract content of must positively affects the organoleptic indices of obtained winemakings material and wines. The highest degustation evaluation obtained winemakings material, fermented on the pulp during the first 7-10 days. The content of the given extract in these wines varies in the limits of 25-27 g/l. Further continuation of the persistence of must on the pulp negatively impacts on the quality of obtained winemakings material .

Mineral Substances are localized in the solid parts of the pulp - seeds, the skin and the pulp of grapes. In the must and the wine they are found in the form free ions or enter into the composition of complex compounds, playing the significant role in the processes, which take place with the fermentation of must and subsequent endurance of winemakings material.

In Fig.7 are shown the curves of the dynamics of the ash contents and its alkalinity in winemakings material, obtained from the different types of grapes - Cabernets-Sauvignon and Saperavi, grown in one and the same microzone of Kakheti - Teliani.

Accumulation of mineral substances in winemakings material is the especially physical process of the extraction of inorganic substances from the solid parts of the grapes by the straying must, which washes the surface of skin, to pulp and the seeds of grapes. Despite the fact that this process has nothing in common with the oxidation processes, polymerization and condensation of organic matter of grapes, the curves, which describe the process of the accumulation of mineral substances in winemakings material, take the same form, which above was shown for, the description of the process of accumulation in winemakings material of tannin, anthocyan and other substances of the organic complex of the grapes. This fact makes it possible to conclude that the accumulation in winemaking material both of organic and inorganic matter in essence occurs due to the physical process of their extraction from the solid parts of the straying pulp it obeys the law of the process of the extraction of substances from solid body into the liquid. The basic parameters, which influence the given process, are temperature extractions, with high values of which (thermo-winification) the destruction of the cells of plant tissue, mechanical agitation of the straying medium, etc, simultaneously occurs.

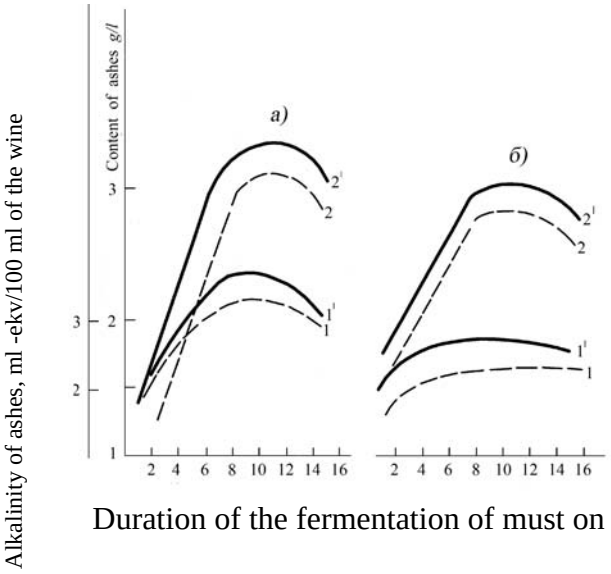

Fig.7. **Change in the ash contents (2,2^1) and of alkalinity of ashes (1,1^1) in the straying must during mixing of medium (-) and without its mixing (---):**

a) Cabernet Sauvignon; b) Saperavi.

Oxidizing transformations, with which are connected the reactions of polimerization and condensation of the organic matter of grapes falling out into the sediment, compose insignificant part in the general process of the accumulation solutes in the end products of processing grapes - winemaking material and the wine. Meanwhile the precisely quantitative content of organic matter in the red wines and Kakhetian type white wines, and subsequently and their oxidizing transformations determine the quality of the end products of processing grapes - winemaking material and wine. Actually, the greatest degustation evaluation, as a rule, obtains winemakings material and wines, prepared from that intermixed in the process of the alcoholic fermentation on the pulp of medium. Mixing the straying medium in essence influences the intensification of the process of the extraction of organic matter from the solid parts

of the grapes into the straying must, increasing thus the content both of organic and inorganic matter in the end product.

In the present work the information about the processes presented, which take place with the fermentation of must and following maintain winemakings material on the pulp, to the certain degree they reveal the essence of the biochemical and physico-chemicaltransformations, critical for the formation the organoleptic indices of the Georgian (Kakhetian) types of the table wines, which do not have analogs in the world practice of the wine making.

In Georgian Scientific Research Institute of food industry are carried out comparative studies of the physical chemistry indices of wines, obtained by European and Kakhetian methods from the industrial and little encountered types of the grapes / 1,2/. Data analysis of table. 1 show that there is a clearly expressed regularity in the physical chemistry indices of the Kakhetian wines, which distinguish them from the European types of wines.

Table 1

Physico-chemical indices of the white Georgian wines

Type of the grapes	White wine type	Strength, % vol.	*g/l*				Degustation evaluation in the 8-point scale
			Titrate acidity	tartaric acid	Tannin	Extract	
Rkatsiteli	Khakh.	13,4	5,13	2,56	2,97	28,36	7,7
Rkatsiteli	Eur.	12,1	6,04	2,98	0,64	22,94	7,5
Kakhuri mtsvane	Kakh.	13,8	5,29	2,26	2,85	29,21	7,8
Kakhuri mtsvane	Eur.	12,0	5,89	2,76	0,91	24,81	7,7
Upipko mtsvane	Kakh.	13,0	5,53	2,34	2,66	27,99	7,7
Upipko mtsvane	Eur.	11,3	5,11	2,32	0,84	23,52	7,6
Goruli mtsvane	Kakh.	13,8	4,97	1,82	1,87	26,74	7,3
Goruli mtsvane	Eur.	12,3	6,90	3,33	0,79	19,06	7,3
Chinuri	Kakh.	11,9	5.02	2,00	1,91	27,10	7,3
Chinuri	Eur.	10,7	5,48	2,26	0,70	23,80	7,4
Aligote	Kakh.	11,8	5,62	2,23	1,79	18,36	6,8
Aligote	Eur.	11,5	6,45	2,30	0,54	18,66	7,1
Buera	Kakh.	9,5	5,70	2,62	1,32	17,72	6,8
Buera	Eur.	10,9	6,98	3,17	1,23	15,29	6,6
Khikhvi	Kakh.	14,1	5,29	2,56	2,35	31,49	7,4
Kisi	Kakh.	13,22	4,97	2,52	3,95	27,24	7,4
Shaba	Kakh.	13,3	5,29	2,73	4,81	31,02	7,3
Sapena	Kakh.	13,6	4,92	2,02	1,75	25,62	7,1
Mkhargrdzeli	Kakh.	12,8	5,37	1,77	2,91	28,74	7,0
Saperavi	Red	11,5	6,1	2,13	3,2	25,4	-
Cabernets- Sauvignon	Red	11,4	6,1	2,14	4,1	25,9	-

BIBLIOGRAPHY

1.Bagathura N. 2007, Natural wines, juices and beverages. Tbilisi, Publishing House “Paragraph”

2. Giashvili d., 1967, Georian Institute of Food industry, Work collection, Moscou, Publishing House “Pischevaia promishlennost”, pp. 98-137.

Technology of the production of Esnault- dye from the red types of the grapes

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Abstract

Nontoxicity and their harmlessness for the human organism is the basic requirement, presented to the food dyes. Unfortunately, the majority of artificial dyes do not possess this property and in the scientific literature their harmful action on human health repeatedly was noted.

Work depicts the new technology of obtaining and the physical chemistry indices of the Esnault-dyes, obtained from the Georgian types of the grapes.

It's remaked of receiption technologies from the red types of grapes with bioactive materials of wealty natural red dye (of wines, grapes juice).

It's used for reception grapes natural dyes of functional destinition (antiooxidantial) food products (juices, confectionery products).

It's investigated influence of technological factors in the grapes juice by substitution of materilas.

From the ancient times, natural plant pigments were used for the dyeing of foodstuffs. However, the further development of organic chemistry gave the great (wide) opportunity to the production of artificial coloring substances, low price of which almost, for some time, stopped the investigation in the field of receiving and using of the natural plant colorings. The industrialization of food production to a considerable extent contributed to the replacement of natural plant dyes by the artificial.

Non-toxicity and harmlessness for the human organism is the basic requirement for the food dyes. Unfortunately, in the synthetic dyes used to colorize the food products there were found the substances harmful for human health, therefore usage of such kind of colorings in recent years is limited.

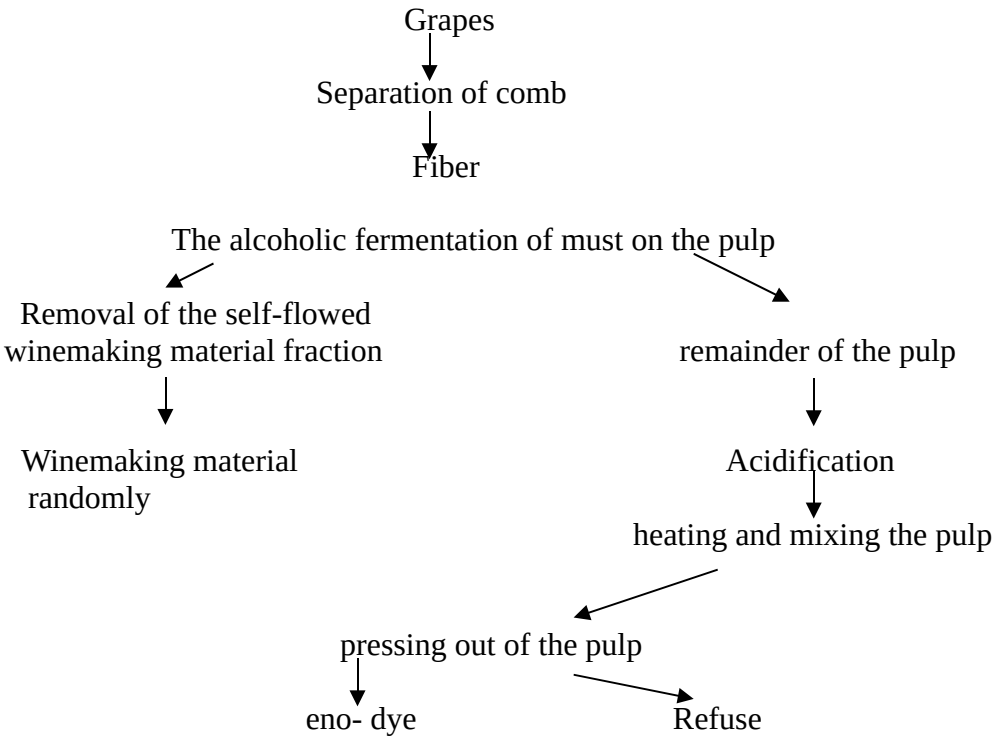
In 2007 Euro-parliament made a decision about the limitation of the using of artificial dyes as a result of their negative effect on the children organism. In connection with the above said the sharp rise of demand on natural colorings is expectable for making wine, alcohol and non-alcoholic drinks.

The industrial production of eno-dyes are established in Italy, where the grape husk (chacha) are used as the source raw material for obtaining the red grape dye. Technology foresees the working out of grape husk with an 0,2% solution of sulfurous anhydride or 0,4% meta-bisulfate of potassium. Mass is kept for 48-72 h, then extract is taken off and presses out remainder. The obtained extract is concentrated by steaming in the vacuum- apparatuses at a temperature of 40-45°C, 3-5 or more times. Received dye is used to make wine, fruit syrups, table vinegar etc.

In the literature there are described the methods of obtaining the eno-dye, which foresees the extracting of grape sweet fermented husk by the acidic aqueous or water-alcohol solutions and concentration of the obtained extract. General deficiencies in the described methods of obtaining the eno-dye is the fact that with the concentration of the extract eno-dye enin gradually loses the glucose, converts into the badly water-soluble aglucone enidin and falls out into the sediment.

The conception of the high-quality wines production proposed by us, first of all foresees the usage of only self-flowing fraction of wine receiving, which is not more than 50% of worked out grapes amount. The pressed fractions, which is 15-20% of whole mass we use for obtaining the eno-dye according to the diagram shown below.

The process chart of the receiving technology of eno-dye from the red type grapes



New technology for obtaining the eno-dye foresees the processing of red type grapes by “red” method. Obtained pulp is moved to drain in order to separate the self-flowed fraction. Juice is used to make high-quality wine. Remained husk is moved to thermal-fermentation

and heats up to 55-60^{0}C, constant mixing during 3-4 hours, after that it is cooled to the room temperature and is moved to press. The pressed out liquid phase is dye for the nonalcoholic drinks. It should be noted that new eno-dye contains entire complex of the bioactive and nutrients of grapes; therefore the nonalcoholic drinks, prepared on this dyes get antioxidant properties, they are enriched with all useful organic and inorganic matters of grapes and this differs it from the synthetic dyes harmful for the human organism.

Wines obtained from the grapes of Saperavi are characterized by intensive painting, because of the high content of the coloring and tanning substances in the processed raw material. The extraction of phenol compound from the rind of grapes berries occurs because of high temperature of alcohol fermentation (25-30^{0}C) and the presence of essential oil remainder in leach (winemaking material).

Eno-dye can be obtained also from the juice of the red types grapes, by their concentration.

The natural juice, obtained from the grapes of Saperavi by pressing, is characterized by weak painting feature that is caused of difficulty of the extraction the coloring substances from the grapes rind. In view of this during obtaining the natural juice without raw material's preliminary working the basic number of phenol compounds remains in grape refuse (husk).

Grape juice with the antioxidant activity can get closer or considerably exceed the red wine in case of maximal moving of bioactive substances from rind to grape juice.

In connection with the above said, were carried out the investigation in order to intensify the extraction process of phenol compounds from grapes to natural juice. Investigations were conducted in laboratory conditions. The berries separated from crests were processed by the water hot vapor with the pressure 1,5 for 5 min. The heat-treated raw material underwent for splitting by cooling to fermentation temperature, after which into the pulp was introduced pectolic fermentation preparation Nigrine- PK by the activity of 3600 un/g.

Chemical composition, %

Titrate acidity ……. 0,9
Common sugar …….16,0
Saccharose ……. 0
Invert sugar ……... 16,0
Pectin:
Dissolved …… 0,19
not dissolved ……. 0,24
common …… 0,43
Vitamin C (mg%) …… 11,4
Content of the coloring substances
in the skin of the grapes……3,95

Specifications analysis

Average weight of the crest, g……. 3,1
Average quantity of grains
on one cluster, p …… 67
Weight of one grain, g …….. 1,4
Specific weight of the skin, % ……... 5
Specific weight of the grape
seed, % ……3,2
Humidity, % …… 90

Fermentation was conducted at a temperature of 45-55^{0}C. Fermentation preparation was applied in 0,05% of raw material and 0,1% amount. The duration of fermentation was 2,4 and 6 h. For control there were taken juices, which were obtained from thermally worked and not worked crushed pulp pressing.

Yield of juice with the different conditions of the fermentation is shown in table 1 The analysis of received data shows that the yield of the juice, obtained in laboratory conditions with pressing out of pulp by hand on the basket press, is considerably lower than it could be expected in the production conditions, where basket presses with the power drive are used and also screw presses of the continuous action.

In spite of this, according to the laboratory investigation data it is possible to judge perspectives of the investigated versions of obtaining the juices.

Data analysis of table 1 shows that with the simple mechanical pressing the yield of juice from the processed raw material does not exceed 45%. The heat working of pulp makes it possible to increase the yield of grape juice by 10% (experiment 2), whereas the fermentative working of the heat-treated pulp makes it possible to bring the yield of juice to 65-66%.

In table 1 are brought the results of the chemical analysis of juices, obtained by different experimental variants, analysis of which shows that the preliminary working out of raw material before pressing out of juice has an essential influence on the content of phenol substances in the juice. The greatest content of tanning substances are found in juice, obtained by the fermentation of the heat-treated pulp for 2 hours. This juice is enriched by both the tannin and by coloring substances.

The comparison of received data makes it possible to conclude that for the maximal moving of phenol compounds into the juice from the rind of grape berries it is necessary both the heat working of pulp and its following fermentative working out. As can be seen from the presented data, during the fermentative processing of the crushed pulp the yield of juice increases and is equal to the same with the juice pressing out from the heat-treated fermented pulp; however, the given juice contains less phenol compounds than the juice, obtained by the fermentation of the heat-treated pulp. This indicates to the significant role of fermentation in the process of the extraction of organic matter from the rind of grape berries into the must.

Table 1

Designation of experimental variant	Temperature of fermentation, h	Duration of fermentation, h	%Dose of the introducing ferment to the weight of pulp,	Dry matter on the refractometer, %	Yield of juice, %	Yield of juice in the conversion to the dry matter of control juice (№2). %	Rate of filtration and 5 min, ml
Juice, obtained by the pressing of crushed pulp (control room)	-	-	-	18,5	45,0	-	9
Juice, obtained by the pressing of the heat-treated pulp (control room)	-	-	-	18,0	55	-	7
Juice, obtained by the fermentation of the heat-treated pulp	45	4	0,05	19,0	66,6	70,4	25
Same	45	6	0,05	19,0	63,3	70,4	18
«	45	2	0,1	19,4	66,6	77,8	10
	45	4	0,1	18,8	60,0	70,0	10
«	45	6	0,1	19,5	60,0	65,0	16
	55	2	0,05	18,5	55,0	56,5	10
«	55	4	0,05	2,0	66,6	74,1	20
«	55	6	0,05	19,5	66,6	72,2	12
«	55	6	0,1	20,0	65,0	72,2	17
Juice, obtained by fermentation of crushed pulp (not thermally processed)….	55	4	0,1	18,1	66,6	70,4	10

Same	……	55	6	0,1	18,2	59,0	62,5	12

The significant role to outcome and composition of grape juice plays the duration of the pulp fermentation. It can be supposed that the longer is the fermentative working out of pulp, the higher is crushing level of plant tissue and accordingly increases the yield of juice from pulp. However, from the data of tables 1 and 2 it seems that there is no directly proportional dependence between the duration of the pulp fermentation and the yield from it the juice, enriched by organic matter. So, with 45^0C and the duration of the pulp fermentation for 6 hours (experiment 4 tables 1) the yield of juice was 63,3%, with the same temperature and duration of the fermentation for 2 h, juice is obtained more - 66,6%. Moreover in the juice itself is contained more both the tannin and coloring substances (experiments 4 and 5 in table 1).

The fermentative processing of raw material differently influences on outcome and composition of natural juice and natural wine during the processing of the same type of grapes, the comparison of data of tables 1 and 2 testifies this. In particular, the fermentative working out of pulp before the must fermentation influences not so essentially as on wine outcome on its chemical composition as well. However, in this case noticeably increases the outcome of the self-flowed must.

Table 2

Titrate acidity, *g/l*	Extractivness by desiccation, *g/l*	% Content of alcohol,	Flying acidity, *g/l*	Common sugar, %	Vitamin C, *mg* %	*g/l*Tanning substances,	Tartaric acid, *g/l*	Pectin, *z/n*	*Mg/l*Coloring substances,
6,7	19,2	0,8	0,9	17,5	1,8	2,8	5,1	1,9	432,5
7,1	19,5	0,8	1,0	17,7	4,6	3,8	5,2	2,4	515,8
8,0	19,8	1,0	0,65	17,8	6,7	3,9	5,4	1,6	732,8
7,6	19,7	0,8	0,84	17,8	6,7	4,1	5,4	1,2	855,2
7,9	19,6	1,0	0,9	17,6	6,2	4,5	5,6	1,3	926,3
7,3	20,3	0,9	0,85	18,1	6,7	4,2	5,3	2,0	953,8
8,3	20,4	1,0	0,84	18,2	5,1	4,3	5,7	1,0	784,2
7,3	19,6	0,9	0,72	17,6	5,1	4,2	5,2	1,8	793,1
8,6	21,3	0,8	0,72	18,8	5,2	4,2	5,7	1,5	983,2
7,8	20,2	0,9	0,84	18,1	4,6	4,1	5,6	1,0	906,2
6,3	20,8	1,0	0,83	18,4	4,7	3,8	4,9	0,5	894,2
5,8	19,7	0,8	0,75	17,8	3,0	2,9	4,4	0,9	442,3
7,8	19,6	0,9	0,83	17,6	3,1	3,5	5,6	0,8	501,4

With the vacuum- concentration of the red types grapes juice can be obtained the natural eno-dye, enriched by the bioactive substances of grapes. We have used this kind of dye

for obtaining the nonalcoholic drinks and the pastry of functional designation (antioxidant foodstuffs of nourishment).

Tannins and off-flavours in relation to the wood selection and processing in the oak alternative production

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ABSTRACT

The planning of a processing to obtain oak alternative products for wine aging, as chips, demands the adaptation of the oenological quality criteria, normally applied in the wood selection to barrels, and processing, avoiding the increasing of defect risk in wines. Because of the growing demand, the possibility of manufacture these products from wooden remains obtained in the stave cut, and also from trees with small diameter or defects, inadequate to obtaining of staves, is being regarded. In this case the wood is not usually as much carefully selected as for staves, and often there is a tendency to simplify the processing. This work shows the influence of the wood grain, presence of knots or another defects, and removal of the brushing (habitual in staves), on the tannic and off-flavour composition, as possible responsible of sensorial defects in wines.

RESUMEN

La planificación de un procesado para la obtención de productos alternativos de roble para envejecimiento de vinos, requiere una revisión de los criterios de calidad enológica utilizados en la selección de madera para barrica y del procesado, sin que se incrementen los riesgos de defectos en vinos. Ante la gran demanda, se ha contemplado la posibilidad de fabricar los alternativos a partir de restos del corte de duelas, o de árboles de pequeñas dimensiones o con defectos; en cuyo caso la madera no es tan cuidadosamente seleccionada como para duelas, y además se tiende a simplificar su procesado. Este estudio presenta los resultados obtenidos sobre influencia del grano de la madera, presencia de nudos u otros defectos, y eliminación del cepillado (habitual en duelas), en la composición tánica y off-flavours, posibles responsables de defectos sensoriales en vinos.

INTRODUCTION

The new tendencies in oenology, such as the introduction of new sources of oak wood, the reducing of seasoning times of wood in cooperage, the preference of light or medium toasting instead of strong one by part of the oenologists, and the extensive use of new barrels in substitution of the old ones, have contributed to increase the potential and the quality of the wine aging process, but they also suggest new questions related with the risk of defects in wines that should be resolved. The possibility of defect appearance in wines related with the wood, takes a special relevance with the recent authorization in European Union about the use of alternative treatments to wine aging in barrels (1507/2006 regulation, October 11, 2006), since these elaboration techniques are especially sensitive to the presence of defects in the wood.

The planning of a special processing to obtain oak wood alternative products, such as chips, staves and others, involves a previous adaptation of the criteria of oenological quality applied during the wood selection to barrel production, and also, of the wood processing, avoiding the risk of defects in wines related with the wooden use. Habitually, the oak alternative products are obtained starting from staves, rejected during barrel production, because of defects, although they are small (deformations, knots, cracks, etc.), shown especially at the end of seasoning process. However, the growing demand of these alternatives has led us to think on the possibility of manufacture them also starting from the wooden remains from cut of staves, and from wood of small diameter oaks or with defects that are not adequate to obtain staves for barrels (Fernández de Simón et al., 2006, Chatonnet, 2007). In this case the wood usually is not as carefully selected as to staves and also, its processing in cooperage tends to be simplified.

The criteria of wood oenological quality are generally supported on the quantitative evaluation of compounds of special importance by their implication in the oxide-reduction processes that take place in wine during its interaction with the wood, or by their aromatic potential. Phenolic (tannins and low molecular weight polyphenols) and volatile compounds (phenols, lactones, furanic derivatives, etc) are analyzed in the fresh wood free of defects (Fernández of Simón et al., 1996, 2006; Chatonnet and Dubourdieu, 1998), and also during its processing in cooperage (Chatonnet et at., 1994; Fernández of Simón et al., 1999; Cadahía et al., 2001a, b; 2007; Martínez et al., 2008). However, the new tendencies in oenology demand the addition of other criteria on those that to base the prevention of defects in wines related with the wood.

The objective of this work has been to know the influence of defects in the initial wood, and simplification of its processing in cooperage on the chemical composition, focussing us on the study of tannins and off-flavour compounds, possible responsible of sensorial alterations in wines.

MATERIALS AND METHODS

The study about relation between wood grain size and chemical composition was carried out with American oak (*Q. alba* from Missouri) and French oak (*Q. petraea* from Vosges). Six staves of each species showing areas with different grain sizes were selected. Wood samples were obtained from each ones and classified in two lots according to the grain size. The study about defects was carried out with staves of French oak (*Q. petraea* from Vosges) and Spanish oak (*Q. pyrenaica* from Castilla y León). Wood samples were taken from areas with and without defects (knots, fissures, dark coloration, etc.), of 20 staves or wooden pieces, which were separately mixed before its analysis. The study about effect of the brushing or cleaning of the staves after seasoning was carried out using 4 staves of American oak (*Q. alba* from Missouri) and 4 of French oak (*Q. petraea* from Vosges). The staves were cut in four sheets, the external ones of greyish colour were considered representative of the wood fraction that is eliminated during stave brushing, and the interior ones of the wood fraction that remind after brushing.

The wood tannins were analyzed by liquid chromatography, using a diode array detector (HPLC-DAD) and according to the methodology previously described (Fernández de Simón et.al., 1999). The volatile compounds were evaluated by gas chromatography with a mass spectrometry detector (GC-EM) (Cadahía et al., 2003), focusing specially our attention on the off-flavour compounds implicated in the "sawdust character" (Chatonnet and Dubourdieu, 1998).

RESULTS AND DISCUSION

Effect of wood grain size. The grain of the wood is defined as average width of the growth rings of the tree. Traditionally, grain size has been used as an important criterion of quality in the selection of oak wood destined for wine barrel manufacturing. However, this should not be looked, as the only parameter to evaluate the oenological quality. We have classified the samples in two lots according to grain size, obtaining from French wood a lot showing fine grain (1.1-1.7 mm) and other coarse grain (2.2-3.2 mm), and from American wood one of fine grain (1.0-1.6 mm) and other one of coarse grain (3.1-4.4 mm).

The results obtained in previous studies (Esteruelas et al., 2008) showed a low correlation among the composition of phenolic aldehydes and acids, volatile compounds (volatile phenols, w-lactones, pyranones, and others), and grain size. These compounds are usually considered as chemical parameters of oenological quality for oak wood. In the same way, the levels of ellagitannins (Fig. 1), and off-flavours (Fig. 2) associated with "sawdust character" (Chatonnet and Dubourdieu, 1998), shown low correlation with the grain size of the wood.

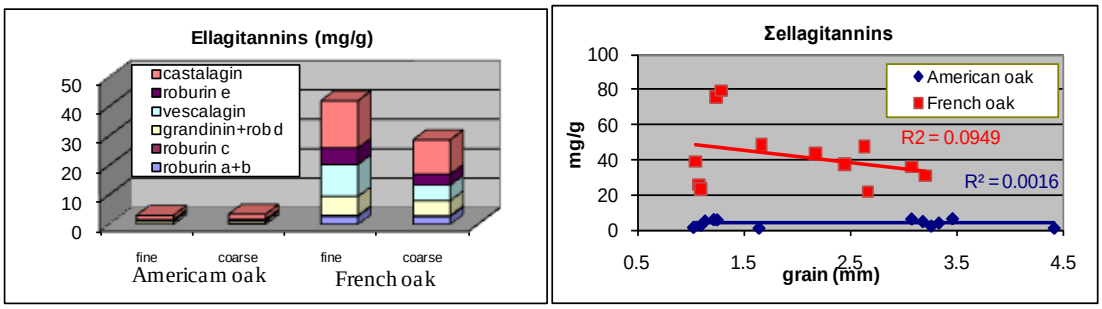

Fig. 1. Concentration (mg/g) of ellagitannins in heartwood of American and French oak of different grain sizes. The values are average for six samples in each wood type.

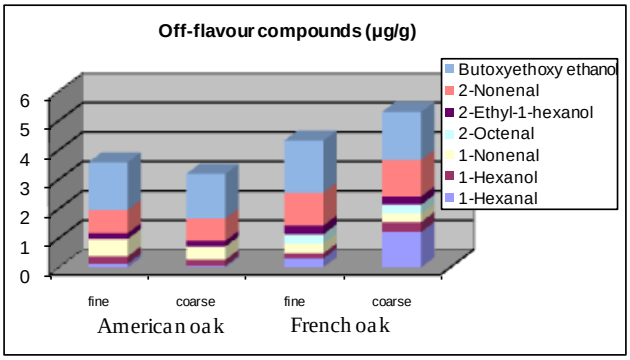

Fig. 2. Concentration (µg/g) of off-flavour compounds in heartwood of American and French oak of different grain sizes. The values are average for six samples in each wood type.

The differences observed between fine and coarse grain have not been significant. The exception was 1-hexanal that showed concentration slightly superior in coarse grain wood of French oak, but because of the high variability of the values shown the differences have not statistically significant. The species, geographic origin, population type (growth conditions, forest treatment, etc.) and the processing wood in cooperage have more influence on its chemical composition. The difference in wine tertiary aromas and other sensorial characteristics due to oak, frequently attributed by cooperages to the wood grain size, are more related with the mentioned parameters. For example, in European oak, the

predominance of each oak species (*Q. petraea* and *Q. robur*) according to the origin of the wood can influence in its characteristics.

Effect of knots and other defects. The chemical changes observed in the oak heartwood with defects comparing with the one without defects, show in addition to a loss of aromatic potential that is especially important for its use in oenology (Esteruelas et al., 2010), a decrease of the ellagitannins contents, in French as well as Spanish wood (Fig. 3). It involves a decrease of the protection against biological contaminations, as well as of its anti-rust potential. Simultaneously, in the wood with defects an increasing of the concentration of some volatile compounds, considered off-flavours such as phenols, cresols, and others whose sensorial properties are not known, has been observed, increasing the risk of sensorial defects in wines (Fig. 4). However, the majority of compounds related with "sawdust character" (Chatonnet and Dubourdieu, 1998), have decreased in the wood with defects (Fig. 4 and 5).

In general, the chemical changes shown in the oak wood with defects can be described as harmful, from oenological point of view. So, it is convenient the elimination of these defects for an appropriate planning of the manufacture and use of the alternative products, focused to elaboration and aging of wines.

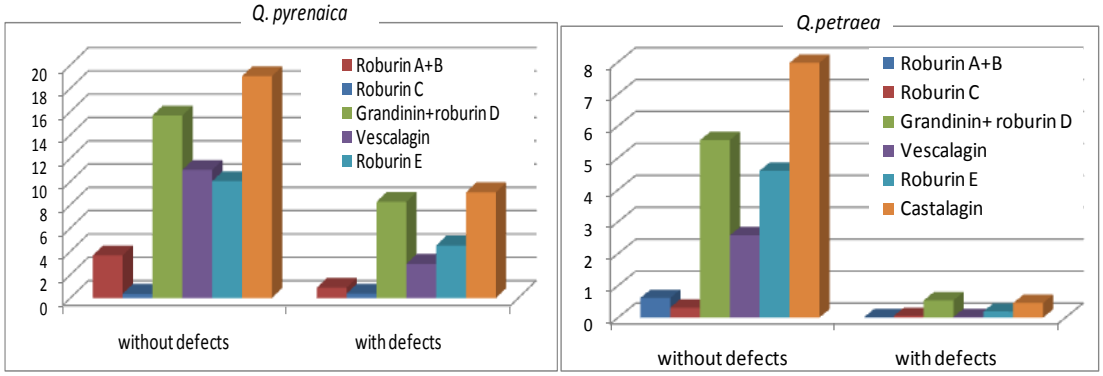

Fig. 3. Concentration (mg/g) of ellagitannins in heartwood of Spanish (*Q. pyrenaica*) and French oak (*Q. petraea*), without and with defects (knots, fissures, dark coloration, etc.). The values have been obtained from the analysis in duplicate of one sample of each one, obtained mixing samples from 20 staves.

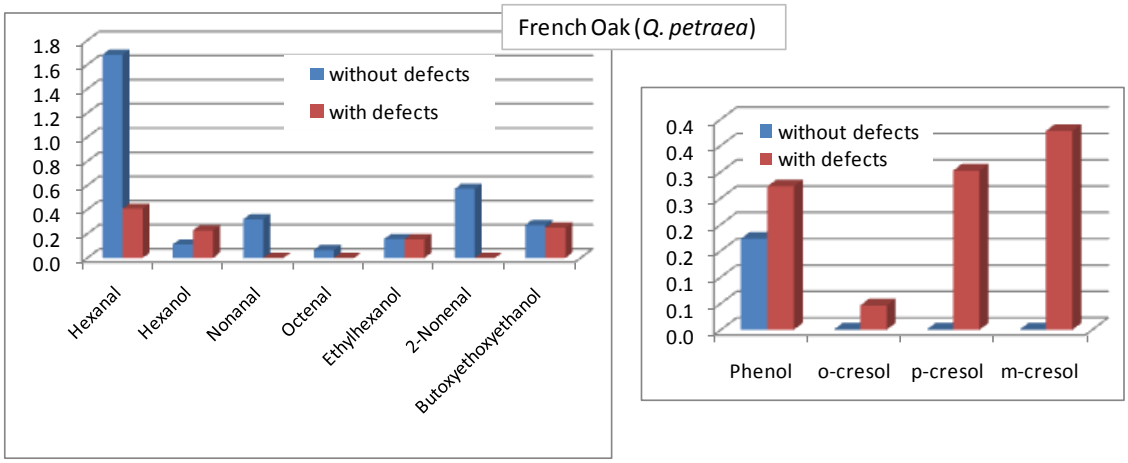

Fig. 4. Concentration (µg/g) of off-flavours in heartwood of French (*Q. petraea*), with and without defects (knots, fissures, dark coloration, etc.). The values have been obtained from the analysis in duplicate of a sample obtained mixing samples from 20 staves.

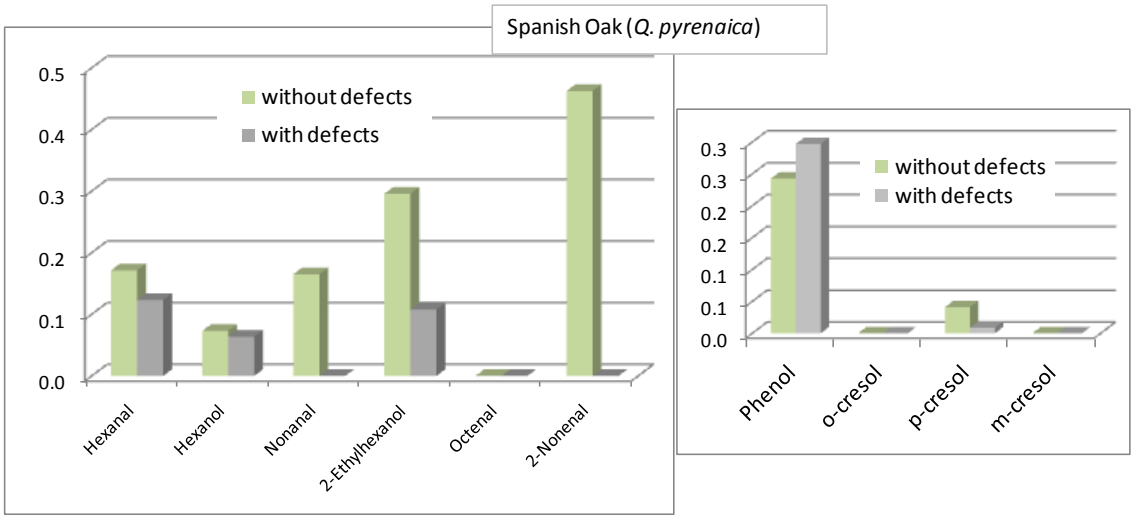

Fig. 5. Concentration (µg/g) of off-flavours in heartwood of Spanish oak (*Q. pyrenaica*), with and without defects (knots, fissures, dark coloration, etc.). The values have been obtained from the analysis in duplicate of the sample obtained mixing samples from 20 staves.

Effect of the brushing or cleaning of staves. The ellagitannins content was higher in the interior sections of the staves comparing to the external ones, as much in American as French oak wood, being the differences something more accused for the French one (Fig. 6), although the variance analysis did not show statistically significant differences, possibly because of the high variability showed by the values. During seasoning, the wood is subjected to the action of the natural conditions of the cooperage, being favoured the natural lixiviation of the soluble compounds and the processes of oxidative polymerization, that contribute to decreasing of ellagitannin concentration. These processes, as it was to be expected, are more evident in the superficial layers of the wood, that change to grey coloration during seasoning, and that are eliminated by brushing before the curved of the staves and toasted of the barrels. The off-flavour levels have shown small differences between the external and internal layer. However, they never overcame the values usually shown by oenological good quality woods (Fig. 7).

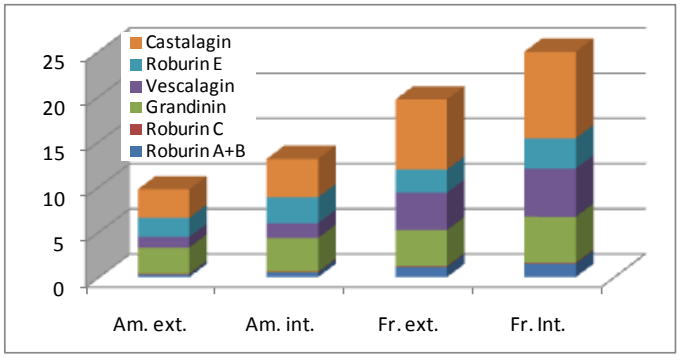

Fig 6. Concentration of ellagitannins (mg/g) in different stave layers (external and internal) of French (*Q. petraea*) and American (*Q. alba*) oak wood. The values are average of four samples, obtained from four staves in each wood type.

These results together with those observed in the polyphenolic and aromatic profile of the wood (Esteruelas et al., 2009) can be qualified neither as beneficial nor harmful, and so an

external brushing during the manufacture of alternatives is not considered as necessary, so much if they are going to be used toasted as non-toasted.

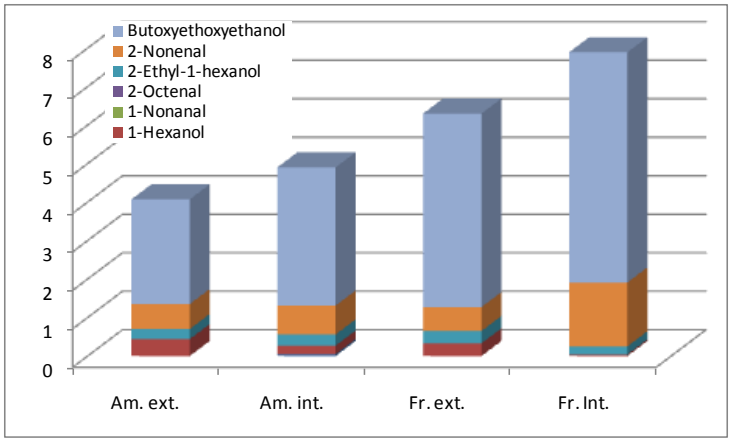

Fig. 7. Concentration (µg/g) of volatile compounds related with off-flavour in American and French oak wood of different layers of staves. The values are average of four samples, obtained from four staves in each wood type.

CONCLUSIONS

The grain of the oak wood should not be considered the only and decisive parameter to define its oenological quality, since correlation has not been observed between grain size and chemical composition with oenological significance.

The chemical changes observed in the oak wood with defects can be described as harmful, so it is convenient their elimination to a correct management during the manufacture and use of the oak alternative products, destined to the elaboration and aging of wines.

The chemical changes observed in the internal layers of the staves in relation to the external ones can be qualified neither as beneficial nor harmful, so an external brushing during the production process of alternative is not necessary.

ACKNOWLEDGEMENTS

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BIBLIOGRAPHYC REFERENCES

Cadahía E.; Muñoz L., Fernández de Simón B., García–Vallejo M. C.,2001a. Changes in low molecular weight phenolic compounds in Spanish, French and American oak Woods during natural seasoning and toasting. *J. Agric Food Chem.* 49: 1790–1798.

Cadahía E; Varea S., Muñoz L., Fernández de Simón B., García–Vallejo M.C.,2001b. Evolution of ellagitannins in Spanish, French and American oakwoods during natural seasoning and toasting. *J. Agric. Food Chem.* 49: 3677–3684

Cadahía, E.; Fernández de Simón, B.; Jalocha, J., 2003. Volatile compounds in Spanish, French and American oak woods after natural seasoning and toasting. *J. Agric. Food Chem.*, 51: 5923–5932.

Cadahía, E.; Fernández de Simón, B.; Vallejo, R.; Sanz, M.; Broto, M.. 2007. Volatile compounds evolution en Spanish oak Wood (*Quercus petraea* and *Quercus pyrenaica)* during natural seasoning. *Am. J. Enol. Vitic.* 58: 163–172.

Chatonnet P., Boidron J. N., Dubourdieu D., Pons M. 1994. Evolution of oakwood polyphenolic compounds during seasoning. First results. *J. Int. Sci. Vigne Vin*. 28: 337–357.

Chatonnet, P.; Dubourdieu, D., 1998. Identification of substances responsible for the sawdust aroma in oak wood. *J. Sci. Food Agric*., 76: 179–188.

Chatonnet, P., 2007. Productos alternativos a la crianza en barrica de los vinos. Influencia de los parámetros de fabricación y de uso. Informe técnico. En: Jornadas sobre: crianza en barricas y otras alternativas. Encuentro enológico, Fundación para la Cultura del Vino, Madrid.

Fernández de Simón, B.; Cadahía, E.; Conde, E.; García–Vallejo, M.C., 1996. Low molecular weight phenolic compounds in Spanish oakwoods.*J. Agric. Food Chem*. 44: 1507–1511.

Fernández de Simón, B.; Cadahía, E.; Conde, E.; García–Vallejo, M.C., 1999. Evolution of phenolics compounds of Spanish oak Wood during natural seasoning. First results. *J. Agric. Food Chem*., 47: 1687–1694.

Fernández de Simón, B.; Sanz, M.; Cadahía, E.; Poveda, P.; Broto, M., 2006. Chemical Characterization of Oak Heartwood from Spanish Forests of *Quercus pirenaica* (Wild.). Ellagitannins, Low Molecular Weight Phenolic, and Volatile Compounds. *J. Agric Food Chem*., 54: 8314–8321.

Esteruelas, E.; Sanz, M.; Muñoz, A.M.; Fernández DE Simón, B.; Cadahía, E., 2008. El grano y la calidad de la madera de roble destinada a la fabricación de barricas. *Viticultura/Enología Profesional*, 114: 17-23.

Esteruelas, E.; Muñoz, A.M.; Sanz, M.; Fernández DE Simón, B.; Cadahía, E., 2009. Reflexiones sobre la madera de roble destinada a la fabricación de productos alternativos. *Enoviticultura*, 1: 1-7.

Martínez, J.; Cadahía, E.; Fernández de Simón, B.; Ojeda, S:; Rubio,P., 2008. Effect of the seassoniing mhetod on the chemical composition of oak heartwood to cooperage. *J. Agric. Food Chem*.,56: 3089–3096.

The Collection of *Saccharomyces* Strains Isolated from the Different Soil-Climatic Zones of Eastern Georgia

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ABSTRACT

The well known regions of viticulture in Georgia, Kakheti and Qartli are characterized by temperate, corresponding to dry continental climate. The multiplicity of soil-climatic zones, rich in fruits and grapevine varieties determines the diversity of fermentative yeasts with different indices of alcoholic fermentation and pesticide resistance. Over 150 yeast strains of the genus *Saccharomyces* were isolated from Kakheti and Qartli regions at the stages of blossoming, early ripeness, and complete ripeness.

In 50 strains, the intensity of alcoholic fermentation exceeded 12%. The yeast screening has shown that the doses of three different widespread pesticides introduced into the vineyard have various impacts on the yeast growth. The highest toxicity is characteristic for Sakozeb M-45. Only 5% of tested yeasts grew intensively on Sakozeb M-45, 13% – on Cihom Blue and 40% – on Neoram. Some strains resistant to all three pesticides were also revealed.

RESÜMEE

Georgiens beste Traubenregionen von Kachetien und Kartli zeichnen sich durch das gemäßigte, trockene und kontinentale Klima aus. Die Vielfalt von Boden-Klima-Zonen einerseits sowie Obst- und Weintraubensorten andererseits ist gute voraussetzung für großes Spektrum von Gärungshefen mit unterschiedlichen Gärungsparametern und Stabilität gegen die Pestiziden. Von uns wurde in Kachetien und Kartli in der Blute-, Halbreife- und Vollreifephasen der Weintrauben mehr als 150 verschiedene Stammen von *Saccharomyces*-Familien isoliert. Davon übertraf in 50 Stammen die Intensität von alkoholischer Gärung die 12%-Marke. Die Untersuchungen haben gezeigt, dass die Wirkung von den in Weinberg einzusetzenden Pestiziden auf das Wachstum von Hefen ist unterschiedlich. Mit höchster Toxizität lässt sich Sakozeb M-45 charakterisieren. Intensives Wachstum wurde nur in 5%

von untersuchten Hefen auf Sakozeb M-45 festgestellt, auf Cihom-blue 13% und auf Neoram 40%.

INTRODUCTION

Viticulture and winemaking are among the chief and traditional fields of Georgian economics descending from the ancient ages (Javakhishvili, 1986; Ketskhoveli *et al*., 1960). The well-known regions of viticulture in Georgia Kakheti and Qartli are characterized by temperate corresponding to continental dry climate. Cinnamonic forest, raw humus calcareous, alluvial calcareous, alluvial forest calcareous, chestnut, and black soils are found in above mentioned viticulture regions. The multiplicity of soil-climatic zones and grapevine varieties determine the diversity of fermentative yeasts, which are characterized by specific physiological properties and high metabolic potential (Kirtadze, Kurdadze, 1992). Southern Caucasus is considered as homeland of yeasts (Jackson, 2000).

Unique endemic varieties of grapevine together with national technologies of winemaking perform Georgia a unique wine producing country. Besides, while producing of ecologically clean wine, vine diseases revealing at different stages of vegetation should be taken into consideration. During the entire period of vegetation, vine culture is treated several times by different pesticides. Part of the pesticides penetrates into grape juice, and as a result of fermentation appears in wine material, essentially influencing its quality. Thus, nowadays production of ecologically clean wine remains one of the most important problems of modern wine industry (Cabras *et al*., 1999; 2001).

In natural populations of yeasts indigenous strains adapted to pesticides and heavy metals are revealed (Kadagishvili *et al.*, 2006; Machavariani *et al.*, 2006). Presumably, exactly such strains are characterized by oxidative degradation of pesticides. Alcoholic fermentation, conducted by applying of their metabolic potential is a way to obtain ecologically clean products.

The goal of the present work is isolation of yeast strains belonging to the genus *Saccharomyces* at various stages of vine vegetation from the regions of Eastern Georgia (Kakheti, Qartli) and selection of yeasts capable of high alcoholic fermentation and pesticide detoxification potential among them.

MATERIALS AND METHODS

The samples were taken from phylosphere and rizosphere of different cultivars (cv) of *Vitis vinifera*: cv Rkatsiteli, cv Manavis mtsvane, cv Aladasturi, cv Saperavi, cv Goruli mtsvane, cv Gorula.

The sampling was conducted at the stages of blossoming, early ripeness and complete ripeness and from the sediments of wine produced in peasant farms.

In order to isolate yeasts from samples, the methods of limited dilution and enrichment culture were used. In addition, samples of phylosphere were directly placed on Petri dishes on the surface of solid nutrient medium. The perfect medium for yeasts, g/l – KH_2PO_4 – 2; $MgSO_4$× $7H_2O$ – 1; $(NH_4)_2SO_4$ – 1; glucose – 20; yeast extract – 2, (agar – 20), pH 6.5 (Zakharov *et al*.,, 1984) was used both in solid and liquid forms to isolate yeasts. The incubation of yeasts on solid medium was performed at 25°C in thermostat for 2-3 days. The incubation of yeasts in liquid medium was conducted in 750 ml flasks at rotary shaker (180 revolutions per minute) at 25°C for 3 days. After multiple purification and plating, pure

cultures of yeasts were obtained. The growth intensity of yeast strains in liquid medium was measured by spectrophotometer at wavelength of 660 nm.

In order to determine the intensity of alcoholic fermentation, the yeast biomass was placed into the medium of the same composition under anaerobic conditions; the amount of glucose was equal to 23%. The fermentation was conducted at 25% in thermostat for two weeks. To estimate the concentration of alcohol accumulated during fermentation, the amount of residual glucose was determined (Dahlgvist, 1961). The concentration of ethyl alcohol was established in terms of the amount of glucose applied by yeasts (K=0.58). Ethyl alcohol was determined at HPLC by distillation method– MA-E-AS312-01-TALVOL.

The pesticides: Sakozeb M-45R (reactant – Mankoceb 800 g/kg), Neoram (reactant – copper chloroxide 377 g/kg), Cihom Blue (reactant – Cineb 340 g/kg and copper oxicloride – 170 g/kg) sanctioned by the Ministry of Agriculture of Goergia were used for research. The amount of pesticieds introduced into the media was determined by the standards of their charges. Particularly, Sakozeb M-45 – 3,5 g/l, Neoram – 4 g/l, Cihom Blue – 5 g/l. The cultivation of yeasts was conducted on solid nutrient media containing one of the above mentioned pestisides at 25°C in thermostat for a week. The yeast growth intensity was estimated visually by 4-point system: +++ – intensive growth; ++ – limited growth; + – supressed growth; - – growth inhibition.

RESULTS AND DISCISSION

From phylosphere and rizosphere of different cultivars (cv) of *Vitis vinifera* (cv Rkatsiteli, cv Manavis mtsvane, cv Aladasturi, cv Saperavi, cv Goruli mtsvane, cv Gorula) spread in the regions of Eastern Georgia (Qartli and Kakheti) have been isolated the following pure yeast cultures:

- 9 pure yeast cultures at blooming stage
- 70 pure yeast cultures at the stage of early ripeness
- 67 pure yeast cultures at the stage of complete ripeness
- 37 pure cultures from the sediments of wine produced in peasant farms (Fig. 1).

Fig. 1. Pure yeast cultures isolated from wine sediments and different stages of vine vegetation: a – blooming, b –early ripeness, c – complete ripeness

At the stages of early ripeness and complete ripeness, the yeasts were mainly isolated from phylosphere; the amount of isolated yeasts is in correlation with concentration of sugar in grapes.

The screening to determine pesticide (Sakozeb M-45, Neoram, Cihom Blue) detoxification potential of yeasts freshly isolated at the different stages of vine vegetation has shown that the influence of required treating dosage on yeast growth and development is different. The

highest toxicity is characteristic to Sakozeb M-45. Only 45 strains of tested yeasts were grown in Sakozeb M-45-containing medium. Among them 7 strains exposed intensive growth. In case of Neoram, 114 strains grew; among them 58 strains exposed intensive growth. 80 strains grew at Cihom Blue-containing medium; 19 strains revealed intensive growth, i.d. only 5% of 146 strains isolated at different stages of vine vegetation are characterized by intensive growth on Sakozeb M-45, 13% – on Cihom Blue and 40% – on Neoram (Fig. 2).

16 strains out of 37 ones isolated from wine sediment grew at Sakozeb M-45-containing medium. Among them 2 strains displayed intensive growth. In case of Neoram, 26 strains grew; 6 strains revealed intensive growth. 24 strains grew on Cihom Blue-containing medium. Among them 3 strains displayed intensive growth.

Fig. 2. Different growth intensity of yeast strains on the media containing various pesticides: a – Sakozeb M-45, b–Neoram, c– Cihom Blue

The yeast cultures while growing on Neoram-containing medium decolorize the nutrient medium. In some cases, blue color of the nutrient medium caused by the pesticide proceeds to yeast biomass. Presumably, the ions stipulating color of the nutrient medium penetrate to biomass and color it (Fig.3).

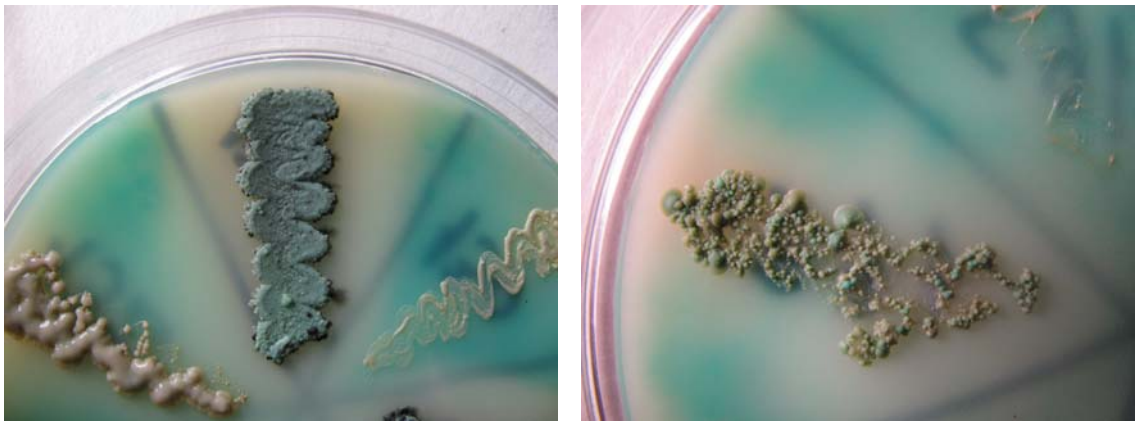

Fig. 3. Color change of Neoram-containing medium caused by yeast strains

Sakozeb M-45 and Cihom Blue influence on form and color of yeast colony. In some cases, the smooth surface of yeast colonies transform into rugose one (Fig. 4: a, b); sometimes so-called secondary growth takes place (Fig. 4: c).

Fig.4. The impact of Sakozeb M-45 (a) and Cihom Blue (b, c) on the form of yeast colonies

The screening of freshly isolated strains at different stages of vine vegetation resistant to the pesticides was conducted according to high fermenting potential.

The fermentation results for strains isolated at blooming stage show that none of them displayed high intensity of fermentation and accumulated amount of alcohol was equal to 2-4%.

The screening of yeasts isolated at the stages of early ripeness and complete ripeness to reveal high capability of alcohol fermentation showed that the intensity of alcohol fermentation ranged in 7-13%. The fermentation process continued for 16 days. During the first four days, the process was conducted at 30Cº that was unfavorable for fermentation process. As the temperature decreased to 25Cº, the fermentation process was intensified and the amount of accumulated alcohol for 12 days exceeded 9% in strains under the following conditional numbers: 91, 99, and 112. In all tested strains the fermentation process actually completed and alcohol concentration insignificantly changed further (Fig. 5). Some strains under conditional numbers - 91, 112, and 143 are shown in Fig.5.

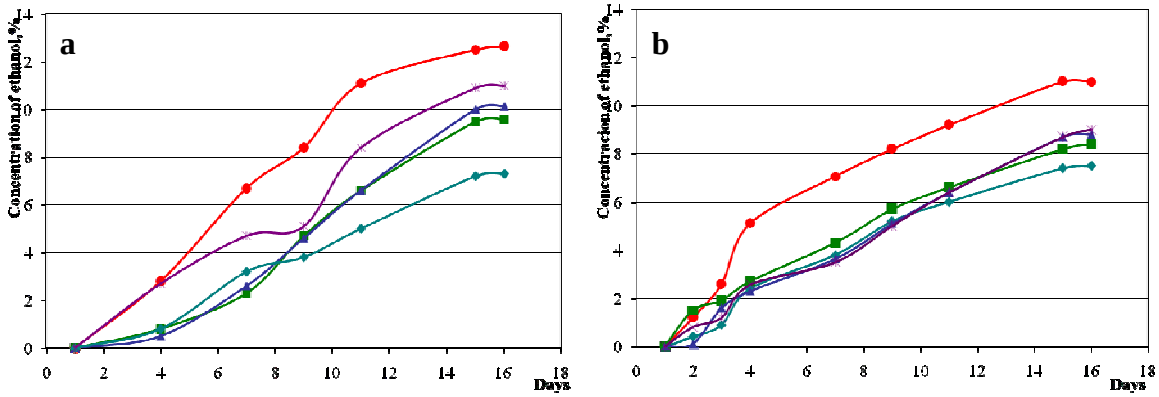

Fig. 5. The intensity of alcohol fermentation of yeasts isolated at the stages of early ripeness (a) and complete ripeness (b): a - -●- 91; -■- 95; -▲- 99; -◆- 103; -×- 112; b - -●- 143; -■- 171; -▲- 188; -◆- 141; -×- 237

(The conditional numbers belong to the yeast strains isolated from the different cultivars of Eastern Georgia: 91 – cv Saperavi, village Anaga, Sighnaghi district, 95 – cv Rkatsiteli, village Vaqiri, Sighnaghi district, 99 – cv Rkatsiteli, village Kardenakhi, Gurjaani district, 103 – village Manavi, cv Rkatsiteli, Sagarejo district, 112 – village Manavi, cv Saperavi, Sagarejo district, 141 – cv Rkatsiteli, village Tibaani, Sighnaghi district; 171 – cv Rkatsiteli, village Chandari, Gurjaani district; 143 – cv Aladasturi, village Tibaani, Sighnaghi district; 188 – cv Saperavi, village Nikozi, Gori district; 237 – cv Saperavi, village Qvemo Khodasheni, Telavi district).

It was found that 50 strains among 183 revealed intensive alcoholic fermentation: the concentration of formed ethanol exceeded 12%.

The intensity of alcohol fermentation was especially high in the yeasts isolated from the sediments of wine produced in peasant farms. In most strains, the concentration of alcohol formed by yeasts exceeded 12%; it could be explained by screening of strains tolerant to high alcohol concentrations that naturally takes place in wine.

On the base of the experiments, alcohol fermentation was more intensive in the yeasts isolated from Kakheti region in comparison with that of isolated from Qartli region; it must be the result of natural selection process. Grapes of vine cultivars from Qartli region are characterized by less sugary compared with those of Kakheti region that is clearly reflected on the yield of ethyl alcohol.

CONCLUSIONS

As a result of conducted works it might be concluded:

- The yeast collection including 146 strains isolated from Kakheti and Qartli regions at the stages of blooming, early ripeness and complete ripeness and 37 strains isolated from wine sediments has been created.
- Only 5% of strains out of 146 isolated at different stages of vine vegetation are characterized by intensive growth on Sakozeb M-45, 13% – on Cihom Blue and 40% – on Noeram. 6% of strains out of 37 isolated from wine sediments are characterized by intensive growth on Sakozeb M-45, 7% – on Cihom Blue and 16% – on Neoram.
- In the yeasts isolated from Kakheti region, alcoholic fermentation is more intensive in comparison with that of isolated from Qartli region; it must be the result of natural selection process.
- Alcohol fermentation is more intensive in the yeasts isolated from wine sediments, where take place the naturally screening of strains tolerant to high alcohol concentrations.

ACKNOWLIDGMENTS

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BIBLIOGRAPHY

Cabras P., Angioni A., Garau V., Pirisi F., Farris G., Madau G., Emonti G. 1999. Pesticides in fermentative processes of wine. *J. Agric Food Chem.* 47, 9:3854-386.

Cabras P., Angioni A., Garau V., Pirisi F., Cabitza F., Pala M., Farris G. 2001. Fenhexamid residues in grapes and wine. *Food Additives & Contaminants*: Part A, 18, 7:625-629.

Dahlgvist A. Determination of maltase and isomaltase activities with a glucose-oxidase reagent. *Biochem. J.*, 1961, 80, p. 547-551.

Jackson. R. 2000. Wine Science: Principles, Practice, Perception. Academic Press. Elsevier.

Javakhishvili I. 1986. The History of Georgian Economics. v. II, In: Works in 12 volumes. Metsniereba, Tbilisi, Ed. D. Chkhikvishvili.

Ketskhoveli N. Ramishvili M., Tabidze D. 1960. Georgian Ampelography. Georgian

Academy of Sciences, Tbilisi.
Kirtadze E., Kurdovanidze T. 1992. Biochemical Features of the Secondary Alcohol Fermentation. Metsniereba, Tbilisi. Ed. G. Kvesitadze.
Machavariani N., Shatirishvili A., Jorbenadze Ts. 2006. Strains resistant to heavy metal ions in the natural populations of wine yeast. *Proc. Georgian Acad. Sci., Biol. ser. B*. 4, 4:74-78.
Kadagishvili E., Sadagishvili T., Menabde M., Zarnadze T., Shatirishvili A. 2006. Study of genetic alterations induced by the pesticide kurzat in artificial and natural populations of yeast. *Proc.of the Georgian Acad. Sci., Biol. ser. B*. 4, 3:77-80.
Zakharov I., Kozhin S., Kozhina T., Fedorova I. 1984. Methods in yeast *Saccharomyces cerevisiae* genetics. Nauka, Leningrad. Ed. M. Meisel.

XXXIIIème CONGRÈS MONDIAL DE LA VIGNE ET DU VIN
8ème ASSEMBLÉE GÉNÉRALE DE L'O.I.V.
Sous-thème 2-B (Oenologie)
Méthodes d'analyse : Innovations et perspectives

Instabilité protéique : 1- Évaluation et gestion des risques de casse protéique. 2 - Détection spécifique par électrophorèse & par un nouveau test : l'ImmunoTest $^{\pi}$®.

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RÉSUMÉ

Les protéines présentes dans les vins proviennent majoritairement des raisins. La casse protéique est un trouble qui se produit parfois en bouteille lorsque le vin blanc ou rosé contient ces protéines. Il existe de nombreux tests permettant d'évaluer la stabilité protéique. Mais ils ne sont pas satisfaisants comme l'indique leur nombre. Pour évaluer les risques de casse protéique, nous avons étudié ce phénomène. Grâce à l'électrophorèse SDS-PAGE et l'Immunologie, nous avons pu déterminer les protéines impliquées dans la casse protéique naturelle des vins.

Grâce à des anticorps spécifiques dirigés contre ces protéines (Chitinase et Thaumatin-like), nous avons réalisé un test basé sur l'Immunologie et la technique du dot-blot. Nous espérons que ce test nommé ImmunoTest $^{\pi}$® devienne l'outil de référence parce que spécifique et fiable pour déterminer l'instabilité protéique des vins blancs et rosés.

ABSTRACT

The proteins present in wines mainly come from grapes. The protein haze is a disorder that sometimes occurs when the bottled white wine or rosé contains these proteins. There are many tests to check the protein instability. But they are not satisfactory as indicated by their large number. To assess the risk of proteic haze, we have studied this phenomenon. Thanks to SDS-PAGE and Immunology, we have been able to determine the proteins involved in the case of natural protein wines.

With specific antibodies against these proteins (Chitinase and Thaumatin-like) we have realized a test based on the Immunology and dot-blot technique. We hope that this test called ImmunoTest $^{\pi}$® will become the reference tool due to its specificity and reliability to determine the protein instability of white wines and rosés.

INTRODUCTION

Le trouble ou casse protéique est un problème qui peut se produire dans toutes les boissons à base de fruits (pomme, poire, banane, orange, etc.) et dans les boissons fermentées (bière, cidre, vin) (Manteau et Poinsaut, 2010a).

Dans les vins, les protéines présentes proviennent majoritairement des raisins et sont des protéines de défense (Waters *et al*., 1996 ; Ferreira *et al*., 2002).

La casse protéique est un trouble qui se produit parfois en bouteille lorsque le vin blanc ou rosé contient ces protéines (Manteau et Poinsaut, 2010a). Les années, cépages, et les process de vinification semblent avoir une incidence sur les concentrations en protéines présentes dans les vins et le risque de casse protéique. Afin d'éviter ceci, il existe de nombreux tests permettant d'évaluer la stabilité protéique des vins blancs et rosés. Cependant, ceux-ci ne sont pas spécifiques des protéines instables. Dans les vins, Pocock *et al*. (2006) ont dénombré par moins de 17 tests différents dont 14 sont basés sur le chauffage du vin. Ces tests donnent des résultats qui ne sont pas cohérents les uns avec les autres (Esteruelas *et al*., 2009). L'interprétation de leurs résultats conduit souvent à un mauvais dosage de bentonite.

Notre travail sur la comparaison de 5 tests de stabilité protéiques - couramment utilisés en œnologie (chaleur, chaleur + tanin, Bentotest®, Prostab, acide trichloroacétique) montre que ces tests ne sont pas fiables (Manteau *et al*., 2006 et 2010b). Les résultats de ces tests font que l'occurrence d'une casse protéique en bouteille est sans nul doute le risque le plus mal évalué. La concentration et la qualité des protéines, les tanins et le pH seront également détaillés ici vis-à-vis du risque de casse protéique.

Afin de mieux évaluer les risques de casse protéique, nous avons étudié ce phénomène. Grâce à l'électrophorèse SDS-PAGE (technique très fiable pour séparer et mettre en évidence les protéines) et l'Immunologie. Nous avons pu ainsi déterminer les protéines impliquées dans la casse protéique naturelle des vins.

Grâce à des anticorps spécifiques dirigés contre ces protéines (Chitinase et Thaumatin-like), nous avons réalisé un test basé sur l'Immunologie et la technique du dot-blot : l' ImmunoTest$^{\pi}$®.

MATÉRIELS ET MÉTHODES

1-Vinothèque

Nous avons réalisé une vinothèque contenant plus de 200 vins (au moins 12 cépages de pays différents), stockés dans différentes conditions (température ambiante et 4°C) et bouchés avec des bouchons lièges de différentes qualités et des bouchons synthétiques.

2- ImmunoTest $^{\pi}$®

Basé sur la technique du dot-blot, nous avons développé ce test avec la société Bio-Rad. En accord avec les recommandations de Bio-Rad, 5 µL des différents vins + un vin Témoin positif (vin très instable) et d'un Témoin négatif (vin stable) sont déposés sur la bandelette de nitrocellulose. Après séchage, la bandelette est saturée, mise à réagir avec les anticorps primaires, lavée, mise à réagir avec les anticorps secondaires, lavée à nouveau, puis révélée en accord avec les recommandations du fabricant.

3-Le matériel et les autres méthodes utilisées pour cet article sont décrits dans les différentes publications de ces auteurs : Manteau *et al*., 2003, 2006 et 2007 ; Manteau et Poinsaut, 2010a et 2010b.

RESULTATS ET DISCUSSION

La casse protéique est un précipité amorphe et translucide lorsqu'on l'observe en microscopie optique (Manteau et Poinsaut, 2010a). Dans les vins contenants des protéines, la température et le bouchon ont une grande importance comme nous pouvons le voir sur la Figure 1. Comme nous pouvons le voir sur la Figure 1A, le froid (4°C) freine l'apparition du trouble. Tout comme la température, le bouchon est également sous-estimé dans la casse protéique (Figure 1B). Un bouchon de liège est totalement imbibé par le vin au bout de quelques mois comme l'indiquent González-Adrados *et al*. (2008). La présence d'une forte concentration en tanin dans le bouchon entraîne toujours la précipitation des protéines du vin à son contact, comme sur cette photo (Figure 1B).

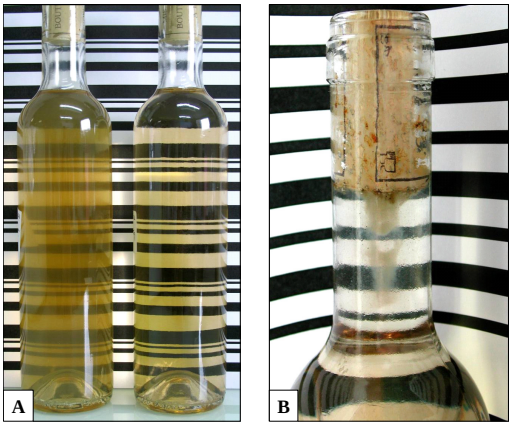

Figure 1 : Photos de casses protéiques en bouteille. (A) : Importance de la température. Ces deux bouteilles contiennent le même vin. A gauche, la bouteille est stockée à 20°C et à droite, l'autre bouteille est stockée à 4°C. (B) : Importance du bouchon. Le vin blanc est riche en protéines et celles-ci précipitent au contact des tanins relargués par le bouchon en liège.

Le rôle majeur des protéines et des tanins dans la casse protéique peut être très facilement observé, comme nous l'avons fait ici avec un vin de Sauvignon riche en protéines (environ 500mg/L), une solution de bentonite et une solution de tanin de pépins (Figure 2). La casse qui se produit dans ces conditions après 24H à température ambiante n'est pas différente de la casse protéique naturelle et spontanée observée en bouteille (données non montrées). Plus un vin riche en protéine est traité avec une concentration importante en bentonite et moins il se trouble (Figure 2A). De façon similaire, plus on ajoute de tanins dans un vin riche en protéine et plus le trouble sera important. Ceci démontre donc que tanins et protéines sont d'une importance cruciale dans ce phénomène.

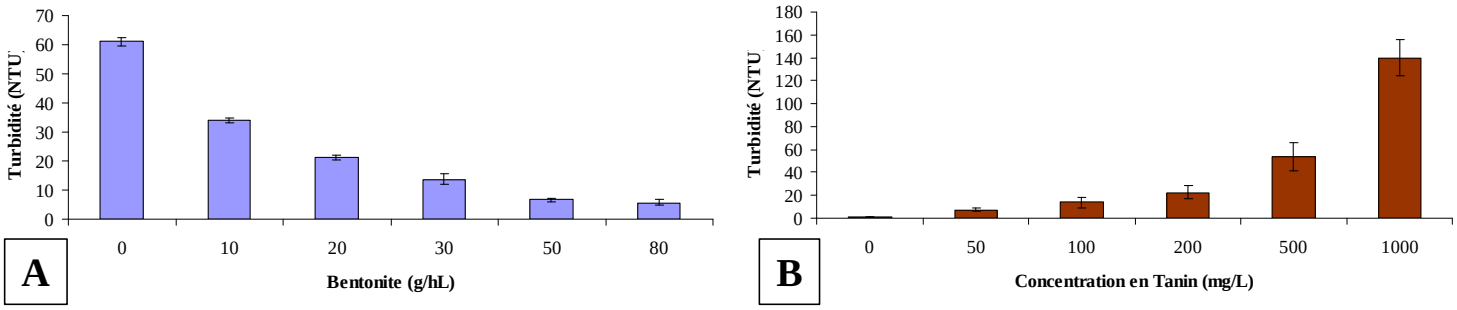

Figure 2 : Mesure de la turbidité d'un vin instable. (A) en fonction de sa richesse en protéine ; (B) en fonction de sa richesse en tanins. (Les barres d'erreurs correspondent à l'Ecart type.)

Les tests qui sont classiquement utilisés en Œnologie pour la détermination de l'instabilité protéique ne sont ni fiables ni spécifiques comme nous l'avons montré (Manteau et Poinsaut, 2010b). Afin de caractériser les protéines impliquées dans ce phénomène, nous avons utilisés l'électrophorèse SDS-PAGE. Cette technique est très utilisée dans les laboratoires de recherche en Biochimie car très fiable. Ceci permet de mettre en évidence et de séparer les protéines en fonction de leurs masses molaires. Nous avons pu ainsi caractériser les protéines instables impliquées dans la casse protéique (Manteau *et al*., 2003 ; 2006 et 2007). Deux protéines de défense du grain de raisin, représentant 50 à 90% des protéines du vin, pouvaient être considérées comme des protéines « marqueurs » : Chitinase et Thaumatin-like. Comme nous pouvons le voir sur cette Figure 3, ces deux protéines représentent une forte proportion des protéines du vin et de la casse protéique naturelle.

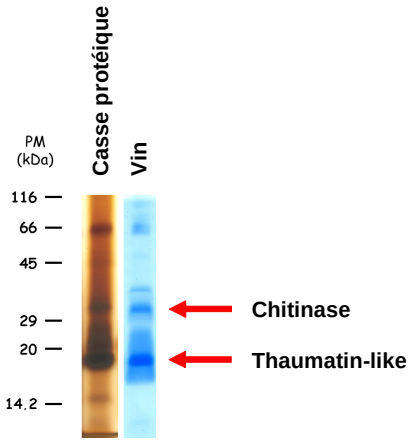

Figure 3 : Electrophorèse SDS-PAGE d'une casse protéique naturelle (gauche) et d'un vin très riche en protéines (droite).

Ces deux protéines ciblées, nous avons donc réalisé des anticorps spécifiques puis un test Immunologique sur la technique du Dot-blot. Cette technique est également une technique très fiable et robuste pour séparer et mettre en évidence les protéines dans les laboratoires de recherche. Nous avons ainsi validé la technique pour mettre en évidence spécifiquement les protéines instables dans les vins (données non montrées). Grâce à la société Bio-Rad, ce test a pu être optimisé et développé à grande échelle. Vous pouvez voir sur la Figure 4, le protocole de ce test nommé : ImmunoTest $^{\pi}$®. La simple observation visuelle de spots sur une bandelette permet d'indiquer si un vin est stable ou non. Un témoin positif riche en protéine et instable (produisant un spot très coloré) et un témoin négatif stable (absence de spot) servent de contrôles internes à l'expérimentation. Les vins instables peuvent être traitées à l'aide d'une solution de bentonite qui peut être fournie pour que les laboratoires puissent réaliser une gamme plus facilement. Le vin ainsi traité peut être déposé de manière similaire et une dose optimale et raisonnée de bentonite peut être ainsi définie (données non montrées).

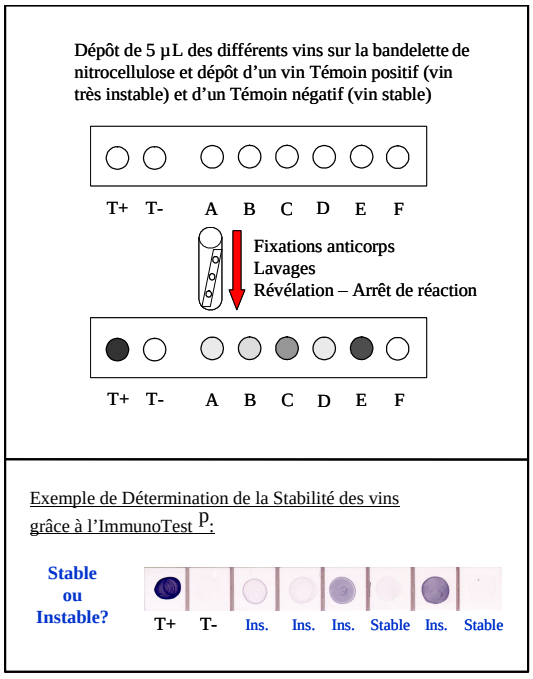

Figure 4 : Protocole de l' ImmunoTest $^{\pi}$® (Dépôts A, B, C, D, E et F = différents vins ; dépôt T+ = Témoin positif et T- = Témoin négatif).

CONCLUSIONS

La richesse en protéines et en tanins d'un vin, sa température de stockage, son bouchage (Figure 1 & 2) mais également son pH (Manteau et Poinsaut, 2010b) sont des points clefs à maîtriser ou bien évaluer si on veut gérer au mieux le risque de casse protéique.

Bien que de nombreux travaux scientifiques ont pour but de trouver une alternative, la bentonite reste le seul traitement efficace pour stabiliser un vin vis à vis des protéines (Manteau et Poinsaut, 2010a). Malheureusement, comme nous l'avons démontré et comme le démontrent un grand nombre de laboratoire d'analyses qui utilisent 2 voire 3 tests différents,

les tests de détermination de la stabilité des vins blancs et rosés ne sont pas fiables. Ils amènent souvent à des mésestimations de la dose de bentonite à apporter au vin. S'il y a un sur-traitement en bentonite, le vin risque de perdre des composés aromatiques et s'il y a sous-traitement, il y a risque de casse protéique.

Comme nous l'indiquent Manteau *et al*., (2007), Chitinase et Thaumatin-like ne sont pas les seules protéines impliquées dans la casse protéique. Mais elles sont majoritaires et représentent souvent plus de 70% des protéines impliquées dans ce phénomène (données non montrées). C'est pourquoi nous considérons qu'elles sont des « marqueurs » et que nous les avons ciblées.

Cette nouvelle méthode de détermination de l'instabilité protéique des vins a fait l'objet d'un dépôt de brevet. De plus, la profession semble reconnaître un fort intérêt pour l'ImmunoTest $^{\pi}$® puisque nous avons obtenus le Trophée d'Argent Vinitech 2008, le Prix à l'Innovation 2009 dans la catégorie « Internationale » VITeff 2009 et très récemment un « Prix spécial Innovation 2010 » au congrès INTERVITIS INTERFRUCTA.

Cette technique est simple, robuste car elle n'est pas sensible aux produits œnologiques et ne nécessite ni que le vin soit filtré, ni de matériel particulier. De plus, l'interprétation en est très facile car elle est visuelle. Grâce à une gamme de bentonite, ce test fiable et spécifique des protéines instables permet de déterminer une dose de bentonite optimale et raisonnée.

Nous espérons que l'ImmunoTest $^{\pi}$® deviendra la méthode de référence pour déterminer l'instabilité protéique des vins blancs et rosés.

REMERCIEMENTS

Les auteurs de ce poster tiennent à remercier les laboratoires d'Œnologie et Stress, Défense et Reproduction des Plantes de la Faculté des Sciences de Reims pour le don des anticorps qui ont permis de débuter ce travail.

BIBLIOGRAPHIE

Esteruelas, M., Poinsaut, P., Sieczkowski, N., Manteau, S., Fort, M.F., Canals, J.M., Zamora, F. (2009) Comparison of methods for estimating protein stability in white wines. *American Journal of Enology and Viticulture* **60**: 302-311.

Ferreira, R.B., Picarra-Pereira, M.A., Monteiro, S., Loureiro, V.B., Teixeira, A.R. (2002) The wine proteins. *Trends in Food Science & Technology* **12**: 230-239.

González-Adrados, J.R., González-Hernández, F., García de Ceca, J.L., Cáceres-Esteban, M.J., García-Vallejo, M.C. (2008) Wine absorption by cork stoppers. *Spanish Journal of Agricultural Research* **6**: 645-649.

Manteau, S., Poinsaut, P. (2010a) Instabilité protéique des vins blancs et rosés. Partie 1/2 : États des lieux des connaissances. *Revue des Œnologues* **134**: 22-24.

Manteau, S., Poinsaut, P. (2010b) Instabilité protéique des vins blancs et rosés. Partie 2/2: Comparaison des tests de stabilité protéique dans les vins blancs et rosés et mise au point d'un nouveau test: l'ImmunoTest $^{\pi}$. *Revue des Œnologues* **135**: 23-27.

Manteau, S., Lambert, B., Jeandet, P., Legendre, L. (2003) Changes in chitinase and thaumatin-like pathogenesis-related proteins of grape berries during the Champagne winemaking process. *American Journal of Enology and Viticulture* **54**: 267-272.

Manteau, S., Poinsaut, P., Scotti, B., Vidal, F., Sieczkowski, N. (2006): Comparative study of methods useful for protein measurement in Enology. In *Macromolecules and Secondary Metabolites of Grape and Wine*. Eds. Jeandet, P., Clement, C., Conreux, A., Intercept Publishers - Lavoisier, Reims, pp. 191-195.

Manteau, S., Poinsaut, P., Sieczkowski, N., Calegari, G., Scotti, B. (2007) De nouvelles protéines impliquées dans la casse protéique "naturelle" des vins blancs et rosés. *Infowine.com, Revue Internet de Viticulture et Oenologie* **13**: 1-4.

Pocock, K.F., Waters, E.J. (2006) Protein haze in bottled white wines: How well do stability tests and bentonite fining trials predict haze formation during storage and transport? *Australian Journal of Grape and Wine Research* **12**: 212-220.

Waters, E.J., Shirley, N.J., Williams, P.J. (1996) Nuisance proteins of wine are grape pathogenesis-related proteins. *Journal of Agricultural and Food Chemistry* **44**: 3-5.

INFLUENCE OF GLUTATHIONE ADDITION ON VOLATILE PROFILE OF TREBBIANO AND BOMBINO BIANCO WINES

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KEYWORDS: volatile compounds, *Trebbiano*, *Bombino Bianco*, glutathione

ABSTRACT

Volatile compounds play an important role in wine quality owing to their contribution to wine sensory properties. However, they undergo to oxidation processes leading to a significant decrease in characteristic aromas and to the formation of new undesirable ones. Glutathione protects must against oxidation, taking part into processes including the prevention of free radicals formation, the detoxification of cells, as well as the inhibition of enzymatic and nonenzymatic mechanisms involved in browning; it traps caftaric acid quinones, and also acts as a general reducing agent.

The objective of this work was to evaluate the effects of glutathione addition during winemaking on the volatile composition of *Trebbiano* and *Bombino Bianco* wines. Volatile fraction has been evaluated by gas-chromatography coupled to mass spectrometry together with the main oenological parameters. Results have shown that the addition of glutathione allowed a better preservation of aroma compounds, likely preventing their oxidation.

RIASSUNTO

I composti volatili svolgono un ruolo importante sulla qualità del vino contribuendo alle caratteristiche organolettiche complessive del prodotto finito. Tuttavia, tali componenti sono sottoposti a processi di ossidazione che portano ad una significativa diminuzione di aromi caratteristici e alla formazione di nuovi composti indesiderati. Il glutatione protegge il mosto dall'ossidazione, partecipando a processi quali la prevenzione della formazione di radicali liberi, la detossificazione delle cellule, così come l'inibizione dei meccanismi enzimatici e non enzimatici coinvolti nel fenomeno dell'imbrunimento; tale composto intrappola i chinoni dell'acido caftarico e agisce come agente riducente.

L'obiettivo di questo studio è stato valutare gli effetti dell'aggiunta di glutatione sulla composizione volatile dei vini *Trebbiano* e *Bombino Bianco*. La frazione volatile dei vini è stata analizzata mediante gas-cromatografia accoppiata alla spettrometria di massa. I risultati hanno mostrato che l'aggiunta di glutatione ha comportato una migliore conservazione dei composti aromatici, prevenendo la loro ossidazione.

INTRODUCTION

Aroma is one of the most important factors determining wine’s character and quality. Consequently, oxidation of white wine by molecular oxygen and the consequent loss of its characteristic aroma are of great concern in winemaking. Glutathione (GSH) is a major

natural component of many plants and foods (Friedman, 1994) with many properties, including preventing the formation of free radicals and detoxifying cells, as well as inhibiting enzymatic and nonenzymatic mechanisms involved in browning processes (Lavigne *et al*., 2007).

The addition of GSH has been successfully reported in combination with oxygenation of musts (Vaimakis, Roussis,1996), of wine under accelerated oxidation (El Hosry *et al*., 2009), in combination with other antioxidants (Papadopoulou, Roussis, 2008), in model wine medium (Roussis, Sergianitis, 2008). Wines produced have shown a significant quality improvement preventing the disappearance of aromatic esters and being lacking in the distinctive oxidation flavor.

On the other hand, literature on the applicability of GSH addition at the beginning of a standard winemaking procedure is scant. Furthermore, the success of some winemaking technology is well known to be greatly affected by grape variety. Therefore, more investigation on different cultivars is desirable.

The objective of this work was to evaluate the effects of glutathione addition during winemaking on the volatile composition of *Trebbiano* and *Bombino Bianco* wines produced in Apulia region. Volatile fractions have been evaluated by gas-chromatography coupled to mass spectrometry, with previous sample preparation.

MATERIAL AND METHODS

Winemaking

Grapes from *Trebbiano* and *Bombino Bianco* vines were harvested at the technological maturation based on optimum juice composition and have been subjected to microvinification. Grapes were crushed, destemmed, separated from the skins by pressing and added with one hundred milligram per litre of potassium metabisulfite. The juice obtained was divided in three 100 L tanks for triplicate fermentation, and inoculated with S. cerevisiae *(Zymasil, AEB),* previously rehydrated according to manufacturer instructions. The following winemaking technologies were tested: traditional and with the use of glutathione (5g/hl, Fermoplus Energy Glu, AEB) added directly to destemmed grapes. Fermentation was carried out at a controlled temperature (18°C). After the end of fermentation (residual sugars <2 g L−1), the wine was racked, cold-settled for 4 weeks at 5 ◦C, racked again and bottled. Samples were stored at 14 ◦C until analysis.

Preparation of volatile extracts and GC–MS analysis

Volatile compounds of wines were recovered by solid phase extraction (SPE), according to the method proposed by Pineiro *et al*., 2004. Separation and quantification were performed on an Agilent Technologies 6890N gas chromatograph equipped with a DB-WAX fused silica capillary column (60 m*0.25mm*0.25µm) and a 5975 mass spectrometer detector (GC-MS). Separation conditions were as follow: injector temperature 250°C; GC column temperature 40°C (3 min) at 3°C min-1 to a final temperature of 200°C (20 min); carrier gas He at 1 mL min-1; volume of injection 1 µL in splittless mode.

The identification of the volatile compounds was achieved by comparing mass spectra with those of the data system library (NIST 02, P>90%) and retention indexes with published data. Concentration of each volatile compound is expressed as mg internal standard equivalents L^{-1} wine, obtained by normalizing the compound peak area to that of the internal standard and multiplying by concentration of the internal standard (1.2 mg L^{-1}).

Oneway analysis of variance (ANOVA) using the Duncan test at level of significance $p<0.05$ was used for statistical analysis (Statsoft 6.0).

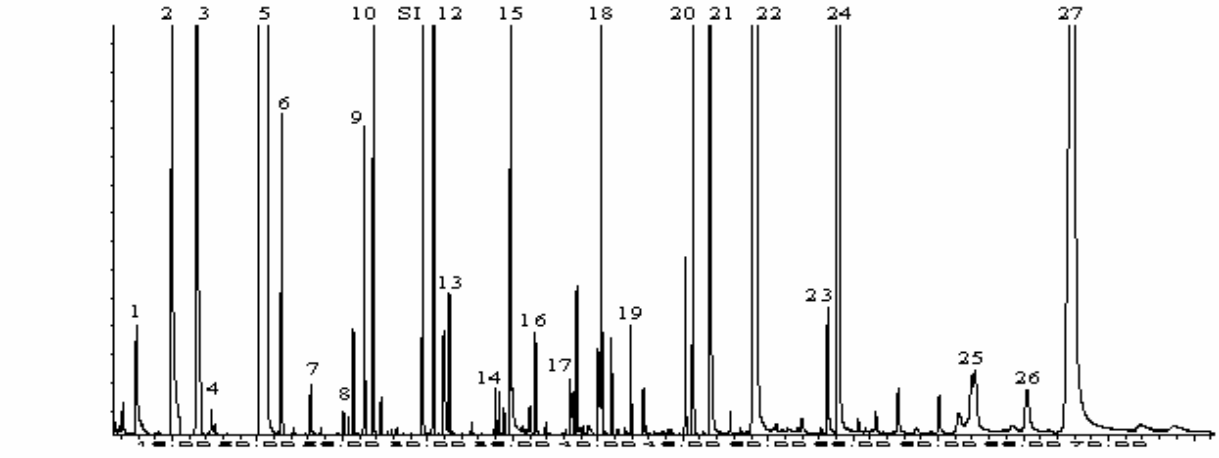
RESULTS AND DISCUSSION

The winemaking technology using GSH addition for *Trebbiano* and *Bombino Bianco* white wines has been evaluated with respect to the traditional one as regards the main oenological parameters, and volatile profile. The results obtained are presented and discussed in the following. Tab. 1 shows the main oenological parameters related to *Trebbiano* control wine (TCW), GSH-added *Trebbiano* wine (GTW), *Bombino Bianco* control wine (BCW) and GSH-added *Bombino Bianco* wine (GBW). Both GTW and GBW showed a higher content of total phenols and lower values of absorbance at 420 nm suggesting that the added GSH preserved wine from oxidation of phenols and slowed down browning processes. All the other parameters did not change significantly.

Tab. 1 Main oenological parameters of Trebbiano control wine (TCW), GSH-added *Trebbiano* wine (GTW), *Bombino Bianco* control wine (BCW), and GSH-added *Bombino Bianco* wine (GBW)

Oenological parameters	TCW	GTW	BCW	GBW
Total Phenols (mg/L)	156a ± 12	193b ± 9	164a ± 8	205b ± 10
Absorbance at 420 nm	0.112b ± 0.003	0.095a ± 0.002	0.170d ± 0.002	0.121c ± 0.003
Ethanol % (v/v)	11.06a ± 0.12	11.1a ± 0.2	11.23a ± 0.10	11.27a ± 0.10
pH	3.30a ± 0.11	3.35a ± 0.12	3.14a ± 0.06	3.16a ± 0.08
Total acidity (g tartaric acid L)	5.0a ± 0.2	4.6a ± 0.2	5.88b ± 0.11	5.64b ± 0.10
Volatile acidity (g acetic acid L)	0.16a ± 0.03	0.16a ± 0.05	0.156a ± 0.013	0.16a ± 0.02
Total dry extract (g/L)	16.6a ± 0.3	16.46a ± 0.19	17.72a ± 0.09	16.9a ± 0.5

Within aroma compounds, esters, giving to wines fruity and floreal notes, and terpenes, giving fragrance to several grape varieties and wines, are of particular significance. Terpens were not found in *Trebbiano* and *Bombino* wines, irrespectively of the winemaking technology used, probably being at very low concentrations.



I.S.:internal standard, 1: 1propanol;2: 2-methyl-propanol; 3:isoamylacetate; 4: 1-butanol; 5: isoamyl alcohols; 6: hexanoic acid, ethyl-ester; 7: acetic acid, hexyl ester; 8: 1-pentanol, 4-methyl; 9: ethyl lactate; 10: hexanol; 11: t-2-hexenol; 12: octanoic acid, ethyl ester; 13: 1-heptanol; 14: benzaldehyde; 15: 2,3 butandiol R,R; 16: 2,3 butandiol R,S; 17: decanoic acid, ethyl ester; 18: diethyl succinate; 19: methionol; 20: acetic acid, 2-phenylethyl ester; 21: Hexanoic acid; 22: phenylethanol; 23: diethylmalate; 24:octanoic acid; 25: decanoic acid; 26: etil 9-deconoate; 27: ethyl hydrogen succinate

Fig.1 Gas-chromatogram of GSH-added *Trebbiano* wine, volatile fraction

As regards esters compounds, which are produced by yeast metabolism during alcoholic fermentation, their determination in TCW, GTW, BCW and GBW is reported in Tab. 2.
Ethyl butanoate, hexanoic acid ethyl ester, octanoic acid ethyl ester, ethyl 3-hydroxybutyrate, diethyl succinate and decanoic acid ethyl ester have been observed in GTW at higher concentration than in TCW. In contrast, the addition of glutathione on *Bombino Bianco* grapes does not seem to affect volatile esters composition of the wine.
Therefore, results showed that some esters compounds were protected from oxidative degradation by means of GSH addition, in *Trebbiano* winemaking.

Tab. 2 Esters compounds determined in Trebbiano control wine (TCW), GSH-added *Trebbiano* wine (GTW), *Bombino Bianco* control wine (BCW), and GSH-added *Bombino Bianco* wine (GBW). *Data, means of 3 replicates, are expressed as mg internal standard equivalents L^{-1} wine ± standard deviation; nd, not detected.

COMPOUNDS	RETENTION TIME	TREBBIANO		BOMBINO BIANCO	
		TCW (mg/L)*	GTW (mg/L)*	BCW (mg/L)*	GBW (mg/L)*
Esters					
ethyl butanoate	12.61	0.217a ± 0.008	0.25b ± 0.01	0.28a ± 0.07	0.21a ± 0.02
1-butanol 3-methyl acetate	16.58	3.07a ± 0.03	2.88a ± 0.04	2.19a ± 0.25	1.74a ± 0.05
hexanoic acid ethyl ester	22.25	0.716a ± 0.006	0.77b ± 0.01	0.51a ± 0.01	0.55a ± 0.02
acetic acid hexyl ester	24.29	0.114b ± 0.006	0.104a ± 0.004	0.071a ± 0.003	0.072a ± 0.005
ethyl lactate	26.27	0.33a ± 0.004	0.32a ± 0.01	0.53a ± 0.08	0.47a ± 0.01
octanoic acid ethyl ester	32.81	1.03a ± 0.04	1.11b ± 0.02	0.82a ± 0.07	0.77a ± 0.03
ethyl 3-hydroxybutyrate	37.02	0.076a ± 0.002	0.092b ± 0.002	0.09a ± 0.01	0.083a ± 0.002
decanoic acid ethyl ester	42.74	0.422a ± 0.01	0.503b ± 0.005	0.45a ± 0.05	0.47a ± 0.05
etil 9-decenoate	40.81	0.068b ± 0.008	0.042a ± 0.002	0.17b ± 0.02	0.068a ± 0.002
diethyl succinate	44.73	0.378a ± 0.002	0.390b ± 0.02	0.56b ± 0.06	0.27a ± 0.01
acetic acid 2-phenylethyl ester	50.91	0.35a ± 0.02	0.34a ± 0.01	0.31a ± 0.01	0.32a ± 0.01
diethylmalate	60.06	0.13a ± 0.02	0.13a ± 0.01	0.11a ± 0.02	0.18a ± 0.11
ethyl hydrogen succinate	72.32	6.10b ± 0.09	5.52a ± 0.15	5.68a ± 0.93	4.34a ± 0.17

CONCLUSIONS

Results have shown that the addition of glutathione before the standard winemaking procedure is a promising tool for the preservation of phenols of *Trebbiano* and *Bombino Bianco* wines. In particular *Trebbiano* showed also an increase in some esters compounds contributing to floreal and fruity notes. Further investigation should be done as regards sensory properties of these wines.

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BIBLIOGRAPHY

Di Stefano R. 1991. Proposal for a method of sample preparation for the determination of free and glycoside terpenes of grapes and wines. *Bulletin de l'ÓIV*, 721: 219-223.

El Hosry L., Auezova L., Sakr A., Hajj-Moussa E. 2009.Browing susceptibility of white wine and antioxidant effect of glutathione. *International Journal of Food Science and Technology*, 44: 2459–2463.

Friedman M. 1994. Improvement in the safety of foods by SH-containing amino acids and peptides. *Journal of Agriculture and food chemistry*, 42: 3-20.

Lavigne V., Pons A., Dubourdieu D. 2007. Assay of glutathione in must and wines using capillary electrophoresis and laser-induced fluorescence detection. Changes in concentration in dry white wines during alcoholic fermentation and aging. *Journal of Chromatography A,* 1139: 130–135.

Papadopoulou D., Roussis I.G. 2008. Inhibition of the decrease of volatile esters and terpenes during storage of a white wine and a model wine medium by glutathione and N-acetylcysteine. *International Journal of Food Science and Technology*, 43:1053–1057.

Pineiro Z., Palma M., Barroso C.G. 2004. Determination of terpenoids in wines by solid phase extraction and gas chromatography. *Analytica Chimica Acta*, 513: 209-214.

Roussis I.G., Sergianitis S. 2008. Protection of some aroma volatiles in a model wine medium by sulphur dioxide and mixtures of glutathione with caffeic acid or gallic acid. *Flavour and Fragrance Journal,* 23: 35–39.

Vaimakis V., Roussis I.G. 1996. Must oxygenation together with glutathione addition in the oxidation of white wine. *Food Chemistry*, Vol.57, 3: 419-422 .

PRE-FERMENTATIVE COLD MACERATION FOR THE AROMA ENHANCEMENT OF AGLIANICO AND MONTEPULCIANO WINES

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KEYWORDS: Aroma compounds, dry ice, pre-fermentative cold maceration

ABSTRACT

Wine aroma is the result of several chemical and enzymatic reactions, together with odour-active compounds directly deriving from the grape. In non-aromatic grape varieties, the varietal characters of wines originate mainly from the transformation of odourless precursors, such as glycosidically bound volatile compounds, into odour-active compounds during winemaking. Therefore, research on enological techniques for the improvement of wine quality by increasing its varietal potential is of great interest.

Cold maceration of grapes allows the production of typical wines, that are characterised by an aromatic profile strongly connected to the zone of production and to variety. The effects of pre-fermentative cold maceration technique on the aroma of *Aglianico* and *Montepulciano* were addressed in this work. The volatile composition of wines have been evaluated by gas-chromatography coupled to mass spectrometry, with previous sample preparation. Results have shown that cold maceration was able to improve the extraction of aromatic substances.

RIASSUNTO

L'aroma del vino è il risultato di diverse reazioni chimiche ed enzimatiche e della presenza di composti odorosamente attivi che derivano direttamente dall'uva. Nelle varietà non aromatiche i composti volatili liberi provengono principalmente dalla trasformazione di precursori inodori, quali i componenti volatili glicosilati, in composti sensorialmente attivi. Le ricerche sullo sviluppo di tecniche enologiche idonee a migliorare la qualità del vino, aumentandone il potenziale varietale, sono perciò di grande interesse. La macerazione prefermentativa a freddo consente la produzione di vini tipici, caratterizzati da un profilo aromatico strettamente legato alla zona di produzione e alla varietà.

L'obiettivo di questo lavoro è stato valutare gli effetti della macerazione pre-fermentativa a freddo sull'aroma dei vini *Aglianico* e *Montepulciano*. La frazione volatile libera del vino è stata analizzata mediante gas-cromatografia abbinata alla spettrometria di massa, previa estrazione del campione. I risultati hanno mostrato che la macerazione a freddo ha migliorato l'estrazione delle sostanze aromatiche.

INTRODUCTION

Grape aroma compounds constitute a large and complex group of chemicals which are present in free or glycosidically bound forms in the mesocarp vacuoles and in the pericarp, immediately under the skin of the berry (Esti, Tamborra, 2006). They greatly affect wine aroma due to the persistence of some of them throughout the entire process of winemaking. Also climate and soil characteristics play a decisive role in the sensory quality and regional character of wines (Kotseridis, Baumes, 2000). Moreover, the type of wine produced is affected by fermentation and maceration conditions, clarification treatments and fining (Marais *et a*l., 1986). Several studies regarding aromatics extraction in some varieties during winemaking have been carried out (Zimman *et al*., 2002; Gomez-Mıguez *et al*., 2007). Cold maceration of grapes allows the production of typical wines, that are characterised by an aromatic profile strongly connected to its zone of production and to its variety. The temperature reduction could be obtained using an heat exchanger or by direct addition of a cryogen to the grapes crushed. The cryogen, because of its very low temperature, causes the cellular crash of berries promoting the release of aromatic compounds in the must, and also, greatly prevents its oxidation.

The objective of this work was to evaluate the effects of pre-fermentative cold maceration technique on the aroma of *Aglianico* and *Montepulciano*. Volatile composition of wines was evaluated by means of gas-chromatography coupled to mass spectrometry.

MATERIAL AND METHODS

Grapes from *Aglianico* and *Montepulciano* vines were manually harvested, placed in 20 kg plastic boxes and transported to an experimental wine-production centre. Approximately, 700 kg of grapes were processed in each assay as well as in the control wine. The grapes were destemmed and crushed and then transferred into a 100 L stainless steel tank for maceration.

Traditional winemaking and pre-fermentative cold maceration (5°C for 24 h) with the use of solid state CO_2, added directly to the destemmed grapes, were tested. The wines were elaborated without any further treatment. During the alcoholic fermentation, temperature and solid soluble were measured daily and punching was carried out twice a day. At the end of fermentation, the wines were bottled and stored for 3 months at about 14°C until the analysis.

Volatile compounds of wines were recovered by solid phase extraction (SPE), according to the method proposed by Piñeiro *et al*., 2004. Determination of volatile compounds was performed using a gas chromatograph (Agilent Technologies 6890N) equipped with a mass spectrometer detector (Agilent Technologies 5975). The chromatographic column was a capillary column DB-WAX (60 m*0.25mm*0.25µm, J&W Scientific) and the oven temperature programme was 40°C (15 min), 2°C/min up to 200°C (20 min). The flow of carrier gas (He) was 1 mL/min. The volume of injection was 1µL in splitless mode. The identification of the volatile compounds was achieved by comparing mass spectra with those of the data system library (NIST 02, P>90%) and retention indexes with published data. Concentration of each volatile compound is expressed as mg internal standard equivalents L^{-1} wine, obtained by normalizing the compound peak area to that of the internal standard and multiplying by concentration of the internal standard (1.2 mg L^{-1}).

Oneway analysis of variance (ANOVA) using the Duncan test at level of significance $p<0.05$ was used for statistical analysis (Statsoft 6.0).

RESULTS AND DISCUSSION

Concentrations of volatile compounds identified and quantified in *Montepulciano* and *Aglianico* wines produced with traditional winemaking (MTW, *Montepulciano* wine; ATW,

Aglianico wine) and with pre-fermentative cold maceration (MCMW, *Montepulciano* wine; ACMW, *Aglianico* wine) are shown in Tab.1.

Tab. 1 Concentration of volatile compounds in *Montepulciano* and *Aglianico* wines made by different winemaking technologies. *Data are expressed as means of 3 replicates ± standard deviation; nd, not detected. Different letters within the same row for each variety mean significant differences (P<0.05)

COMPOUNDS	**MONTEPULCIANO**		**AGLIANICO**	
	MTW **(mg/L)***	MCMW **(mg/L)***	ATW **(mg/L)***	ACMW **(mg/L)***
esters				
ethyl butanoate	0.28b ± 0.04	0.16a ± 0.04	0.21a ± 0.03	0.21a ± 0.02
1-butanol, 3-methyl acetate	0.69a ± 0.06	0.61a ± 0.10	0.94a ± 0.08	1.84b ± 0.07
hexanoic acid, ethyl ester	0.24a ± 0,03	0.23a ± 0.02	0.19a ± 0.03	0.36b ± 0.03
acetic acid, hexyl ester	nd	nd	0.039a ± 0.005	0.06b ± 0.01
ethyl lactate	0.37a ± 0.05	0.56b ± 0.04	0.13a ± 0.01	0.31b ± 0.04
octanoic acid, ethyl ester	0.20a ± 0.04	0.24a ± 0.01	0.15a ± 0.04	0.26b ± 0.03
ethyl 3-hydroxybutyrate	0.08a ± 0.01	0.09a ± 0.01	0.05a ± 0.01	0.07a ± 0.01
decanoic acid, ethyl ester	0.09a ± 0.01	0.12a ± 0.02	0.11a ± 0.01	0.14a ± 0.02
diethyl succinate	0.94a ± 0.10	1.11b ± 0.02	1.16b ± 0.07	0.71a ± 0.04
acetic acid, 2-phenylethyl ester	0.07a ± 0.01	0.07a ± 0.01	0.19a ± 0.01	0.37b ± 0.02
diethylmalate	0.10a ± 0.02	0.11a ± 0.01	nd	nd
ethyl hydrogen succinate	6.5a ± 0.5	6.6a ± 0.4	7.2b ± 0.5	6.0a ± 0.3
alcohols				
1-propanol	0.28b ± 0.02	0.21a ± 0.02	0.22a ± 0.01	0.19a ± 0.02
1-propanol, 2-methyl	3.3a ± 0.5	3.23a ± 0.10	2.25b ± 0.03	1.74a ± 0.08
1-butanol	0.07a ± 0.01	0.09a ± 0.01	0.09b±0.01	0.06a ± 0.01
1-butanol, 3-methyl	52a ± 7	55a ± 3	44.9a ± 0.8	47.3a ± 1.8
1-pentanol, 4-methyl	0.06a ± 0.01	0.06a ± 0.02	0.036a ± 0.005	0.050b ± 0.003
1-pentanol, 3-methyl	0.12a ± 0.01	0.13a ± 0.01	0.077±0.003	nd
1-hexanol	2.2a ± 0.3	2.3a ± 0.2	0.88a ± 0.01	2.63b ± 0.09
trans - 3-hexen-1-ol	0.04a ± 0.01	0.04a ± 0.02	0.05a ± 0.01	0.06a ± 0.01
cis - 3-hexen-1-ol	0.34a ± 0.04	0.35a±0.03	0.023a ± 0.001	0.019a ± 0.002
1-pentanol	nd	nd	0.030a ± 0.002	0.031a ± 0.005
2,3-butanediol R,R	0.33a ± 0.04	0.36a ± 0.04	0.37a ± 0.05	0.66b ± 0.06
methionol	0.43a ± 0.07	0.56b ± 0.03	0.25a ± 0.03	0.30a ± 0.03
2,3-butanediol R,S	nd	nd	0.23a ± 0.01	0.18a ± 0.01
benzyl alcohol	2.0a ± 0.3	2.04a ± 0.15	0.15a ± 0.03	0.15a ± 0.02
phenylethanol	40a ± 5	51a ± 5	50a ± 4	50a ± 2
acids				
acetic acid	0.74a ± 0.09	0.99b ± 0.03	1.04b ± 0.02	0.53a ± 0.04
propanoic acid, 2-methyl	0.23a ± 0.04	0.27a ± 0.02	0.110a ± 0.004	0.22b ± 0.01
butanoic acid	0.13a ± 0.02	0.12a ± 0.01	0.09a ± 0.01	0.08a ± 0.01
butanoic acid, 3-methyl	0.88a ± 0.13	1.06a ± 0.05	1.15b ± 0.03	0.84a ± 0.11
hexanoic acid	1.76a ± 0.20	1.60a ± 0.07	1.39a ± 0.19	1.8a ± 0.3
octanoic acid	1.34a ± 0.10	1.17a ± 0.09	0.93a ± 0.07	1.61b ± 0.13
benzoic acid	0.32a ± 0.04	0.34a ± 0.03	0.14 ± 0.02	nd

The esters 1-butanol 3-methyl acetate, hexanoic acid ethyl ester, ethyl lactate, octanoic acid ethyl ester and acetic acid 2-phenylethyl ester have been observed in ACMW at higher

concentration than in ATW. Also the alcohols 1-pentanol 4-methyl, 1-hexanol, 2,3-butanediol R,R and the acids 2-methyl propanoic acid and octanoic acid were observed in ACMW at higher concentration than in ATW. Formation of esters and alcohols is related to the metabolism of aminoacids; as a consequence, their concentration, depending on the decomposition of the skins, increase with the use of dry ice (Alvarez *et al*., 2006), although discordant results are reported in literature (Peinado et al., 2004; Pineiro *et al*., 2006). Alcohols and acids are known to be characterized by a negative odor impact and relatively high odor thresholds; on the contrary, esters show a positive odor impact being described with fruity notes and have much lower odor thresholds in the range 0.0015-0.8 mg/L. Therefore, a positive contribution of the increased esters to the wine aroma is reasonable.
Differently, no much changes were observed in MCMW with respect to MTW. Also, in both wines MCMW and ACMW, no terpenoids were observed probably being at very low concentrations. These preliminary results supported the generally accepted concept that the improvement in quality, owing to cold maceration, depends on the investigated variety.
Relationships between volatile composition of grape and the related pre-fermentative macerated wine, together with the contribution of each specific compound to the wine aroma, in relation to its odour threshold, need to be further invesigated.

CONCLUSIONS

Results have shown that pre-fermentative cold maceration caused the increase of the content of some volatile compounds, especially esters, in *Aglianico* wine. No much changes were observed for *Montepulciano* wine. Further investigation should be done as regards the contribution of each specific compound to the wine aroma.

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BIBLIOGRAPHY

Alvarez I., Aleixandre J.L., Garcia M.J., Lizama V. 2006. Impact of prefermentative maceration on the phenolic and volatile compounds in Monastrell red wines. *Anal Chim Acta* 563:109-115.

Di Stefano R. 1991. Proposal for a method of sample preparation for the determination of free and glycoside terpenes of grapes and wines. *Bulletin de l'ÓIV*, 64:219-223.

Esti M., Tamborra P. 2006. Influence of winemaking techniques on aroma precursors. .*Anal Chim Acta* 563**:**173-179.

Gomez-Miguez M., Gonzàlèz Miret M.L., Heredia. F.J. 2007. Evolution of colour and anthocyanin composition of Syrah wines elaborated with pre-fermentative cold maceration *J. Food . Food Engineering* 70: 271-278.

Marais J. 1986. A reproducibile capillary gas chromatographic technique for the determination of specific terpenes in grape juice and wine. *S. Afr. J. Enol. Vitic*. 7: 21-25.

Kotseridis Y., Baumes. R. 2000. Identification of Impact Odorants in Bordeaux Red Grape Juice, in the Commercial Yeast Used for Its Fermentation, and in the Produced Wine. *J. Agric.Food Chem.* 48: 400-40.

Peinado A.R., Moreno J., Bueno E.J., Moreno A.J., Mauricio C.J. 2004. Comparative study of aromatic compounds in two young white wines subjected to pre-fermentative cryomaceration. *Food Chem.* 84: 585-590

Piñeiro Z., Palma M., Barroso G.C. 2004. Determination of terpenoids in wines by solid phase extraction and gas chromatography. *Anal. Chim. Acta.* 513: 209-214.

Piñeiro Z., Natera R., Castro R., Palma M., Puertas B., Barroso G.C. 2006. Characterisation of volatile fraction of monovarietal wines: influence of winemaking practices. *Anal. Chim. Acta.* 563: 165-172.

Zimman A., Joslin W.S., Lyon M.L., Meier J., Waterhouse A.L. 2002. Maceration Variables Affecting Phenolic Composition in Commercial-scale Cabernet Sauvignon Winemaking Trials. *Am. J. Enol. Vitic.* 53:2:93-98.

COLOR AND AROMA OF AGLIANICO AND MONTEPULCIANO WINES AS AFFECTED BY AGEING ON LEES IN BARREL

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ABSTRACT
During ageing wine matures since several chemical reactions involving sugars, acids and phenolic compounds modify its aroma, color, mouthfeel and taste. Oak ageing is often coupled to the use of lees, which release polysaccharides, proteins and peptides, aminoacids and lipids improving tartaric and protein stabilization, protecting wine color against oxidation, and changing the final aroma balance. However, it is well known that not all grape varieties are suited to the ageing technology using barrels and lees, depending on wine characteristics.
The aim of this study was to assess the suitability of two Italian red wines, Aglianico and Montepulciano, to be aged in barrel on lees. The ageing in barrique on lees was compared with the ageing in stainless steel tanks, investigating on color (dAl%, dAT%, dTAT%) and changes in volatile fraction. Results have shown that the wood-lees-ageing technology applied to Aglianico and Montepulciano wines improved color stability and flavor properties. Changes in Aglianico and Montepulciano wines were different both from a qualitative and quantitative point of view.

RIASSUNTO
Durante l'affinamento, il vino matura, poiché diverse reazioni chimiche che coinvolgono zuccheri, acidi e composti fenolici, modificano il suo aroma, colore, e gusto. L'affinamento in botte è spesso accoppiato all'uso delle fecce, le quali rilasciano polisaccaridi, sostanze proteiche e lipidi, migliorando la stabilizzazione tartarica e proteica, proteggendo il colore dall'ossidazione e modificando l'aroma. Comunque, è ben noto che non tutte le varietà di uva sono adatte alla tecnologia di affinamento che utilizza contenitori in legno e fecce. Lo scopo del presente lavoro è stato valutare l'adattabilità di due vini rossi, Aglianico e Montepulciano, ad essere affinati su fecce in legno. L'affinamento in barrique su fecce è stato confrontato con l'affinamento in acciaio inossidabile valutando il colore e i cambiamenti nella frazione volatile. I risultati hanno dimostrato che la tecnologia di affinamento su fecce in legno ha migliorato la stabilità del colore e le proprietà di flavor dei vini. Le modifiche introdotte da questa tecnologia sono state differenti nei vini Aglianico e Montepulciano sia dal punto di vista qualitativo che quantitativo.

INTRODUCTION
Wine ageing, defined as the period between alcoholic fermentation and bottling, is a fundamental step for obtaining high quality wines.
During this period the wine matures since several chemical reactions involving sugars, acids, and phenolic compounds modify its aroma, color, mouthfeel, and taste. In particular, the wine acquires a peculiar aromatic complexity (Camara, Alves, Marques, 2006), reduces its astringency (Ribéreau-Gayon, Glories, Maujean, Dubourdieu, 2000b) and stabilizes its color (Bakker, Picinelli, Briddle, 1993) as a result of important chemical modifications deriving from

esterification, hydrolysis, and redox reactions, spontaneous clarification, CO_2 elimination, slow and continuous diffusion of oxygen. During ageing, also significant changes in wine aroma occur. Their interpretation is complicated because wine aroma results from several substances deriving from grape, fermentation, and ageing, which are involved in several reaction pathways.
Oak ageing is often coupled to the use of lees, the residue forming at the bottom of containers after alcoholic fermentation, with the aim to improve wine quality since lees release many compounds. Polysaccharides, nitrogen compounds and lipids are released into the wine, enriching it from nutritional, organoleptic (Bautista, Fernàndez, Falquè, 2007) and technological (Waters, Wallace, Tate, Williams, 1993) points of view. Furthermore, the contact between wine, wood and lees can cause an increase of color stability of pigments due both to wood microxigenation (Ribéreau-Gayon *et al.* 2000b) and to the establishment of interactions between color matter and lees-deriving glycoproteins acting as protective colloids (Escot, Feuillat, Dulau, Charpentier, 2001; Salmon, Fornairon-Bonnefond, Mazauric, 2002).
Oenologist experience showed that not all grape varieties lend themselves to winemaking with wood-lees-ageing technology (Ortega-Heras, González-Huerta, Herrera, González-Sanjosé, 2004). Grape, as well as the deriving wine, should have a complex polyphenolic structure to sustain wood and a certain aromatic fineness to blend well with wood-derived flavors and also to not be too much spoiled of floreal and fruity notes by lees. It is well known, indeed, that ordinary wines can not be turned into quality wines by oak aging (Ribéreau-Gayon *et al.* 2000b).
The aim of this study was to investigate on relationship between chemical composition and the suitability of two typical Italian red wines, Aglianico and Montepulciano, to be aged in wood on lees. The ageing in barrique on lees was compared with the ageing in stainless steel tanks, investigating on color (dAl%, dAT%, dTAT%) and changes in volatile fraction.

MATERIAL AND METHODS

Red winemaking of Aglianico and Montepulciano grapes
Aglianico and Montepulciano wines (vintage 2008) were produced by De Majo Norante winery (Campomarino, CB, Italy) as described in the following.
Aglianico and Montepulciano grapes were destemmed, crushed and added with 10g/hL of SO_2. Successively, 20g/hL of selected yeasts were inoculated and alcoholic fermentation was carried out at 27-28°C, together with maceration in stainless steel tanks, with periodical pumping over. After 14 days of maceration, the wine was drained out by pressing to make a separation of wine and solid parts. Then, both the wines were divided into 2 subsets: the first was raked and stored in stainless steel tanks (Aglianico wine, A_Control; Montepulciano wine, MP_Control); the second was left with lees and stored in barrique (Aglianico wine, A_BL; Montepulciano wine, MP_BL). For each winemaking three replicates were performed. All wines aged on lees were stirred every two/three days in order to re-suspend the lees and homogenize the wine. The sampling was performed after stirring the wine, at six months of ageing.

Color parameters (dAl%, dAT%, dTAT%)
Color was measured using a Varian UV-visible spectrophotometer (Mod. Cary 50 SCAN, Palo Alto, Calif., U.S.A.). The color parameters calculated were the followings: i) dAl%, the contribution of monomeric anthocyanins to 520 nm absorbance; ii) dAT%, the contribution of anthocyanin-tannin pigments bleachable by SO_2 to 520 nm absorbance; iii) dTAT%, the contribution of SO_2 non-bleachable anthocyanin-tannin pigments to 520 nm absorbance. Total

red pigment color was measured at 520 nm, after wine acidification with hydrochloric acid to pH 1. Afterwards, 20% $Na_2S_2O_3$ (w/v, in water) was added to wine, followed by absorbance measurements at 520 nm. The percentages of TAT, AT and Al to total pigments were estimated according to the following equations:

TAT (%)= (Al + AT) (%) - Al (%)

TAT (%) = (Absorbance of wine at 520 nm after the addition of SO_2)/(Absorbance of wine at 520 nm without the addition of SO_2) x100

(Al + AT) (%) = (Absorbance of wine at 520 nm without the addition of SO_2) - (Absorbance of wine at 520 nm after the addition of SO_2)/(Absorbance of wine at 520 nm without the addition of SO_2) x100

Al (%) was determined measuring 520 nm absorbance after passing of the wine sample through a PVPP (polyvinylpolypyrrolidone) resin able to retain anthocyanin combined with tannins

All measurements were undertaken at room temperature as a single measurement of replicated treatments.

Determination of volatile compounds

Volatile compounds were recovered by solid phase extraction (SPE) according to Lòpez *et al.* (2002), with some modifications. Briefly, LiChrolut EN resins prepacked in 200mg cartridges (Merck, Darmstadt, Germany) were rinsed with 4mL of dichloromethane-hexane (2:3), 4mL of methanol and, finally, 4mL of a water–ethanol mixture (12%, v/v). 50mL of wine, containing 1ppm of 1-octanol as internal standard, were passed through the SPE cartridge at around 2mL/min. Afterwards, the sorbent was dried by letting air pass through it. The analytes were recovered by elution with 1.3mL of dichloromethane-hexane (2:3). A 1µL sample was injected in the gas chromatographic system.

A 6890N series gas chromatograph (Agilent Technologies) with an Agilent 5973 mass selective detector (MSD) and equipped with a HP-INNOWAX capillary column (60m x 0.25mm I.D, 0.25µm film thickness, J&W Scientific Inc., Folsom, USA) was used for the determination of volatile compounds. The carrier gas was helium at a flow rate of 1.0mL/min. The injection was made in the splitless mode, the injector temperature was 250°C. The column oven temperature was initially held at 40°C, then it was programmed to 230°C at 2.5°C/min, with a final holding time of 20min. Spectra were recorded in the electron impact mode (ionization energy, 70eV) in a range of 30–500amu at 3.2scans/s. A solvent delay time of 10min was used to avoid overloading the mass spectrometer with solvent.

The identification of the volatile compounds was achieved by comparing mass spectra with those of the data system library (NIST 98, P>90%) and retention indexes with published data. Concentration of each volatile compound is expressed as µg internal standard equivalents L^{-1} wine, obtained by normalizing the compound peak area to that of the internal standard and multiplying by concentration of the internal standard.

RESULTS AND DISCISSION

The suitability of Aglianico and Montepulciano wines to be aged on lees in barrique has been evaluated with respect to the stainless steel ageing. To this aim, color and aroma have been studied by means of the evaluation of dAl%, dAT%, dTAT% parameters and volatiles profile.
The results obtained are presented and discussed in the following.

Color parameters dAl%, dAT%, dTAT%

The Fig. 1 shows the contribution of dAl%, dAT%, dTAT% to the color of wines Aglianico (A) and Montepulciano (B), at six months storage. These parameters take into account anthocyanin copigmentation, thus color stability. In the case of Aglianico, the contribution of monomer anthocyanins to color does not significantly change according to the ageing technology, likely as result of the several equilibra involved.

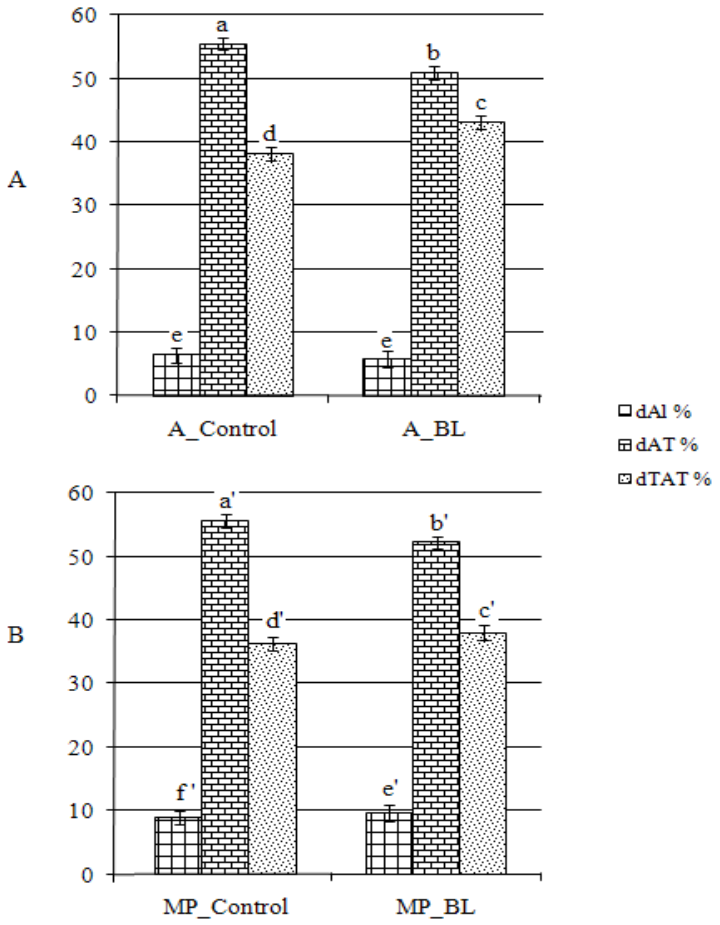

Fig. 1. Bar chart showing the contribution of dAL%, dAT%, dTAT% to the color of wines Aglianico (A) and Montepulciano (B), at six months storage.

Differently, results clearly show that, the contribution of anthocyanin-tannin pigments bleachable by SO_2 decreases in wine aged in barrique on lees with respect to the stainless steel aged wine whereas the contribution of anthocyanin-tannin pigments not bleachable by SO_2 increases. The AT forms disrupted seem to shift equilibria towards the formation of the most stable TAT. This is likely to occur because wood, allowing microxygenation, enhances anthocyanin copigmentation and glycoproteins, released by lees, improve wine color stability interacting with phenolic compounds (Pérez-Serradilla, Luque de Castro, 2008).
Also in the case of Montepulciano, AT and TAT contributions decrease and increase, respectively, in wood lees-aged wine with respect to the stainless steel aged wine. However, Montepulciano wine shows a higher percentage of monomer anthocyanins and a lower percentage of TAT forms than Aglianico wine, suggesting a minor color stability. This could be due to a major availability of tannins in Aglianico variety, entailing a higher presence of precursors in TAT copigmentation.

Volatile compounds
Esters, lactones, aldehydes, and phenols were detected by gas-chromatography coupled to mass spectrometry in Aglianico and Montepulciano wines. These compounds are known to significantly contribute to wine aroma since they have generally low sensory thresholds. Within the compounds investigated, the ones that have showed significant changes according to the ageing technology, as resulted from statistical test ANOVA, have been reported in Tab. 1.
Aglianico wine aged in stainless steel tank is characterized by the highest content in phenethyl acetate, ethyl decanoate and ethyl hexadecanoate, contributing with notes respectively of rose, fresh fruit and mild waxy; Montepulciano wine aged in stainless steel tank is characterized by the highest content in ethyl propionate, methyl salicylate and diethyl succinate contributing with notes respectively of fruit, apple, and apricot. As expected, these wines are poor in volatile phenols, lactones and aldehydes deriving mostly from oak ageing. An increase of the concentration of several volatile compounds has been obtained with the technology of ageing on lees in barrel for both wines. It is worth noting that changes in aroma of Aglianico and Montepulciano wines are different both from a qualitative and quantitative point of view. Aglianico wine showed the main changes in wood-deriving compounds, such as eugenol, phenol 2,6-dimethoxy-4-(2-propenyl), furfural, 5-methylfurfural, syringaldehyde (Chatonnet, Boidron, Pons, 1989). Montepulciano wine showed the main changes in esters compounds, such as ethyl propionate, diethyl succinate and ethyl decanoate. Small changes, with the exception of whiskey lactone isomers, occurred in volatile phenols, chetones and aldehydes. The different behaviour of Aglianico and Montepulciano is probably related with wine characteristics affecting the extraction from wood (Garde-Cerdàn, Torrea-Goni, Ancìn-Azpilicueta, 2004). In particular, Ortega-Heras *et al.* 2004 founded that the grape variety has an important role on the extraction of compounds from the barrel by the wine, in agreement with our results. For both Aglianico and Montepulciano, a few cases of loss in compounds, and at a very low extent, occurred, showing that no spoilage of wine was caused by lees.

Tab. 1 Compounds detected in all wines aged in barrique on lees whose concentration is significantly higher (One-way variance analysis, $P<0.05$) than in stainless steel made wine.

Compound	Peak	RT (min)	A_BL-A_Control			MP_BL – MP_Control			Odor descriptor
Esters									
Isobutyl acetate	1	11.75	0.058 [a]	±	0.029	0.071 [a]	±	0.011	sweet
Isoamyl acetate	3	16.58	0.054 [a]	±	0.040	-0.230 [b]	±	0.037	fruity
Ethyl proprionate	6	28.10	1.068 [b]	±	0.144	1.353 [a]	±	0.307	fruity
Ethyl octanoate	7	32.80	-0.041 [b]	±	0.011	-0.003 [a]	±	0.012	fresh fruit
Ethyl dl-2-hydroxycaproate	13	38.29	-0.008 [c]	±	0.006	0.060 [a]	±	0.015	green fruit
Ethyl 2-furoate	15	42.24	0.005 [a]	±	0.002	0.005 [a]	±	0.003	fruity
Ethyl decanoate	16	42.75	0.022 [c]	±	0.003	0.122 [a]	±	0.012	fruity
Diethyl succinate	17	44.75	4.905 [b]	±	0.630	6.526 [a]	±	1.121	apple
Methyl Salicylate	19	49.26	0.026 [a]	±	0.019	0.049 [a]	±	0.006	minty
Diethyl malate	26	60.09	-0.400 [c]	±	0.057	0.082 [a]	±	0.015	—
Ethyl hexadecanoate	31	67.74	-0.147 [c]	±	0.006	-0.006 [b]	±	0.014	mild
Ethyl 4-hydroxy-3-methoxybenzoate	35	80.65	0.024 [b]	±	0.019	0.172 [a]	±	0.019	vanilla
Phenols									
Guaicol	23	52.83	0.189 [b]	±	0.046	0.438 [a]	±	0.086	wood
Eugenol	27	64.97	0.045 [a]	±	0.002	0.035 [b]	±	0.001	cinnamon
4-Ethyl phenol	28	65.31	0.065 [c]	±	0.001	0.103 [b]	±	0.001	pungent
Phenol, 2,6-dimethoxy-4-(2-propenyl)	34	77.68	0.063 [a]	±	0.002	-		-	roasted
Chetons-Lactons									
Acetoin	5	25.04	0.107 [b]	±	0.009	-0.244 [c]	±	0.006	buttery
Methyl 2-furyl ketone	9	36.59	0.036 [a]	±	0.002	-		-	toasted
3(2H)-Thiophenone, dihydro-2-methyl	12	37.73	0.004 [b]	±	0.003	-0.043 [c]	±	0.002	—
Thiophene, tetrahydro-2-methyl-	21	50.30	-0.021 [b]	±	0.011	0.019 [a,b]	±	0.016	—
trans-Methyl octanolide	24	54.05	0.111 [a]	±	0.006	0.109 [a]	±	0.005	coconut
cis-Methyl octanolide	25	56.88	0.272 [a]	±	0.016	0.192 [b]	±	0.009	coconut
Aldehydes									
Furfural	8	34.55	0.490 [a]	±	0.0066	0.093 [c]	±	0.0030	wood
Benzaldehyde	11	37.50	0.002 [b]	±	0.0054	0.017 [a]	±	0.0078	almond
5-Methyl furfural	14	39.97	0.347 [a]	±	0.0207	0.050 [c]	±	0.0057	caramel
Syringaldehyde	37	94.88	0.336 [a]	±	0.0400	0.217 [b]	±	0.0024	—

CONCLUSIONS

Results showed that the wood-lees-ageing technology applied to Aglianico and Montepulciano wines improved color stability, above all for Aglianico wine, likely due to a major availability of tannins causing a higher presence of precursors in copigmentation.

Changes in aroma of Aglianico and Montepulciano wines, as affected by the investigated ageing process, are different both from a qualitative and quantitative point of view. Aglianico wine showed the main changes in wood-deriving compounds, whereas Montepulciano wine showed the main changes in esters compounds.

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BIBLIOGRAPHY

Bakker J., Picinelli A., Briddle P., 1993. Model wine solutions: colour and composition changes during ageing. Vitis, 32: 111-118.

Bautista R., Fernàndez E., Falquè E., 2007. Effect of the contact with fermentation-lees or commercial-lees on the volatile composition of white wines. Eur Food Res Techn, 224: 405-413.

Camara J.S., Alves M.A., Marques J.C., 2006. Evolution of oak-related volatile compounds in a Spanish red wine during 2 years bottled, after aging in barrels made of Spanish, French and American oak wood. Anal Chim Acta, 563: 189-203.

Chatonnet P., Boidron J.N., Pons M., 1989. Effect on heat on oak wood and its chemical composition. Part 2. Variations of certain compounds in relation to toasting intensity. Connais Vigne Vin, 23: 223-250.

Escot S,. Feuillat M., Dulau L., Charpentier C., 2001. Release of polysaccharides by yeast and the influence of released polysaccharides on colour stability and wine astringency. Aust J Grape Wine Res, 7: 153-159.

Garde-Cerdán T., Torrea-Goñi D., Ancín-Azpilicueta C., 2004. Accumulation of volatile compounds during ageing of two red wines with different composition. J Food Eng, 65: 349-356.

Lòpez R., Aznar M., Cacho J., Ferreira V., 2002. Determination of minor and trace volatile compounds in wine by solid-phase extraction and gas chromatography with mass spectrometric detection. J Chromatog A, 966: 167-177.

Ortega-Heras M., González-Huerta C., Herrera P., González-Sanjosé M.L., 2004. Changes in wine volatile compounds of varietal wines during ageing in wood barrels. Anal Chim Acta, 513: 341-350.

Pérez-Serradilla J.A., Luque de Castro M.D., 2008 Role of lees in wine production: A review. Food Chem, 111: 447-456.

Ribéreau-Gayon P., Glories Y., Maujean A., Dubourdieu D., 2000b. Handbook of Enology, Vol. 2. The Chemistry of Wine Stabilization and Treatments. New Jersey: John Wiley & Sons Ltd.

Salmon J.M., Fornairon-Bonnefond C., Mazauric, J.P., 2002. Interactions between wine lees and polyphenols: Influence on oxygen consumption capacity during simulation of wine aging. J Food Sci, 67: 1604-1609.

Waters E.J., Wallace W., Tate M.E., Williams P.J., 1993. Isolation and partial characterization of a natural haze protective factor from wine. J Agric Food Chem, 41: 724-730.

Volatile, aroma-forming compounds in bunch skeleton of Kakhetian white grape vinous (*V. vinifera L.*) breeds

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Abstract

The article presents results of the study of qualitative and quantitative content of volatile, aroma-forming compounds, higher alcohols, fat acids, complex ethers, terpenes, lactones, volatile phenols, aldehydes, hydrocarbons in bunch skeleton of white grapes of Kakhetian vinous vine (*V. vinifera L*.) breeds (Rkatsiteli, Kakhuri Mtsvane, Khikhvi, Kakhuri Mtsvivani). The degree of transfer of aroma-forming compounds (especially terpenes, fat acids, lactones) from the bunch skeleton to wine and role of the bunch skeleton in formation of traditional Kakhetian type wine during 5 months after completion of fermentation is shown. The wine produced from Kakhuri Mtsvivani grapes is characterized by higher content of volatile, aroma-forming compounds compared to other grape varieties.

Resümee

In der Arbeit sind wie qualitative, als auch quantitative Kennzahl der höheren Spiritus, Fettsäure, Ester, Terpene, Laktone, verdunstbaren Phenol, Aldehyde, Kohlenwasserstoff, verdunstbaren, aromabildenden Verbindungen des Traubenkammes der Weinsorte der weißen Rebe (*V.vinifera L.*) von Kakheti dargestellt, ist dargestellt die Übergangseigenschaft der einzelnen Gruppen der aromabildenden Verbindungen (besonders Terpene, Fettsäure, Laktone) vom Kamm im Wein. Die Rolle des Kammes im Prozeß der Formierung des traditionellen kakhetischen Weins, innerhalb 5 Monaten nach der Gärung. Der Wein von kakhetischen „Mtsvivani“, unterscheidet sich im Vergleich zu anderen Weinsorten durch hoches Gehalt der verdunstbaren, aromabildenden Verbindungen.

Introduction

Traditional Kakhetian type of wine is produced by fermentation of the grape sweet with hard parts of grapes (skin, bunch skeleton and pips) in “kvevri” - earthenware buried in soil up to the neck. Upon completion of fermentation wine is formed with chacha during following 5 months. During this period wine is enriched with various volatile and non-volatile compounds of hard parts of the grape.

Among the hard parts of the grape pips and skin are investigated relatively well, while chemical composition of the bunch skeleton is studied at a lesser extent despite the fact that it is impossible to produce the traditional Kakhetian wine without this component. We disagree with the point of view that bunch skeleton may impose to wine some roughness, bitter taste or extended tanning tones and, thus, worsen its quality. It may happen only if the bunch skeleton is of green color, and therefore immature. This is one of the main conditions of making the Kakhetian wine.

Many scientists studied the role of volatile, aroma-forming compounds - high alcohols, fat acids, complex ethers, terpenes, lactones, volatile phenols, aldehydes, hydrocarbons and other compounds - in the process of formation of wines from grapes of various breeds.

According to Ribero Gaioni et al. (1979), there are two types of wine aromas: primary (breed related) and secondary (the intensive smell of wine formed under influence of yeasts). The latter is refined and enriched after completion of fermentation and slacks and disappears during wine formation, substituted with bouquet. The soft bouquet is developed during wine formation as a result of complex biochemical transformation of the primary and secondary aromatic components. According to Rodopulo (1987), the table wines and some technical grape varieties contain terpenoids, aliphatic and aromatic alcohols and their ethers, as well as complex ethers.

The content of terpenes and other aromatic compounds in grape depends on its ripeness. During the ripening of the grape in line with gathering of sugar the content of aromatic compounds, especially of less volatile ones, is raising. The content of these compounds in grape is small however they determine the aroma of different breeds, both in grapes and in sweet. The maximum of aromatic compounds gathers in grapes during various ripeness periods.

According to Pisarnitski et al. (1980 A), significant amount of terpenoids and volatile phenols exist in wines from grapes with strong breed related aroma; amongst them, wines and cognacs of Kakhetian type are characterized with existence of Vanillin and Coniferyle aldehydes. Authors obtained factions of split products of phenols, lactones, terpenoids and hydrocarbons from various wines.

Kakhetian wines are characterized with higher content of split products of volatile phenols and hydrocarbons, while the content of terpenes is small. Also, Kakhetian wines are characterized with phenolic - gwaiacolic tones. Authors conclude, that the type of wine depends on confluence of aromatic groups, however one or another group is playing the leading role for individual wine.

According to Pisarnitski (1980 B), more intensive is the interaction of bulk chacha with the air oxygen (which is consecutive to the technical process), more amounts of aliphatic aldehydes is produced. The products of hydrocarbon split – terpenes, lactones, volatile phenols - form the each wine specific aromas even in minimal concentrations.

Pisarnitski (1980 C) identified Carboxylic and Heterocyclic compounds in wine making products – cyclopentane-1,2-dion; 5-methylcyclopentane-1,2-dion; 2-oxy-3-methyl-2-cyclopentene-1-oni; ethyloxymethylfurfurol; 5-methyl-4-oxy-3(2H)-furanone; 2,5-dimethyl-4-oxy-3-(2H)-furanone; 2-oxy-methyl-5-methyl-4-oxy-3(2H)-furanone; 3-oxy-2-pyranone; 2,3-dihydro-3,5-dioxy-6-methyl-4-pyranone - compounds which are characterized with strong aroma and represent the hydrocarbon split products.

Glonti et al. (2006) studied qualitative and quantitative content of volatile, aroma-forming terpenic alcohols and lactones in Kakhetian wines formed from Khikhvi, Rkatsiteli and Kakhuri Mtsvane grapes. The high content of terpenic alcohols in, particularly the lynalool, was highlighted. High content of lactones is found in Kakhuri Mtsvane wine. The same is true with regard of terpenic alcohols. Increased concentration of aromatic alcohol –triftofole was identified in the samples under investigation.

Given the above mentioned, we have decided to investigate volatile, aroma-forming compounds of vinous breeds of Kakhetian white grapes. These compounds, in combination with other hard parts of the grape should be actively involved in aroma-forming processes.

Material and Methods

As a material for investigation we have selected the bunch skeletons of white grapes of Kakhetian vinous vine (*V. vinifera L.*) breeds of Rkatsiteli, Kakhuri Mtsvane, Khikhvi, Kakhuri Mtsvivani.

Alcoholic-aqueous solution (65%) is added to the 10 g of scattered bunch skeleton with proportion of 10:1 and placed in thermostat at 45^0C for 10 days with periodic shaking of the mixture. After this time, the extract is separated from the bunch skeleton and another portion of alcoholic-aqueous solution (65%) is added to the skeleton with proportion of 5:1. The mixture is again placed in thermostat at 45^0C for 7 days with periodic shaking. Then the extract is separated again, both extracts are united and this mixture is ready for chromatography.

10 ml of extract is mixed with standard 5mg/l Pentanol solution (internal standard) and with extragent - 1ml chlorinemethylene. After two hours of shaking the mixture is separated from chlorinemethylene layer, which is volatized in the stream of about 50 µl pure nitrogen.

The extract analysis is made on chromatograph with mass-spectrometric detector. Identification of the component is done by comparison of the compounds' mass-specters with library mass-specters. The concentrations are calculated by comparison of peaks of identified volatized compounds with the area of Pentanol (5 mg/l) peaks, without the correction coefficient.

"Agilent Technology 6890" chromatograph with mass-spectrometric detector- 5973. Column – quartz capillary FFAP, length - 30m, diameter - 0.25 mm. Temperature - 230^0C. Thermostat temperature - with program from 50^0C to 220^0C, speed - 3minute. Amount of sample - 2 µl. Mass-specters library NISTO5.

Results and Discusison

The content of volatile, aroma-forming compounds in bunch skeleton of Kakhuri Mtsvivani grapes before and after fermentation are presented in the Tab. 1.

As we can see from the table, the sum of volatile, aroma-forming compounds in bunch skeleton after fermentation is increased, which is caused by increase of the content of fat acids, complex ethers and higher alcohols, while the amount of terpenes, aromatic alcohols, lactones, aldehydes and hydrocarbons is reduced.

Tab.1. Volatized, fragranced compounds of Kakhuri Mtsvivani

№	Name of Sample	Higher Alcohols	Fat acids	Complex ethers	Terpenes	Lactones	Aromatic alcohols	Aldehydes	Hydrocarbons
1	Kakhuri Mtsvivani, Bunch skeleton Before fermentation	0.754	94.978	19.01	14.269	11.802	44.694	2.968	0.162
2	Kakhuri Mtsvivani, Bunch skeleton After fermentation	7.661	155.704	37.231	5.794	0.769	20.890	0.613	0.074

9 components are identified in Fat Acids: Caprilic acid, Nonan acid, Laurine acid, Miristin acid, Pentadekan acid, Palmitin acid, Stearin acid, Vinegar acid, Olein acid. After fermentation the bunch skeleton contains Caprin acid, Miristin acid, Stearin acid, Vinegar

acid; out of the fat acids, there is a high concentration of a Palmitin acid (66.237 mg/100ml) and Olein acid (21.959 mg/ 100ml). The quantity of the latter is decreased after fermentation, while the quantity of Palmitin acid is increased.

Fat acids have the highest representation compared to other compounds- 94.978 mg/in 100 ml extract.

The high concentration is the group of aromatic alcohol (4.694mg/ 100ml), Triptopil (25.462 mg/100ml), Pitol (18.628 mg /100ml), Methylkhalikoli (0.604 mg/ 100ml).

After fermentation 2 components passes from bunch skeleton to wine: Triptopil and Methylkalikov, but the amount of Pitol growth in culis.

9 complex ethers are identified in the bunch skeleton before the fermentation: EthylCapronat, Ethylcaprilat, Ethylcaprinat, Dietiloksalat, Ethyllaurinat, Ethylpalmitat, Ethilmiristinat, Ethylpalmitat, Ethylpelargonat. After fermentation the bunch skeleton contains following compounds: Izoamilacetate, Ethyllactate, Monoethylsukcinat, Ethylstearat, Ethyllinoleat. During the fermentation the following compounds were transferred to wine: Dietiloksalat, Diethylmalonat, Ethyllaurinat, Ethylpelargonat.

Tab. 2 represents the indexes of qualitative and quantitative content of terpenes in bunch skeleton of Kakhuri Mtsvivani grapes before and after fermentation.

Before fermentation 15 terpenes are identified in bunch skeleton: Ilangen, β-Burbonen, Germagren-D, Aromadendren, β-Selinen, α –Muuronen, β-Ionone, α –Kalakoren, γ-Elemen, 3-oxo-α–Ionol, Epibicyclosesvifellandren, α –Amorphen, γ-Cadinen, Cariofilen, Oplopenon. After fermentation the bunch skeleton contains the Limonen, β-Cubeben, Valensen. During the fermentation following compounds are completely transferred from skeleton to wine: Aromadendren, β-Selinen, γ-Muurolen, β-Ionone, α –Kalakoren, γ-Elemen, 3-oxo-α–Ionol, Epibicyclosesvifellandren, while other terpenes are transferred partially. Ilangen and Kadiden have the highest concentration in the bunch skeleton, 4.278 mg/100ml and 3.108 mg/100ml correspondingly.

Before fermentation 4 lactones are identified: γ - Etoksibutirolactones, γ- Nonalactones, 6-Pentil-5,6-Dihydro-2H-Piran-2-Oni, Salvinal 4(14)–N-1-Oni. After fermentation following lactones are transferred to wine: γ-Nonalactone, 6-Pentil-5,6-Dihydro-2H-Piran-2-Oni, partially transferred γ – Etoksibutirolactone. In skeleton the 6-Pentil-5, 6-Dihidro-2H-Piran-2-Oni has the highest concentration (9.614mg/100ml).

Before fermentation there are 8 Aldehydes identified: Benzaldehyde, Hexanal, Oktanal, Nonanal, 2-Oktenal, Cis-2,4-Dekadienal, 2-4-Heptadienal, Trans-2,4-Dekadienal. After fermentation Dekanal and Miristin appear in the skeleton. Almost all components are transferred to wine.

The bunch skeletons of Rkatsiteli, Kakhuri Mtsvane and Khikhvi grapes are characterized with relatively high contents of fat acids. Rkatsiteli skeleton has the highest content of fat acids, while content in skeleton of Khikhvi grapes is three times less. The highest concentration of complex ethers is in bunch skeleton of Khikhvi and less in Rkatsiteli and Kakhuri Mtsvane varieties. Khikhvi is also distinguished by content of lactones, while in Rkatsiteli and Kakhuri Mtsvane concentration of these compounds is a little bit less.

Kakhuri Mtsvane has the highest content of aldehydes. 7 terpens are identified in above mentioned three grape varieies: Linalooloxyde, TransLinalooloxide, Cislinalool, α –Terpineol, Ilangen, Kariofilen, β- Cubenen. The amount of Ilangen, Kariofilen, β- Cubenen are relatively high in skeleton of Khikhvi variety.

It should be mentioned, that concentration of the above compounds is higher in Kakhuri Mtsvivani than in any other varieties. It also differs from other varieties by qualitative content.

Tab. 2. Terpens of bunch skeleton of Kakhuri Mtsvivani

#	Name of compound	Bunch skeleton before fermentation mg/100ml	Bunch skeleton after fermentation mg/100ml
1	Ilangen	4.278	2.073
2	β-Bourbons	1.540	0.551
3	Germakrens	0.335	0.164
4	Aromadendren	0.558	-
5	β-Selinen	0.233	-
6	α -Muurolen	0.494	-
7	β-Ionon	0.476	-
8	α -Kalakoren	0.438	-
9	γ-elemeni	0.649	-
10	3-okso-α -Ionol	0.215	-
11	Epibiciklosesvifellandren	0.514	-
12	α -Amorfen	0.890	0.695
13	γ-Kadinen	3.108	1.680
14	Kariofilen	0.333	0.146
15	Kadinoi-sesKv	2.08	-
16	Limonen	-	0.026
17	β-Kubenen	-	0.241
18	Valensen	-	0.218

Organoleptical evaluation of standard 13% alcohol content mixture, prepared by using the extract (65%) of bunch skeleton of Kakhuri Mtsvivani, was carried out. It was identified that the extract of skeleton is characterized with light-amber color, pleasant, grape aroma and taste, strong specific herb tones more displayed in aroma rather than tones, harmonic, soft and confluenced tanning tones. It should be noted, that wine produce from Kakhuri Mtsvivani without using the chacha is not characterized with specific taste and aroma, as well as bunch skeletons of Rkatsiteli, Kakhuri Mtsvane and Khikhvi grapes.

Conclusions

Following analysis of the above mentioned results one can conclude that:

1. The bunch skeleton of Kakhuri Mtsvivani is rich with volatile, aroma-forming compounds, which determines its active role in the process of alcoholic fermentation of grape sweet, their active participation in on-going complex biochemical transformations as well as formation of the aroma and taste of the future wine.

2. Qualitative and quantitative changes of the sum of aroma compounds, as well as individual compounds representing various groups, from one hand can be explained by transfer of these compounds to the area of fermentation, and from the other, by formation and concentration of lactones and volatile phenols, which are created as a result of etherification, split of terpenes and hydrocarbons during fermentation.

3. Kakhetian wine of Kakhuri Mtsvivani is distinguished with specific, pleasant aroma and taste, which is not observed in wine made without chacha. At the same time, similar taste and aroma can not be found in case of wines formed from other Kakhetian grape varieties. We

think, that such specific aroma and taste are caused bunch skeleton, in particular by volatile, aroma-forming compounds.

4. The above examples confirm the importance of grape bunch skeleton in formation of aroma and taste specific to Kakhatian type of wine, especially during 5 months formation of wine in kvevri after completion of the process of fermentation.

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Bibliography

1. Glonti T., Buishvili G., Makhatadze M., 2006 Comparative characteristics of the wine materials aromatic volatiles compounds, produced from Khikhvi, Rqatsiteli and Kakhuri Mtsvane brands. R. "Metsniereba da Technologiebi" ("Science and Technologies"); Monthly scientific-reviewed magazine of Georgian Academy of Sciences; #10-12, s. 110-113 (in Georgian)

2. Pisarnitsk A., 1980, Aroma making materials of Wine sort cognac, Author reference of doctoral dissertation, pp.10-21, 39 (in Rassian)

3. Pisarnitsk A.P, Rodopulo A.K, Egorov I.A., Egoparova R.Kh., 1980, about typical aromatic materials of wine and cognac. The International All-Union Institute of wine-making, USSR №3, pp. 30-32 (in Rassian)

4. Ribero-Gaion Zh., Pein E., Ribero-Gaion N., Smordo; 1979. Theory and Practice of wine-making. M.: "Pishepromizdat", p. 48

5. Rodopulo A.K., 1987, Aroma making compounds of wine and juice of wine, The International All-Union Institute of wine-making, USSR №4, pp. 53-54 (in Rassian)

Phenolic sources of wine made from grapes of "Kakhuri Mtsvivani" and some other vine (*V. vinifera L.*) breeds

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Abstract

Content rates of phenol compounds (Catechins**,** Proanthocyanidins**,** Flavanols, Phenolcarbonic acids) in wines produced from grapes of "Kakhuri Mtsvivani" and some other vine (*V. vinifera L.*) varieties (Rkatsiteli, Kakhuri Mtsvane, Khikhvi) through fermentation with complete chacha (grape skin, bunch skeleton and pips) are presented in this article. Phenol compounds are analyzed using the chromatographic methods. The role of grape skin, bunch skeleton and pips in formation of traditional Kakhetian type of wines are presented. The wine of "Kakhuri Mtsvivani" has highest content of phenol compounds and is distinguished by specific, developed aroma and taste. Compared to wines of other breeds, it possesses high antioxidant activity which indicates on high nutritious, dietary and therapeutic value of this wine.

Resümee

In der Arbeit sind wie qualitative, als auch quantitative Kennzahl der höheren Spiritus, Fettsäure, Ester, Terpene, Laktone, verdunstbaren Phenol, Aldehyde, Kohlenwasserstoff, verdunstbaren, aromabildenden Verbindungen des Traubenkammes der Weinsorte der weißen Rebe (*V.vinifera L.*) von Kakheti dargestellt, ist dargestellt die Übergangseigenschaft der einzelnen Gruppen der aromabildenden Verbindungen (besonders Terpene, Fettsäure, Laktone) vom Kamm im Wein. Die Rolle des Kammes im Prozeß der Formierung des traditionellen kakhetischen Weins, innerhalb 5 Monaten nach der Gärung. Der Wein von kakhetischen „Mtsvivani", unterscheidet sich im Vergleich zu anderen Weinsorten durch hoches Gehalt der verdunstbaren, aromabildenden Verbindungen.

Introduction

In 19th century and and XX centuries vine (*V.vinifera L.*) breed of Kakhuri Mtsvivani was spread in Telavi and Kvareli districts of Kakheti. According to Tabidze (1954), Kakhuri Mtsvivani is a very perspective vine breed and its further dissemination and trial in various microzones of Kakheti and Kartli is necessary.

Based on the abovementioned, investigation of the chemical and technological characteristics of Kakhuri Mtsvivani is of current importance. Resolution of this issue will facilitate the recovery and extension of Georgian vine gene pool, as well as broadening of the assortment of quality Georgian wines. In line with this, our task is development and refinement of technological methods for formation of various types of high quality wines from Kakhuri Mtsvivani.

Also, in order to better define breed specific characteristics of Kakhuri Mtsvivani, wines of other Kakhetian vine (*V.vinifera L.*) breeds – Rkatsiteli, Kakhuri Mtsvane and Khikhvi were investigated.

The study aimed at assessment of Phenolic compounds of wines, since these compounds are at great extent responsible for formation of aroma and taste which are typical to Kakhetian type of wines. Hence, these compounds represent very important component for wine formation and various well known researches paid due interest to investigation of these compounds (Durmishidze, 1955), (Valuiko, 1973), (Rodopulo (1971).

Phenolic compounds are actively participating in oxidation-reduction transformations which take place at different stages of wine formation, and have great influence on organoleptic characteristics of wines. This is particularly true for wines with high content of phenolic compounds. Kakhetian type wines serve as a good example of such products.

During alcoholic fermentation of Kakhetian type wines phenol carbonic acids are accumulated in the bulk of fermented mass from the hard parts of the grape. Acids are transformed through split of the aromatic rings and formation of fat acid ethyl ethers. It should be noted, that Vanilla and Violet acids are not undergoing such transformation and are transferred from the hard parts of grapes to the fermentation mass (Nutsubidze, Bezhuashvili. 1999).

Phenolic compounds at a great extent define not only the organolepthical characters of wine but also the degree of its utility. High level of antioxidant activity of Proanthocianidins, Catechins, Flavonols, Stilbens, Phenolcarbonic acids are defining the healing and prophylactic value of wine (Lijima et al. 2002), (Renaud et.al. 1991), (Chantal et al. 2005).

Materials and Methods

The research aimed at investigation of main Phenolic compounds in wines of Kakhuri Mtsvivani vine (*V. vinifera L.*) breed formed with and without chacha, wines of other kakhetian vine (*V. vinifera L.*) breeds (Rkatsiteli, Kakhuri Mtsvani and Khikhvi) and in hard parts of the above mentioned grapes (skin, bunch skeleton and pips).

The extracts (65%) from hard parts of grapes with proportion of 1:40 were prepared as a samples at the temperature of 45^0 C during 10 days.

The photo-spectrometric assessment of general Phenolic compounds was carried out by using the Pholin-Chokalteu's reagent. The ethylacetate fraction was distilled from wine for measuring the content of Catechins and polymeric Proanthocyanidins. Flavonols were tested by using the lemon-boric acid reagent. The qualitative and quantitative content of Pheniolcarbomic acids and Catechins were determined by highly effective liquid chromatograph "Varian". Components were split in column "Microsorb 100 C18", 250 mm /4,6mm/5nm (length/diameter/size of interstice) in the gradient mode. Eluent A-water/H_3PO_4 , 99, 5/05, eluent B – Acetonitrile (water) H_3PO_4 , 50/49.5/05. Detection was made on UW / Visible spectrometers at 280 nm wave length.

Sample was filtered by 0.45 M membrane filter, 20 ml was injected into the device for analysis. The antioxidant activity was determined by using the methodology of P. Gardner et al. (1998).

Results and Discussion

Wines of Kakhuri Mtsvivani grape prepared by fermentation with or without chacha appeared to have very rich phenolic content, both in terms of content and qualitative diversity (Tab.1).

The study of some groups of phenolic compounds - Proantocianidins, Catechins, Flavonols- allows comparing the Kakhuri Mtsvivani wine with wines of other grape varieties.

Compared to Kakhuri Mtsvane, wine of Kakhuri Mtsvivani contains more Catechins. Kakhetian type wine of Kakhuri Mtsvivani is also distinguished by higher content of

Flavonols (50.0 mg/l) compared to wines made without chacha (25.0 mg/l). There is relatively smaller amount of Flavonols in Kakhetian type wines of Rkatsiteli and Kakhuri Mtsvane (38.5 mg/l and 37.5 mg/l, correspondingly). Interesting results are obtained in terms of content of oligomeric and polymeric proanthocyanidinins. In Kakhuri Mtsvivani and Kakhuri Mtsvane wines oligomeric proanthocyanidins are exceeding the content of polymeric ones, while in Rkatsiteli wine both components are presented in equal amounts (+15% Mtsvane).

Wine of Kakhuri Mtsvivani, where concentration of Phenolic compounds is high, has soft, harmonic taste, with minimal feeling of bitter or tanning tones. Existence of such organoleptical conditions are attributed to the chemical composition of wine and are well explained by high content of proanthocyanidins, especially oligomeric proanthocyanidins.

Tab. 1. Content of Phenolic compounds in wines of Kakhuri Mtsvivani and grapes of other vine breeds

№	Name of Compound	Kakhuri Mtsvivani made without chacha	Kakhuri Mtsvivani made with chacha	Kakhuri Mtsvane made with chacha	Rkatsiteli made with chacha (15% Mtsvane)
1	Strength, vol. %	11.8	11.9	14.2	14.68
2	Titric acidity g/l	7.5	6.0	5.8	5.92
3	Total extract g/l	20.9	32.6	28.25	33.4
4	Total phenoles g/l	1.1	4.2	3.45	3.3
5	Proanthocianidins g/l	0.824	3.51	2.925	2.57
6	Among them: Oligomeric mg/l	436.8	1.8	1.7	1.3
7	Polimeric mg/l	387.5	1.71	1.225	1.27
8	Catechins mg/l	7.32	375.4	273.7	372.2
9	Flavonols mg/l	25.0	50.0	37.5	38.5

As it is shown in Tab. 2, Kathetian type of wine of Kakhuri Mtsvivani is characterized by high concentration of (+) Catechins (116.07 mg/l) compared to similar indexes for the wine products of Rkatsiteli from micro-zones of Kardenakhi and Kvareli (53.454 mg/l and 51.701 mg/l, correspondingly).

As well, summary quantitative content of Phenolcarbonic acids is higher in wines of Kakhuri Mtsvivani compared to wines of other varieties. For instance, content of Phenolcarbonic acids in Kakhuri Mtsvivani wines (45.497 mg/l) several times exceeds the content of these acids in

Rkatsiteli wine from Kvareli micro-zone (6.285 mg/l) and is also higher than in Rkatsiteli wine from Kardenakhi micro-zone (29.148 mg/l). The wine of Kakhuri Mtsvivani is also distinguished by higher content of Coffee, Vanilla and p-Cumar acids compared to wines of other varieties. It also contains relatively higher amounts of Vanilla (4.343 mg/l and 3.356 mg/l).

Tab. 3 represents the indexes of content of Phenolic compounds in hard parts of Rkatsiteli and Kakhuri Mtsvane grapes.

In hard parts of the grape the highest content of Proanthocianidins is found in bunch skeleton (54.3%) followed by pips (40.6%). Such difference is even more distinct in case of bunch

Tab. 2. Contents of Catechins and Phenolcarbonic acids in wines from grapes of Kakhuri Mtsvivani and other vine *(V.vinifera L.)* breeds

#	Name of compound	Kakhuri Mtsvivani European 2007	Kakhuri Mtsvivani Kakhetian 2007	Kakhuri Mtsvivani Kakhetian 2008	Rkatsiteli 2008	Kakhuri Mtsvane 2008	Khikhvi 2008	Kakhuri Mtsvivani Kakhetian 2009	Rkatsiteli Kvareli, Kakhetian 2009	Saperavi 2008
1	(+) Catechin	10.531	44.644	169.238	53.454	38.734	24.802	116.027	51.701	-
2	Chlorogen Acids	21.105	31.623	44.795	25.241	28.698	8.876	24.456	2.644	-
3	Coffee acids	8.334	23.336	11.123	1.667	3.028	2.041	14.101	1.784	-
4	Vanilla	0.25	0.437	4.343	0.383	0.693	0.221	3.356	1.321	-
5	Vanilla acids	0.506	0.783	1.264	-	-	0.945	0.375	0.151	-
6	p-Cumar acids	7.382	19.167	7.143	1.857	2.085	0.892	3.209	0.385	-
7	Total content without (+) Catechins	37.577	75.346	68.668	29.148	34.504	12.975	45.497	6.285	-
8	Antioxidant activity %	26.5	83.2	91	75	77	69.1	93	76	96.0

skeleton and pips of Kakhuri Mtsvani grape: content of proanthocyanidins in bunch skeleton is 55.7% compared to 33.2% in pips.

Existence of (+) Catechins (12.662 mg/100 ml) and (-) EpiCatechins (0.865 mg/100 ml) were identified in aqua-alcohol solution of bunch skeleton of Kakhuri Mtsvivani grapes.

Tab. 3. Admixtures of Phenolic Compounds in hard parts of grapes on dry material mg/g

#	Name of Sample		General Phenols		Catechins		Proanthocianidins	
			dry material mg/g	%	dry material mg/g	%	dry material mg/g	%
1	Rkatsiteli	Skin	15.6	7.9	1.4	2.8	4	5.1
		Bunch skeleton	88	44.3	20.6	41.7	42.8	54.3
		Pips	95	47.8	27.4	55.5	32	40.6
2	Kakhuri Mtvane	Skin	25.9	13	3.45	8.5	6.6	11.1
		Bunch skeleton	80.6	40.4	18.6	45.5	33.2	55.7
		Pips	93	46.6	18.8	46	19.8	33.2
3	Khikhvi	Skin	22.9	-	4.2	-	6.4	-
		Bunch skeleton	74.5	-	25.2	-	29.8	-
		Pips	-	-	-	-	-	-

Also, it contains Chlorogen, Coffee, P-Kumar acids and Vanilla. Among acids, the the content of Chlorogen is relatively high (0.898 mg/100 ml), followed by Coffee (0.164 mg/100ml) and p-Cumar (0.133 mg/100 ml) acids.

Antioxidant activity of Kakhuri Mtvivani grape skin is lower (21%), whereas it increases in case of bunch skeleton (75%) and pips (88%). Antioxidant activity of grape skin does not change after formation of wine fermented using traditional technology upon full chacha during 5 months, while activity of bunch skeleton and pips reduces (32% and 49%, correspondingly). These data indicate at important role of the bunch skeleton and pips in formation of rich aroma and taste of traditional Kakhetian type wine.

Antioxidant activities of wines fermented in kvevris upon full (grape skin, bunch skeleton and pips) and partial (skin and pips) chacha were compared in this study. Uniform grapes of Rkatsitely vine breed, harvested at similar year and place, were squeezed. Antioxidant activity of wine fermented with complete chacha was 87%, while for wine fermented with partial chacha it was 67%. As we can see, increase of activity from 67% to 87% can be attributed to the bunch skeleton, which indicates on important role of the latter in formation of the high dietary and healing properties of Kakhetian type wine formed in kvevri.

Kakhuri Mtsvivavi wine is characterized with high antioxidant activity: 91% (2008) and 93% (2009) and is close to same index of red grape breed of Saperavi (96%), while the antioxidant activity of wine of Rkatsiteli breed, harvested in the same place (Kvareli), is only 76%; wine of Rkatsiteli grapes harvested in other place (Kardenakhi micro-zone) has almost the same antioxidanyis even lower On the other side (microt activity (75%). Activities of wines of Khikhvi and Kakhuri Mtsvane grapes lay within the range of 69% - 77%. One can conclude that Kakhetian type wine of Kakhuri Mtsvivani vine (*V.vinifera L.*) breed is distinguished from wines of other vine breeds with distinctly high antioxidant activity, which can be considered as Kakhuri Mtsvivani vine (*V.vinifera L.*) breed's characteristic.

By organoleptic comparison of Rkatsiteli, Kakhuri Mtsvanem, Khikhvi and Kakhuri Mtsvivani we have concluded that wines from grapes of Kakhri Mtsvivani vine (*V.vinifera L.*) breed, fermented both with and without chacha should be attributed to the group of wines with high values.

Kakhetian type wine of Kakhuri Mtsvivani is characterized with golden-light tea colors, more developed breed specific aroma and taste, harmonicity, taste and aroma of dry fruits, pleasant tanning tones, orderliness and high quality.

Conclusions

Based on the analysis of experimental data one can conclude the following:

Wine of Kakhuri Mtsvivani vine *(V.vinifera L.)* formed both with and without chacha is distinguished with rich, developed taste and aroma. Wine formed with chacha characterized with specific, pleasant herb tones (which is not felt in wine formed without chacha). Wine gains such specific aroma from the bunch skeleton. This property, as well as other chemical - technological characteristics of Kakhetian type Wine of Kakhuri Mtsvivani distinctly distinguishes it from wines of other vine breeds.

Kakhetian type Wine of Kakhuri Mtsvivani the content of compounds of all Phenolic groups - Catechins, oligomeric and polimeric Proanthocianidins, Flavonoles, Phenolcarbonic acids - is higher compared to grape wines of other vine breeds. This fact could determine at a great extent strong, rich and distinct aroma and taste of Kakhuri Mtsvivani wines.

The main source for enrichment of Kakhetian type wines with Phenolic compounds are the bunch skeleton and pips of the grape. The quantitative contents of total Phenols in bunch skeleton and pips are almost similar. Possibly relative differences could be observed for

individual vine breeds. The content of Proanthocianidins is much higher in bunch skeleton than in pips, which significantly raises importance of the bunch skeleton in formation of the soft, velvet taste and high antioxidant activity of Kathetian type wines.

Antioxidant activity of Kakhatian type wines of Kakhuri Mtsvivani is quite high compared to grape wines of other vine varieties and comes close to antioxidant activity index of Saperavi red wine. This fact increases the dietary, nutritious and healing values of Kakhatian type wines of Kakhuri Mtsvivani.

Making Kakhetian type wines by traditional technology (formation of wine with complete chacha during 5 months after fermentation) determines high antioxidant activity of Kakhuri Mtsvivani wine, its softness, harmonicity, refined specific aroma and taste. It indicates rich and many-sided potential of Kakhuri Mtsvivani vine *(V.vinifera L.)* breeds.

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Bibliography

1. Chantal C.M., Apoldoom A., Bonnefoy Bianca C. H.Lutters, K. Daenens, Theo I.C. van Berkel, M.F. Hoylaeters, Eric A.I. Biessen. Gallic Acid Antagonizm P-Selection-Mediated Platelet-Leukocyte Interactions. Circulation. 2005, vol. 111, pp. 106-112

2. Durmishidze S., 1955, Tanning agent and antocianids in grape and wine. Publication of USSR Science Academy, Moscow, p. 300 (in Rassian)

3. Gardner P., Mc Phail D., Duthie G., 1998,. Elektron spin resonsnce spektroskopie assessment of the antioxidant potential of teas in agueous and organic media. J. Sci Food Agric, 257-262.

4. Lijima K., Yoshizumi M., Ouchi Y., 2002. Effect of red wine polyphenolics on vasculer smooth muscle cell function – mollecularmechanism of the "French paradox". // Mech. Ageing Dev.. 123, pp. 1033-1039.

5. Shalashvili A., Zambakhidze N., Targamadze I., Ugrekhelidze D., 2004, Quantitative Changes of Phenolicic Compounds and their Free Radical Scavening Capaciti at Ageing of Georgian White Wine. Bulletin of the Georgian Academy of Sciences. 2004, 170, #2, pp. 376-378

6. Nutsubidze R., Bezhuashvili M., 1999 Formation of Kakheti Sort Wine Phenolicocarbon Acids. R. "Vazi da Gvino" (Vine and Wine). s. 38 (in Georgian)

7. Renaud S., De Lorgeril M., 1991. Wine, alcohol, plate lets and the French paradoz for coronary heart disease. Lancet. 339, pp. 1523-1526

8. Rodopulo A.,1971, Bio chemistry of wine-making, M. "Nourish Industry", p. 372 (in Rassian)

9. Tabidze D., 1954, Kakhuri Mtsvivani, breeds of Kakhuri vine, Tbilisi, p.204-211 (in Georgian)

10. Valuiko G., 1973, Biochemistry and technologic of red wine, Yalta, p.296 (in Rassian)

MÉTODO DE MEDICIÓN DIGITAL DE LA MADUREZ FENÓLICA DE LA BAYA DE VID. APLICACIÓN EN LA VITIVINICULTURA DE PRECISIÓN PARA ELABORAR VINOS DE EXCELENCIA.

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RESUMEN

La vitivinicultura de precisión se enfrenta al problema de conocer de manera precisa el grado de madurez de la uva y determinar el momento exacto de la vendimia. Se desarrolla un nuevo método basado en el análisis de la imagen digitalizada para la evaluación objetiva de la madurez fenólica de la vid, a través de la evolución del color superficial de la baya. Se trabaja con softwares, evaluando el grado de correlación con métodos tradicionales de laboratorio. El método propuesto es no destructivo, lo que permite que una misma muestra pueda ser evaluada con captura de imagen digital y a continuación, con métodos usuales de laboratorio. La evolución del color superficial en función de tiempo, en *Vitis vinifera var. Cabernet Sauvignon,* generó una curva polinomial con un elevado índice de correlación de *R=0,9985*.

MÉTHODE DE MESURE DE LA MATURITÉ PHÉNOLIQUE DE LA BAIE DE VIGNE. DEMANDE DE PRÉCISION DANS L'ÉLABORATION DU VIN D'EXCELLENCE.

RESUME: La viti-viniculture de précision fait face au problème de connaître de manière a besoin du degré de maturité du raisin et déterminer le moment précis de la vendange. On développe une nouvelle méthode basée l'analyse de l'image digitalisée pour l'évaluation objective de la maturité phénolique de la vigne, à travers l'évolution de la couleur superficielle de la baie. On travaille avec des softwares, en évaluant le degré de corrélation avec des méthodes traditionnelles de laboratoire. La méthode proposée est non destructive, ce qui permet qu'un même échantillon puisse être évaluée avec capture d'image numérique et ensuite, avec méthodes habituelles de laboratoire. L'évolution de la couleur superficielle en fonction de temps, en *Vitis vinifera* cv. *Cabernet Sauvignon*, a produit une courbe polynomial avec un important indice de corrélation de *R=0,9985.*

MEASUREMENT METHOD OF DIGITAL PHENOLIC MATURITY OF THE BERRY VINE. APPLICATION IN WINEMAKING PRECISION TO DEVELOP WINES OF EXCELLENCE.

SUMMARY: Precision viticulture faces the problem to know exactly the grape maturity degree and to determine the exact moment of vintage. A new method based on the digital image analysis for the objective evaluation of the phenolic maturity of the grapevine is developed, through the evolution of the superficial color of the berry. One works with softwares, evaluating the degree of correlation with traditional methods of laboratory (Method of Glories). The proposed method is nondestructive, which allows that a same sample can be evaluated with capture of digital image and next, with usual methods of laboratory. The evolution of the superficial color in *Vitis vinifera* cv. *Cabernet Sauvignon*, generated a polinomial curve with a high index of correlation of *R=0,9985.*

INTRODUCCIÓN

La vendimia implica un conjunto de componentes que afectan al sector vitivinícola, siendo la incertidumbre el sentimiento dominante en el momento de la cosecha, debido fundamentalmente al problema de disponer de información exacta acerca del grado de madurez de la uva. El seguimiento del proceso de maduración por técnicas analíticas de laboratorio es una labor muy compleja, por la diversidad de procesos simultáneos involucrados. Se cuantifican usualmente tres aspectos: madurez fenólica, madurez celular (fragilidad de las estructuras celulares de los hollejos que condiciona la cinética de difusión de los fenoles) y madurez de las pepitas que disminuye la cantidad y la agresividad de los taninos liberados (Flanzy, 2000). Los controles de madurez que deciden la fecha de la vendimia se basan en la determinación del grado alcohólico probable y la acidez (factores fácilmente modificables en la bodega), mientras que la falta de madurez de la piel y de las semillas difícilmente podrán ser compensadas. Existen diversas metodologías para la determinación de la madurez fenólica. Sin embargo, la experiencia práctica en la aplicación de esta metodología indica que existe una gran variabilidad.

Los compuestos fenólicos son la clave del encanto gustativo de los vinos tintos y el conocimiento de su evolución permitiría adoptar criterios adecuados para lograr vinos de excelencia. La concentración de antocianos se incrementa durante la maduración de la baya hasta alcanzar un punto máximo, a partir del cual disminuye ligeramente, mientras que la astringencia de los taninos de la piel disminuye a medida que avanza la maduración.

Los complejos cambios físicos, químicos y enzimáticos, que se producen en las diferentes zonas de la baya de la vid (hollejo, pulpa y semilla) tendrían su correlación en cambios de color y de textura en su superficie durante el período de maduración. Las paredes externas de las células epidérmicas del grano de uva están revestidas por la cutícula y las ceras epicuticulares, delgadas fibrillas que sirven de alojamiento a las levaduras. Las ceras refractan la luz solar e inciden en la coloración de la baya de uva. Se inician como pequeñas plaquetas verticales en el momento de la floración, para alcanzar 0,1µ de diámetro a la madurez, con bordes lobulados, desordenadamente superpuestas formando una capa hidrofóbica de varios micrones de espesor (Formento *et al*., 1998 y 1999; Lúquez y Formento, 2002). El análisis de imagen digital del grano de uva permite analizar su espectro de reflexión, que variará según los pigmentos fotosintéticos (clorofilas y carotenoides) y compuestos polifenólicos presentes. Las clorofilas se degradan durante el momento del envero, lo que coincide con la aparición de los antocianos en las vacuolas, haciendo evidentes los tonos rojos, azul y violeta. Estos cambios superficiales de color son signos externos de profundos cambios químicos y enzimáticos producidos en las células de la piel, de la pulpa y de las semillas de la baya.

OBJETIVOS

El propósito es registrar en forma *objetiva*, a través del análisis de imágenes digitales y utilizando softwares computacionales, los cambios de color que se producen en la vid a medida que avanza el grado de madurez de la baya y correlacionar estos datos con los índices usuales obtenidos. Se busca expresar los diferentes grados de madurez del fruto a través de índices numéricos para determinar con exactitud el período óptimo de cosecha.

MATERIAL Y MÉTODO

Se procedió a seleccionar plantas de vid en un espaldero cultivado con la variedad *Cabernet Sauvignon*, ubicado en la parcela experimental de la Facultad de Ciencias Agrarias, UNCuyo, a 960 msnm (33° 00’ 38,23’’ S; 68° 52´37,58’’ O), en la localidad de Chacras de Coria, Luján de Cuyo, Mendoza, Argentina. Se realizó el muestreo aleatorio de 75 plantas de vid, debidamente identificadas y rotuladas Se extrajeron además muestras en propiedades

particulares del Departamento de Maipú, dentro de la primera zona vitícola de nuestra provincia. Se llevó a cabo un registro de las distintas fases fenológicas del cultivo, así como de la marcha de las labores culturales y tratamientos fitosanitarios realizados. Las muestras fueron recolectadas manualmente de las plantas marcadas y previamente seleccionadas. Se extrajeron a intervalos regulares durante los meses de enero a mayo, cada 10 días durante las primeras fases de desarrollo del grano de uva, para reducir luego el intervalo de tiempo a 5 días en envero y 3 días a medida que se acercaba la madurez tecnológica. Cada muestra se extrajo por triplicado, utilizando tijeras y pinzas, procediendo a colocarlas en recipientes de plástico para evitar cualquier tipo de manipulación que pudiera afectar el estado de la pruina superficial del grano. Se obtuvieron aproximadamente 400 bayas en cada muestra.

Inmediatamente de la cosecha, los frutos se colocan en bandejas, realizando la captura de su imagen digital.Al fragmentar la imagen, el número de pixeles evaluados es de 2 millones (2 x 10^6). Debido a que las unidades experimentales grandes presentan menor variación que las pequeñas, y a que en este caso, el número de datos experimentales disponible es elevado, nos inclinamos por realizar un número de 4 repeticiones para cada bloque. Los ensayos se llevaron a cabo en un laboratorio con temperatura ambiente de 25ºC. De cada imagen digital se obtuvo una *Tabla de Distribución de Frecuencias*, donde los valores de intensidad de reflexión para cada uno de los colores del sistema RGB (rojo, verde y azul), se hallaban discriminados según el sector fragmentado en la imagen original.

Medición objetiva del color

Este nuevo método de mensura refleja de un modo objetivo la evolución del color sin afectar la integridad de la baya y por lo tanto, ésta puede ser utilizada para la valoración de polifenoles según las técnicas tradicionales de laboratorio. El sistema RGB permite desglosar la información contenida en una imagen digital, en tres canales independientes, que representan los tres colores primarios, rojo, verde y azul, cuya adición es el color reflejado por un objeto. Por tratarse de una imagen digital, la intensidad de luz reflejada puede convertirse en valores numéricos, en una escala de cero (reflexión nula) a 255 (reflexión máxima). Estos valores pueden ser graficados y analizados, estableciendo correlaciones entre los diferentes variables del proceso, tales como grado de madurez. La imagen digital capturada es del tipo BMP de 24 bits, de forma rectangular, con 2304 X 1728 pixeles de lado, lo que da un valor de 3.981.312 pixeles evaluados en cada imagen. Debido a que la imagen se fragmenta y se consideran solo una parte de los valores de intensidad de reflexión, el número de pixeles evaluados se reduce a un número de alrededor de *2.000.000 pixeles* en cada imagen analizada.

Se fotografió el material seleccionado en el momento de la extracción de las muestras, utilizando una cámara digital y luego las imágenes se procesaron en computadora.

Segmentación de la imagen.

Se seleccionó un rango de intensidad luminosa que abarca totalmente la superficie dentro de la cual se encuentran las bayas objeto de nuestro estudio, y elimina los reflejos indeseables, que podrían alterar los resultados.

Obtención del Índice de Intensidad de Reflexión.

Se obtiene *Índice de Intensidad de Reflexión* (IIR) que representa la intensidad de cada color en la imagen digitalizada, considerando el número total de pixeles en el rango segmentado 0-150 equivalente a la unidad. Su fórmula es:

$$IIR = \sum_{J=1}^{0-150} [\text{valor de intensidad de reflexión}_{(j)} \bullet \text{área relativa}_{(j)}] \qquad \textbf{(1)}$$

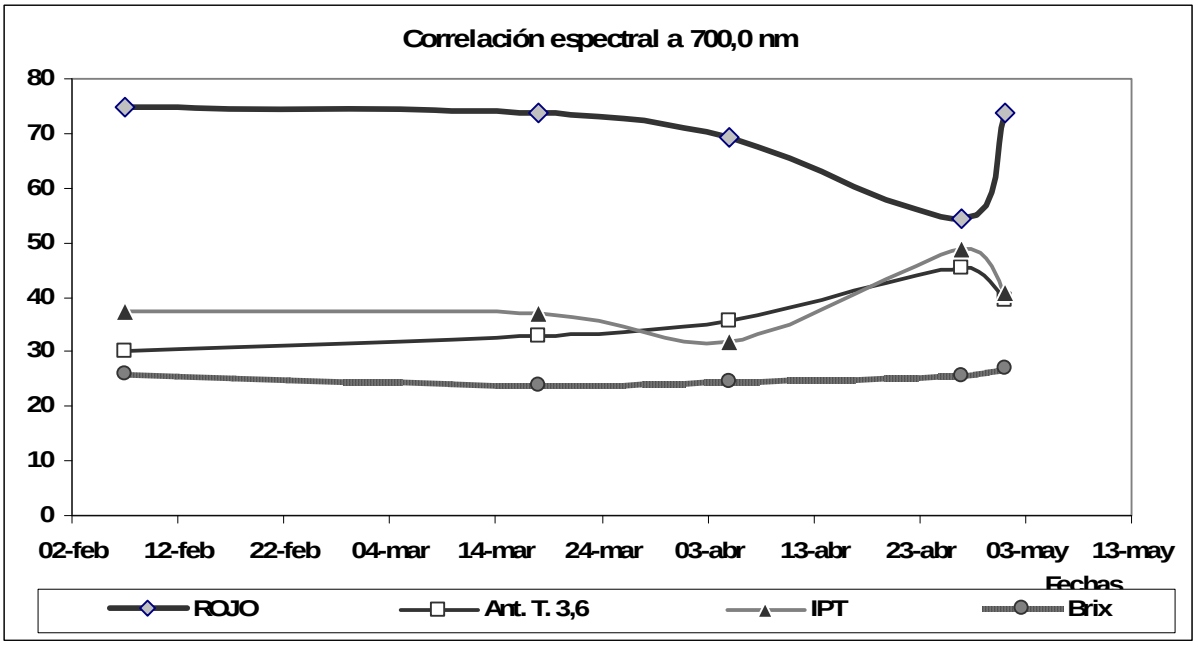
Fotografía N°1: Modelo de Imagen digital obtenida.

Procesamiento de datos.

Se obtuvo una lista de datos para cada una de las imágenes digitales analizadas, con su correspondiente tabla de distribución de frecuencias. Los Índices de Intensidad de Reflexión (IIR) para cada color primario, se correlacionaron con los datos analíticos obtenidos en los métodos tradicionales de análisis de polifenoles. Una vez obtenida la imagen digital, las bayas se analizaron según el *Método de Glories modificado*, a fin de medir antocianos y polifenoles totales extraíbles a pH 1 y pH 3,6.

RESULTADOS:

Fig. 1: Evolución de IIR a 700,0 nm en Bayas de Vid



CONCLUSIONES

La evolución del color superficial en la baya en función de tiempo generó una curva polinomial. El análisis de regresión de los valores del Índice de Antocianos Totales en función de la variación del Índice de Intensidad de Reflexión (IIR) a 546 nm, generó una curva polinomial de 3° grado, con un *elevado índice de correlación de* ***R=0,9966***. El IIR a 435,8 nm produjo un *R=0,9782* y el IIR a 700,0 nm un *R=0,*9568.

El análisis de regresión de la evolución del Índice de Polifenoles Totales (IPT) en función de la variación del Índice de Intensidad de Reflexión (IIR) a 700,0 nm y del tiempo, generó una curva polinomial de 3° grado, con un *elevado índice de correlación de* ***R=0,9985***. El IIR a 435,8 nm produjo un *R=,9072* y el IIR a 546 nm un *R=0,*808.

Se está trabajando en parcelas comerciales de las variedades *Malbec* y *Cabernet Sauvignon*, de dos regiones ecológicamente diferentes, para ajustar el método propuesto a escala industrial.

BIBLIOGRAFÍA

AGUILERA, J.M.; K. OPPERMANN and F. SÁNCHEZ. 1987. Kinetics of browning of Sultana Grapes. *Journal of Food Science* **52** (4): 990-1025.

BARANOWSKY, E. S. y NAGEL, C. W.. 1983. Kinetics of malvidin-3-glucoside condensation in wine model systems. *J. Food Science* **48**: 419.

CASTELLARI, M.; G. ARFELLI; C. RIPONI and A. AMATI. 1998. Evolution of phenolic compounds and red winemaking as affected by must oxigenation. *Am. J. Enol. Vitic.* **49** (1): 91-94.

FLANZY, CLAUDE (Coordinador). 2000. *Enología: Fundamentos Científicos y Tecnológicos*. A. Madrid Vicente Editores y Ediciones Mundi-Prensa, Madrid (España), 800 pp.

FORMENTO, J. C.; RODRÍGUEZ, J.; GALIOTTI, H.; PALADINO, S. y LÚQUEZ, C. 1999. Mosto concentrado tinto. Nueva variedad de vid para su elaboración. *La Semana Vitivinícola* (Sevilla, Valencia, España), **2.763/64** (24/31 de julio).

LEHTONEN P. J., M. M. ROKKA, A. I. HOPIA and I. M. HEINONEN. 1999. HPLC Determination of phenolic compounds in berry and fruit wines and liqueurs. *Vitic. Enol. Sci*. **54** (2-3): 33-38

LITTLE, A. C. Y G. MACKINNEY. 1972. The color of foods. *World Rev. Nutr. Diet.* **14**: 59-84.

LÚQUEZ, C. V. y J. C. FORMENTO. 2002. La flor y el fruto de la vid (*Vitis vinifera* L.).- Micrografía aplicada a la Viticultura y la Enología. *Revista de la Facultad de Ciencias Agrarias, U.N. Cuyo*, **XXXIV** (1): 109-121.

RIBÉREAU-GAYON, P. 1968. *Les composes phenoliques des végétaux.* Dunod, Paris (Francia), 254 pp.

ROBARDS, K.; P. D. PRENZLER; G. TUCKER; P. SWATSITANG and W. GLOVER. 1999. Phenolic compounds and their role in oxidative processes in fruits. *Food Chemistry* **66**: 401-436.

STUDIO DELLE CAPACITÀ COMPETITIVE DI DUE CEPPI DI *SACCHAROMYCES CEREVISIAE* IN DIVERSE CONDIZIONI DI FERMENTAZIONE

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RIASSUNTO

I nuovi metodi molecolari permettono di discriminare il lievito *Saccharomyces cerevisiae* a livello di ceppo. Obiettivo del lavoro è l'applicazione della tecnica di amplificazione multiplex dei microsatelliti e successiva elettroforesi su gel di agarosio e poliacrilamide, nello studio delle capacità competitive di due ceppi di lievito co-inoculati durante la fermentazione alcolica. Preventivamente, la tecnica è stata testata su un gruppo di lieviti selezionati, al fine di verificarne la capacità discriminatoria. È stato saggiato un panel di ceppi commerciali di lievito oltre al ceppo modello di *Saccharomyces cerevisiae,* utilizzato come riferimento. Successivamente sono state allestite fermentazioni in diverse condizioni di temperatura e concentrazione di nutrienti, per verificare l'effetto di tali parametri sullo sviluppo di due ceppi commerciali co-inoculati. I risultati dimostrano che la temperatura è il parametro, tra quelli studiati, che influenza maggiormente lo sviluppo e l'interazione tra i due ceppi anche se la capacità competitiva di uno di essi risulta superiore in ogni condizione di fermentazione.

ABSTRACT

The new molecular methods allow the discrimination of *Saccharomyces cerevisiae* at strain level. Te aim of this work is to study the competition of two strain co-inoculated during the alcoholic fermentation in different conditions, using the multiplex microsatellite PCR technique and electrophoresis with agarose and polyacrylamide gels.

At first, a discriminatory test was carried out. A panel of commercial wine strains plus the *Saccharomyces cerevisiae* type strain were analyzed. Afterward, fermentation trials with different nutrient concentration and temperature were set, to study the effect on the competitive abilities of two co-inoculated strains. The results show that, among the parameters studied, the temperature has the strongest influence on the interaction between the strains. Although this, the competitive ability of one strain results to be higher in all condition tested.

INTRODUZIONE

In vinificazione, i ceppi di lievito selezionati, sono inoculati in un mosto non sterile dove sono presenti numerosi ceppi indigeni di *Saccharomyces* e *non-Saccharomyces*. La dinamica della popolazione dei lieviti selezionati dipende dalla loro capacità di dominare il processo fermentativo ed è influenzata dai fattori ambientali.

Pochi lavori sono disponibili in letteratura riguardanti gli aspetti di tale competizione in particolare sulle interazioni intraspecifiche tra ceppi di *S.cerevisiae*, e come queste sono influenzata dal variare dei parametri chimici del mosto e dalle diverse tecniche di fermentazione. Probabilmente questo è dovuto alla difficoltà nel reperire un metodo affidabile e veloce che permetta la caratterizzazione a livello di ceppo.

Negli ultimi anni, gli studi di discriminazione intraspecifica si sono focalizzati sulle metodologie molecolari basate sul DNA (descritte recentemente nella review di Beh *et al.*

2005) L'analisi dei microsatelliti, basata sul polimorfismo di lunghezza di loci caratterizzati da semplici sequenze ripetute, è stata usata con successo per discriminare ceppi di *S.cerevisiae* (Field e Wills 1998; Gonzalez Techera *et al.* 2001) e, recentemente, la tecnica PCR multiplex di microsatelliti con alto grado di polimorfismo, ha permesso di discriminare un grande numero di ceppi commerciali (Schuller *et al.* 2004; Richards *et al.* 2009). Il potere discriminante, unito alla robustezza ed applicabilità sul DNA grezzo ha reso la PCR dei loci microsatellitari uno dei metodi più popolari ed usati.
In questo lavoro è stata applicata la tecnica di discriminazione intraspecifica di *S. cerevisiae* basata sulla PCR multiplex di tre loci microsatellitari, seguita dall'analisi dell'amplificato con corsa elettroforetica su gel di agarosio e poliacrilamide (Vaudano *et al*. 2008) osservando dapprima la capacità discriminatoria del metodo su di un panel di ceppi commerciali di *S. cerevisiae*. Il metodo è stato successivamente applicato in prove di fermentazione dove è stato osservato l'effetto di tre parametri critici nella fermentazione quali la temperatura, la concentrazione di APA (Azoto Prontamente Assimilabile) e la concentrazione di zuccheri sulla cinetica di sviluppo e sulle capacità competitive di due ceppi co-inoculati.

MATERIALI E METODI

Ceppi di S. cerevisiae e terreni

Sono stati utilizzati ceppi selezionati di *S. cerevisiae*. Questi, denominati con il prefisso ISEIND, sono stati acquistati da fornitori di lieviti vinari ad eccezione del ceppo tipo (type strain) CBS 1171 (ceppo ISEIND30) proveniente dalla collezione conservata presso la sezione di microbiologia enologica del CRA-Centro di Ricerca per l'Enologia di Asti.
I ceppi di lievito commerciali sono stati sottoposti a reidratazione in una soluzione sterile al 5% di saccarosio per 30 min a 40°C. In seguito, i lieviti sono stati diluiti in soluzione fisiologica, distribuiti in piastre contenenti il terreno YMA (yeast malt agar) ed incubati a 24°C per 48 h.

Metodi di estrazione del DNA

Nella prima parte del lavoro, riguardante l'applicazione del metodo nella discriminazione dei ceppi commerciali, il DNA è stato estratto da colonia secondo il metodo descritto da Harju *et al.* (2004).
Durante la seconda parte del lavoro, nella applicazione del metodo per distinguere i ceppi in prove di competizione, la PCR è stata eseguita direttamente su cellule prelevate da colonia mediante il puntale di una micropipetta e risospese nella mix per PCR, preventivamente distribuita in piastre da 96 pozzetti da 0,2 mL.

Analisi PCR

I loci microsatellitari utilizzati in questo studio, SC8132X, YOR267C e SCPTSY7, sono stati scelti per il loro alto grado di polimorfismo (Field e Wills, 1998; Gonzalez Techera *et al.*, 2001). L'amplificazione è stata eseguita utilizzando coppie di primer specifici secondo il metodo di Vaudano *et al.* (2008)
Gli amplificati sono stati analizzati con elettroforesi in gel di agarosio MS-8 (Eppendorf AG, Hamburg, Germany) a 2.5% di dimensioni 15 X 10 cm in buffer TBE (tris borato EDTA) applicando un campo elettrico di 100 V a voltaggio costante per 80 min ed il gel è stato colorato con etidio bromuro.
L'elettroforesi con poliacrilamide è stata eseguita utilizzando un gel pronto all'uso (precast) al 10% in TBE delle dimensioni di 8.6 X 6.8 cm applicando 100V a voltaggio costante per 60 min.; come marcatore molecolare è stato usato il set 100-20 bp (Sigma).
I gel sono stati analizzati con il software di analisi dei cluster Bionumerics (Bionumerics, Applied Maths, Keistraat, Belgium) utilizzando l'indice di similarità Dice e il dendrogramma

è stato costruito con il metodo UPGMA (Unweighted Pair Group Method with Arithmetic Mean).

Fermentazioni

Per le prove di fermentazione a diversa temperatura sono stati scelti i ceppi ISEIND3 e ISEIND5 in quanto possiedono caratteristiche ed applicazioni simili come indicato dai produttori nella scheda tecnica, quali l'utilizzo nella fermentazione in bianco, la tolleranza all'alcool e il fenotipo killer. Le fermentazioni sono state eseguite in duplicato e sono state preparate utilizzando mosto d'uva sterile (Biotta AG CH-8274). Sono stati utilizzati come nutrienti azotati, il solfato di ammonio e il fosfato di ammonio. Il saccarosio è stato addizionato sotto forma di soluzione al 50%, fino a raggiungere le concentrazioni previste di zuccheri totali.

I due ceppi sono stati distribuiti in agar e una colonia per ceppo è stata testata con la tecnica PCR per controllarne l'identità. In seguito la stessa colonia identificata è stata inoculata singolarmente in un mezzo di precoltura costituito da mosto d'uva sterile. Il co-inoculo delle fermentazioni è stato effettuato dopo 24 h di crescita aerobica nel mezzo di precoltura a 20°C in agitazione ed è stato eseguito in ragione di 0.5 X 10^6 cellule/mL di ciascun ceppo. Le fermentazioni sono state eseguite in doppio per ogni condizione. Parallelamente sono state allestite le prove in monocultura in ogni condizione, per verificare la capacità dei due ceppi di completare singolarmente la fermentazione.

I campionamenti sono stati eseguiti dopo 10 min dall'inoculo, al 66% di zuccheri residui, al 33% di zuccheri residui e alla fine della fermentazione (< 2g/L di zuccheri). Per quanto riguarda la fermentazione condotta con una concentrazione zuccherina di 250 g/L, i campionamenti effettuati sono stati 5 (100, 75, 50, 25, 0 % di zucchero residuo) poiché il termine di essa si attesta intorno ai 16° alcolici. La concentrazione zuccherina è stata determinata attraverso l'analisi HPLC utilizzando la colonna Polysphere OAKC Merck, Darmstadt, Germany) con detector rifrattometrico.

I campioni sono stati diluiti distribuiti in piastre contenenti il terreno YMA. Dopo 48 h d'incubazione a 25°C, le colonie sono state scelte in modo randomizzato per l'analisi PCR, campionandole da piastre contenenti da 150 a 300 colonie. Per ogni campionamento, sono state saggiate in PCR 40-60 colonie verificando la quantità relativa dei due ceppi. In totale sono state analizzate circa 2000 colonie.

RISULTATI E DISCUSSIONE

Analisi dei ceppi commerciali

Nelle Fig. 1 è mostrata l'analisi computerizzata effettuata con il software Bionumerics delle corse elettroforetiche su gel d'agarosio e di poliacrilamide, relative ai prodotti di amplificazione dei ceppi oggetto di studio.

Da uno sguardo generale, la corsa elettroforetica con poliacrilamide mostra un maggior numero di bande, soprattutto di dimensioni superiori a 500 bp, indicate solitamente come artefatti (Sambrook *et al.* 1989). Nell'ambito delle dimensioni compatibili con l'amplificazione di microsatelliti, sia nell'elettroforesi con agarosio sia in quella con la poliacrilamide sono presenti bande più o meno intense. Le bande meno intense sono dovute probabilmente ad una amplificazione non specifica. Per la comparazione non sono state considerate le bande con intensità inferiore al 5% dell'intensità massima rilevata e di dimensioni inferiori a 500 bp. In entrambe le analisi é possibile osservare differenze tra i ceppi in numero di bande e relative dimensioni. Alcuni ceppi presentano solamente tre bande intense ma la maggior parte mostra un quadro più complesso, causato, probabilmente, dalla

diversa ploidia e dalla presenza di omozigosi o eterozigosi per i tre locus. (Bakalinsky e Snow, 1990).
L'analisi computerizzata effettuata utilizzando le due corse elettroforetiche insieme (Fig. 1) mostra un grado di similarità dei ceppi ceppo ISEIND2 ceppo ISEIND9 pari al 100%; nella figura è infatti possibile osservare la totale identità del profilo ottenuto con i due gel. Inoltre, è stata rilevata una similarità superiore all'90% tra i ceppi 51-6 che tuttavia sono distinguibili ad una attenta osservazione. In particolare il ceppo 51 presenta, nella corsa su poliacrilamide, due bande più deboli comprese tra 500 e 400, dovute all'amplificazione del locus YOR267C che lo distingue dall'altro ceppo del cluster.
L'analisi mostra ancora una similarità superiore all'90% per il cluster composto da i ceppi 43-44-45-41-42-47. All'interno di questo cluster i ceppi 43-44-45 e la coppia 42-47 risultano identici (similarità 100%). In questo caso, essendo questi ceppi ibridi *S. cerevisiae X S.bayanus* l'omologia tra i profili di ceppi diversi può essere spiegata se il *S.cerevisiae* parentale è sempre lo stesso ceppo. Infatti l'amplificazione avviene solo sul DNA di *S.cerevisiae* e non su *S. bayanus*. Nel caso di un ibrido, supponendo la diploidia, è amplificato solo il DNA sul cromosoma omologo parentale derivante da *S. cerevisiae*.
La totale identità tra i ceppi 2 e 9 potrebbe essere dovuta ad un limite di risoluzione del metodo oppure ad una reale corrispondenza di due ceppi venduti da due case produttrici e aventi nomi commerciali diversi. Questa ultima ipotesi è stata riportata in alcuni lavori (Schuller *et al.* 2004; Fernandez-Espinar *et al.* 2001) come possibile spiegazione in situazioni analoghe di identità tra ceppi.

Effetto della temperatura
Per quanto riguarda le prove di fermentazione a 15°C (Fig. 2a), si nota come il ceppo 5 abbia dominato rapidamente la fermentazione; al secondo campionamento, in corrispondenza del 30% del consumo di zucchero, esso raggiunge già il 70% della popolazione totale e alla fine della fermentazione domina completamente, con il 100% di frequenza relativa mentre il ceppo 3 scompare del tutto. Nella fermentazione a 20°C (Fig. 2b), il comportamento dei due ceppi è differente. Durante la prima parte della fermentazione, i ceppi co-dominano con una leggera prevalenza del ceppo 3 sul ceppo 5, mentre nella seconda parte il ceppo 5 prende il sopravvento nella fermentazione. Nell'ultimo campionamento, quando lo zucchero è esaurito, il ceppo 5 rappresenta la quasi totalità della popolazione anche se il ceppo 3 non sparisce completamente.Nella prova a 25°C (Fig. 2c), sembra riproporsi, nella prima parte della fermentazione, quanto osservato nella fermentazione a 15°C, con una rapida prevalenza del ceppo 5 che raggiunge, al 60% di zuccheri consumati, l'85-90% della popolazione totale. Nell'ultima parte della fermentazione si nota un recupero del ceppo 3 che rappresenta, a fine fermentazione, il 20% circa della popolazione totale.
Si assiste, quindi, in tutte e tre le fermentazioni ad una maggiore capacità competitiva del ceppo 5 che, tuttavia, domina le fermentazione in modo diverso a seconda della temperatura.

Effetto dell'ammonio
Nella figura 3 sono mostrati dati relativi alle prove di fermentazione approntate per verificare l'influenza della concentrazione dell'ammonio sui due ceppi utilizzati. Confrontando la fermentazione in cui sono stati addizionati 300 mg/L di sali ammonici (Fig 3a) con la fermentazione in cui non è stato addizionato ammonio (Fig. 3b) è possibile riscontrare come le frequenze relative dei due ceppi non mostrino differenze sostanziali. Fino al 30% di zucchero consumato, l'assenza di ammonio aggiunto sembra favorire debolmente il ceppo 5, mentre nella seconda parte, la ripartizione tra i due ceppi appare molto simile nelle due prove.

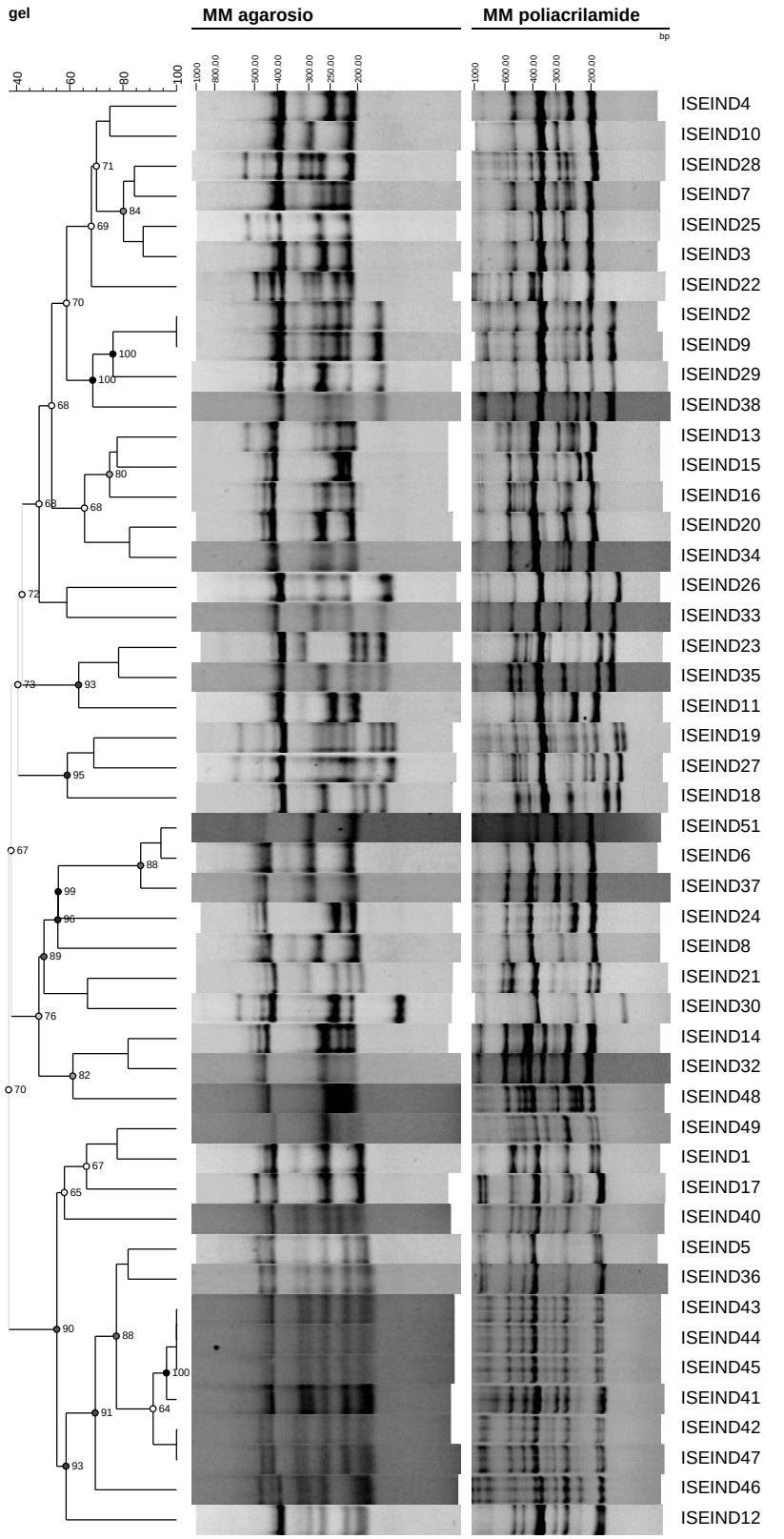

Fig. 1. Analisi delle corse elettroforetiche in agarosio e poliacrilamide con il software Bionumerics.

Al termine della prova, il ceppo 5 risulta essere dominante sul ceppo 3 che comunque non sparisce rimanendo presente nell'ordine di circa il 15-20%.

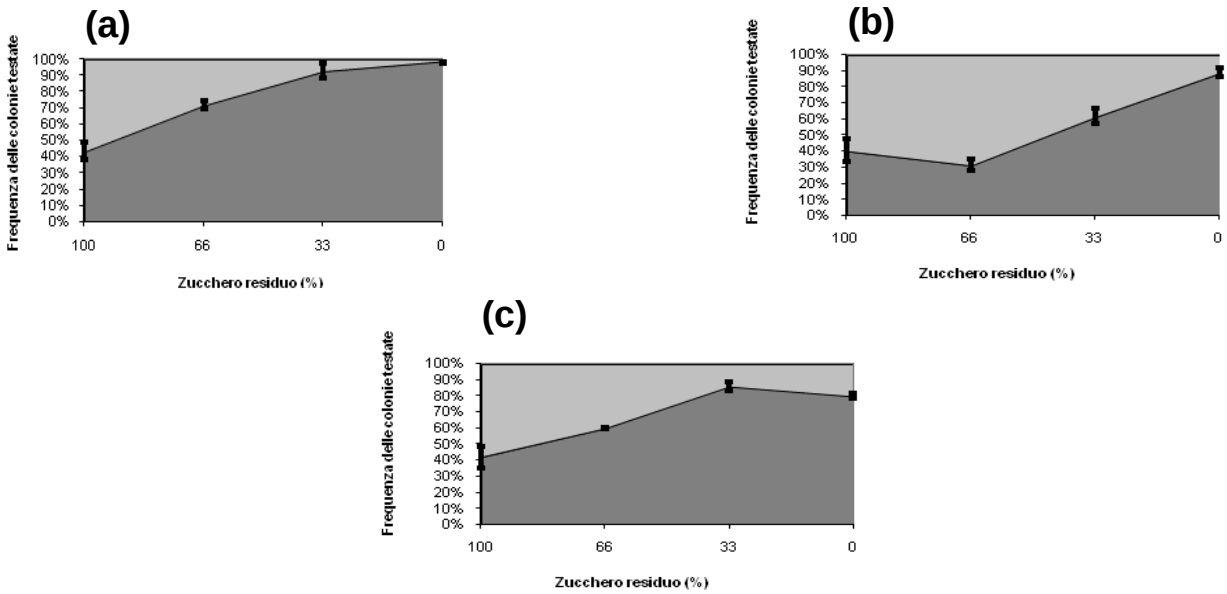

Fig. 2. Fermentazioni condotte con il co-inoculo dei due ceppi. (a) fermentazione a 15°C. (b) fermentazione a 20°C. (c) fermentazione a 25°C. Ceppo 5: ■. Ceppo 3: ■.

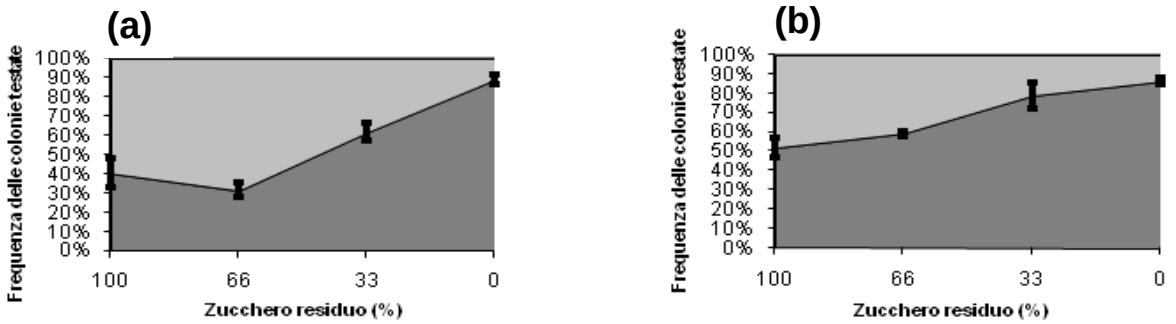

Fig. 3. Fermentazioni condotte con il coinoculo dei due ceppi. (a) fermentazione con 300 mg/L di attivante ammonico. (b) fermentazione senza attivante ammonico. Ceppo 5: ■. Ceppo 3: ■.

Effetto della concentrazione zuccherina

Anche nella prova di fermentazione eseguita a 250 g/L di zucchero (Fig. 4b), è possibile riscontrare una fase di co-dominanza dei due ceppi nella fase iniziale della fermentazione e una dominanza del ceppo ISEIND5 nella fase centrale e terminale di essa. L'interruzione della fermentazione, che si è arrestata a 14° alcolici dopo una lunga fase di rallentamento, sembra avere prodotto, tuttavia, un arresto nello sviluppo del ceppo ISEIND5.

Da uno sguardo generale alle prove di competizione risulta chiara la maggiore capacità competitiva del ceppo ISEIND5 rispetto al ceppo ISEIND3 che tende a soccombere in tutte le prove di fermentazione. Le variabili ambientali studiate sembrano influenzare soprattutto la prima parte della fermentazione. La temperatura sembra il fattore in grado di influenzare

maggiormente lo sviluppo e l'interazione dei due ceppi di *S. cerevisiae*. Per quanto riguarda questa variabile, il ceppo ISEIND5, dominando l'intera prova a 15°C, ha mostrato di essere maggiormente adatto rispetto al ceppo ISEIND3 per fermentazioni condotte a basse temperature. È da notare che la prova di fermentazione a 15°C è l'unica in cui il ceppo ISEIND3 scompare completamente a fine fermentazione.

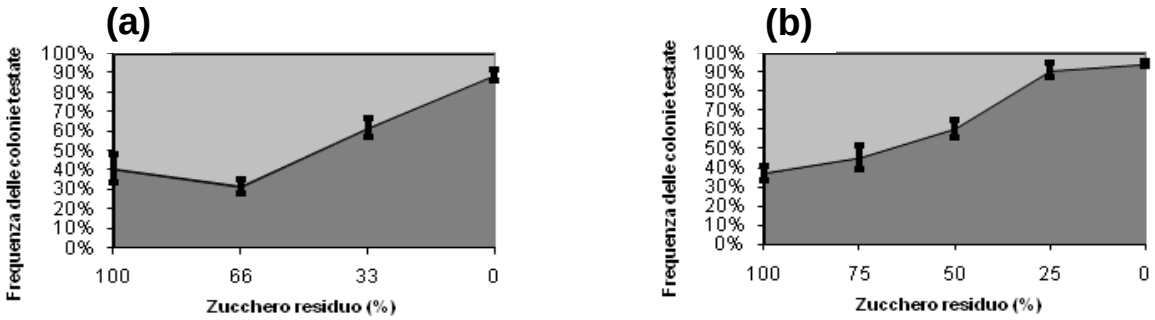

Fig. 4**.** Fermentazioni condotte con il co-inoculo dei due ceppi. (a) fermentazione con 200 g/L di saccarosio. (b) fermentazione con 250 g/L di saccarosio. Ceppo 5:■. Ceppo 3:■ **.**

CONCLUSIONI

È stato verificato, su un panel di ceppi di *S. cerevisiae*, come la PCR multiplex di microsatelliti seguita da elettroforesi su agarosio e poliacrilamide possa essere uno strumento utile ed affidabile per la rapida identificazione e la discriminazione di lieviti vinari a livello di ceppo.

Un'applicazione molto interessante della tecnica può riguardare la verifica del ceppo dominante durante la fermentazione alcolica, permettendo quindi di studiare i fattori che intervengono nella competizione tra lieviti. Attraverso le prove di fermentazione realizzate in questo studio per confrontare due ceppi di *S. cerevisiae*, è stato evidenziato come uno dei due ceppi sia dotato di una superiore capacità competitiva e sia dominante rispetto all'altro in tutte le fermentazioni approntate. Da queste prove, è stato possibile rilevare come, tra i vari parametri studiati, la temperatura sia quello che maggiormente influisce sullo sviluppo e sull'interazione dei due ceppi.

Nell'industria enologica, la capacità di verificare la dominanza o la proporzione dei ceppi selezionati utilizzati durante la fermentazione alcolica, potrebbe essere un utile strumento per ottimizzare l'inoculo dei lieviti e, poiché l'interazione tra il lievito e i composti presenti nel mosto è fondamentale per l'espressione dei caratteri sensoriali, anche per valutare l'impatto sensoriale di diversi ceppi durante la vinificazione.

BIBLIOGRAFIA

1. Bakalinsky, A. and Snow, R. (1990) The chromosomal constitution of wine strain of *Saccharomyces cerevisiae*. *Yeast* **6**: 367-382.
2. Beh, A.L., Fleet, G.H., Prakitchaiwattana, C. and Heard, G.M. (2006). Evaluation of molecular methods for the analysis of yeasts in foods and beverages. *Advances in Experimental Medicine and Biology* **571**: 69-106.

3. Fernández-Espinar, M.T., López, V., Ramón, D., Bartra, E. and Querol, A. (2001) Study of the authenticity of commercial wine yeast strains by molecular techniques. *International Journal of Food Microbiology* **70**: 1-10.
4. Field, D. and Wills, C. (1998) Abundant microsatellite polymorphism in *Saccharomyces cerevisiae*, and the different distributions of microsatellites in eight prokaryotes and S. cerevisiae, result from strong mutation pressures and a variety of selective forces. *Proceedings of the National Academy of Sciences of the United States of America* **95**: 1647-1652.
5. Gonzalez Techera, A., Jubany, S., Carrau, F.M. and Caggero, C. (2001) Differentiation of industrial wine yeast strains using microsatellite markers. *Letters in Applied Microbiology* **33**: 71-75.
6. Harju, S., Fedosyuk, H. and Peterson, K.R. (2004) Rapid isolation of yeast genomic DNA: Bust n' Grab. *BMC Biotechnology* **4**: Http:// www.biomedcentral.com/1472-6750/4/8.
7. Richards, K.D., Goddard, R.M. and Gardner, R.C. (2009) A database of microsatellite genotypes for *Saccharomyces cerevisiae*. *Antonie Van Leeuwenhoek* **96**: 355-359.
8. Sambrook, J., Fritsch, E. F., Maniatis, T., 1989. Molecular cloning. A laboratory manual. CSH Laboratory Press. **1**: 6.44-6.46.
9. Schuller, D., Valero, E., Dequin, S. and Casal, M. (2004) Survey of molecular methods for the typing of wine yeast. *FEMS Microbiology Letters* **231**: 19-26.
10. Vaudano, E. and García Moruno, E., 2007. Discrimination of Saccharomyces cerevisiae wine strains using microsatellite multiplex PCR and band pattern analysis. *Food Microbiology* **25**: 56-64.

ARGUMENTS INDICATING THE PRESENCE OF WINE IN NEOLITHIC POTS FROM GEORGIA USING THE METHOD OF PALYNOLOGICAL AND CHEMICAL ANALYSIS

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ABSTRACT

Palynological investigation of organic remains on the internal side of Neolithic ceramic vessels from the early agricultural settlement of Gadachrili Gora showed the presence of well preserved pollen grains of *Vitis vinifera*. There is also a lot of pollen of weeds characteristic for vineyards. Gadachrili Gora layers were dated to 5815 cal BC and 5783 cal BC. The organic remains from the pot walls were investigated chemically too. Contact of wine with ceramic remains on its walls calcium tartrate, which is precipitated from wine. Fragments of investigated ceramic are characterized by rough, coarsely porous structure. If wine stays in pots with such structure, liquid is penetrating deeply in pores, so calcium tartrate settles not only on the surface, but in pores too. Therefore, amount of settled tartrate will be enough to be analyzed by instrumental methods. Investigating fragments using HPLC, trace amount of tartrate was found. This indicates contact of abovementioned pot with wine.

Durch die palynologische untersuchungen von organischen Reste auf der innere Oberflaeche der neolitischen Tonbehaelter aus juengsten agrokulturellen Schichten von "Gadatschrili Gora" wurden sowohl gut erhaltene Pollen von *vitis vinifera* als auch Pollen des typischen fuer den Weingarten Unkrauts gefunden. Die Schichten von "Gadatschrili Gora" wurden mit 5815 und 5783 vor Chr. datiert. Die organische Reste von Gefaesswaenden wurden auch chemisch untersucht. Weil untersuchten Keramikfragmente grobe und porenhafte Struktur hatten, gelangt der Wein durch den Kontakt mit solcher Keramik tief in Poren, weswegen Kalzium Tartrat nicht nur auf die Oberflaeche, sondern auch tief in die Poren an den Waenden erhalten bleibt. Deswegen erreicht das gesetzte Kalzium Tartrat eine solche Menge, was instrumentell feststellbar ist. Durch die HPLC-Untersuchung von der neolitischen Keramik wurden die Spuren von Kalzium Tartrat gefunden, was auf den Kontakt mit dem Wein hindeutet.

INTRODUCTION

A primitive (manual) technology of pot manufacturing in the Neolithic time results from formation of imperfections and dents especially in the internal part of the vessels. The internal bottom of pots is also rough. In all the aforesaid imperfections and pits an organic material of the food for which the pots were used was accumulated. In the course of time the microscopic food remains were stuck in the pits and cracks and became so hard that could not already be washed from the pots. Moreover, microscopic pores of ceramics were saturated with the liquid that was present in the pots. Here the pots are meant where food was not boiled and that were only used for keeping food or drinks. In this connection, the pot content is very perspective both for palynological investigations and for studies using many methods of natural sciences. The main goal of our research is to reveal the traces of the presence of wine in ceramics of the Neolithic period by simultaneous application of palynological and chemical methods.

MATERIALS AND METHODS

The studied material was taken in 2006-2007 during excavations of the early-agricultural settlement of **Gadachrili Gora** located on the territory of **Lower Kartli** not far from the village **Imiri** of the **Marneuli** region. An absolute altitude of the locality is 360-370 m with the coordinates N – 41° 39' 061; E – 44 ° 82' 100. The monument is divided in two parts by the river **Shulaveris Gele** by a deep canyon. The excavations were performed by the National Museum of Georgia. The monument was revealed in the early 1960s by the workers of the Janashia State Museum of Georgia (the expeditions were headed by A.Javakhishvili and O.Japaridze). However, earlier, no thorough excavations and investigations had been carried out here. During 2006-2007 almost all stratigraphic layers of the monument were excavated and its lowest part according to the ^{14}C dating is OS-63262 – 6910 ±110 BP. cal BC 5815 ±103 (CalPal) 7662-7868 cal BP (remains of small pieces of charcoal) and OS – 63260 – 6890 ±40 BP cal BC 5783 ± 42 (CalPal) 7690 -7775 cal BP (by charred wheat and oats grains).

For the palynological analysis 26 samples were taken from different cultural layers and treated in the laboratory of the Institute of Palaeobiology of the National Museum of Georgia. For comparison the samples from the cultural layers of the monument **Arukhlo** and organic remains from the pot of the monument **Shulaveri Gora** were investigated. At the first stage, the material was boiled for a long time in the 10% KOH solution, then centrifuging in the cadmium liquid and finally acetolysis were performed (Moor et al., 1991). Most interesting were the pot remains. To make a comparison with fossil spectrum, the analysis of recent soil and wine from modern vessels was also made.

Instrumental investigation of pot fragments was performed using High Performance Liquid Chromatographic - HPLC method with UV-detection at 210 nm on the Ion-exchange Hamilton PRP-X200 chromatographic column.

RESULTS AND DISCUSSION

Most part of the samples of the whole studied material was soil formations from the cultural layers. However, four samples are the organics from pots or rather from their fragments. Here, we consider only the ceramic material. The results of its palynological investigation are given in detail in Table 1. These are samples No.16, 17, 18 taken in 2006 and sample No.4 taken in 2007.

In the palynological spectrum of sample 16 (square G-6-02:22) represented by pot fragments 220 palynomorphs were counted of which 120 were pollen and 100 – non-pollen fossils. In the group of arboreal pollen there are many pollen grains of *Fagus, Tilia, Corylus* and *Alnus*. J*uglans, Pterocarya, Quercus, Ulmus*, *Cornus* pollen grains are recorded. *Pinus* and *Picea* pollen grains are met occasionally. Among herbaceous species *Urtica* pollen is predominant. *Cerealia* pollen grains (wheat and barley) are met in large quantities. Pollen of such edible plants as *Chenopodiaceae, Boraginaceae, Polygonaceae, Carduus, Viola,* etc. is also found. Among palynological remains there are many flax fibers. Pine wood cells that may have got there from fire ashes during cooking are recorded. These vessels might have been used for cooking food out of nettle and other herbaceous plants. Large amounts of beech pollen are probably remains of its nuts that are edible and might have been used as a seasoning.

In sample No.17 that also consists of fragments of ceramics in the same square as sample No.16 477 palynomorphs are counted (Pollen 308 and NPP 169). In the palynological spectrum of this sample almost 40% of the total counted pollen falls on *Chenopodiaceae*. There are many pollen grains of *Cerealia* and other edible herbs. These are, for example, *Polygonum, Malva, Urtica, Papaver, Carduus, Psoralea*, etc. In arboreal pollen pine is predominant. Spruce and fir pollen is found in low quantities. Among broad-leaved species *Fagus* and *Carpinus* pollen is predominant. *Quercus, Tilia, Alnus, Pterocarya, Juglans*, *Corylus* is recorded. In the NPP group there are many flax fibers. 4 wool fibers were found. Fungus spores are met in single quantities. Thus, it becomes evident that in the studied pots food out of different herbaceous plants was cooked.

Sample No.18 is the remains of organics from pots found in the cultural layers of the monument **Shulaveri** neighboring **Gadachrili Gora**. This is the lower part of the pot with very thick walls and imprints of textile on the external side of the bottom. In the sample 202 palynomorphs were counted among which 141 falls on the pollen. The palynological spectrum consists essentially of pollen of honey plants. *Lathyrus, Trifolium, Poaceae, Boraginaceae* is predominant. *Filipendula, Symphytum, Fumaria, Knautia, Centaurea, Taraxacum, Polygonum, Fabaceae, Rosmarin* pollen is also recorded. Of great interest is the fact that here bee hairs are revealed. Pollen is extremely well preserved. All this indicates that the pot was used for keeping honey whose microscopic remains were stuck in imperfections of crude ceramics.

Sample No.4 is fragments of thin ceramics with darkened internal walls that were found in the lower layers of **Gadachrili Gora** (square CB-3, inventory No. 66-06:94, 4:90). In this sample 297 palynomorphs were counted. Pollen grains like other palynomorphs are perfectly preserved. There are many flax fibers and fungi spores. However, pollen here is met in low quantities and only 41 pollen grains were counted. In the group of arboreal pollen *Pinus* and *Vitis vinifera* pollen grains are predominant (Fig.1). Altogether, the color of pollen grains including those of *Vitis vinifera* is darker than in other pots. *Juglans* and *Pterocarya* pollen is found. Among herbaceous species *Artemisia* pollen is predominant. *Chenopodium album, Cichorioideae, Polygonum aviculare, Poaceae, Cerealia, Aster* pollen is recorded.

Table1. Pollen content of fossil organic material from Neolithic pots

Gadachrili Gora											
Number of samples	1	4	16	17	18	Number of samples	1	4	16	17	18
Type of samples	Rec.soil	Ceramic	Ceramic	Ceramic	Ceramic	Type of samples	Rec.soil	Ceramic	Ceramic	Ceramic	Ceramic
Square		CB-3	G6-06:22			Square		CB-3	G6-06:22		
Abies nordmanniana	1			1		*Apiaceae*	4		1		
Picea orientalis	2		1	3		*Apium*					1
Pinus	15	7	2	41	4	*Lathyrus*					67
Juniperus					1	*Trifolium*					16
Juglans regia		1	2	1		*Psoralea*				2	
Pterocarya		2	3	1		*Fumaria*					5
Alnus			5	5		*Filipendula*			2		1
Carpinus caucasica			4	8		*Fragaria*					2
Carpinus orientalis				2		*Urtica*			19	1	
Ostrya type				2		*Papaver*				1	
Fagus orientalis			11	2		*Viola*			1		
Quercus			1	2		*Knautia*					1
Tilia caucasica type			4	1		*Plantago m/m*				2	
Ulmus			1			*Malva*				1	
Corylus			5	4		*Convolvulus*				2	
Vitis vinifera		4				*Cyperaceae*				1	
Rosaceae			2			*Lamiaceae*	2				
Rhamnus type			1		3	Undiff. NAP	10	2	5	9	
Cornus type			1			*Polypodiaceae*	1	2	12	5	1
Cerealia	8	1	5	11		*Polypodium vulgare*					1
Triticum	3		1	2		*Polypodium*		1			
Hordeum			3			*Cryptogramma crispa*			1		
Poaceae	5	4	6	9	16	*Dryopteris*			1		
Chenopodiaceae	144		8	76	1	*Sphagnum*			3	1	
Chenopodium album	3	1	1	44		*Undiff. Ascospores*	34	12	6		
Artemisia	19	12	10	8		*Tilletia*				2	
Artemisia annua type			2			*Sordaria*	11	4		1	
Cichorioideae	5	2	1	16		*Podospora*	6	1			
Taraxacum					1	*Sporormiella*	7				
Aster	7	1		3	2	*Chaetomium*	6				
Inula					3	Fibers of flax		201	80	160	53
Cirsium	7					Fibers of wool				4	
Carduus	9		1	10		Undiff.fibres			2		5
Centaurea				2	2	Hair of sheep		1			
Xanthium			3	5		Eggs of parasites of animals	4				
Achillea				2		Hair of lavra *of Dermestidae*	1				
Fagopyrum	1		2			Hair of bee					3
Polygonum		3	1	15	1	Hair of *Acari*	3				
Polygonum aviculare	20	1		4		Zoomaterial		2		2	
Polygonum persicaria			1			Tracheal cells of *Pinus*			6		
Ranunculus			1			Tracheal cells of undiff.wood		35			
Brassicaceae	5					**Total AP**	**18**	**14**	**43**	**73**	**8**
Caryophyllaceae	2					**Total NAP**	**260**	**29**	**94**	**234**	**138**
Boraginaceae	2		3		8	**Total Pteridophyta**	**1**	**3**	**17**	**6**	**2**
Rosaceae	3				4	**Forest elements (AP+Pteridophyta)**	**19**	**16**	**60**	**79**	**10**
Saxifragaceae				2		**Total pollen**	**279**	**46**	**154**	**313**	**148**
Symphytum					3	**Total NPP**	**72**	**256**	**94**	**169**	**61**
Rosmarinus type					2	**Total palynomorphs**	**349**	**302**	**248**	**482**	**209**

Fern spores are present. It should be mentioned that on the territory of Georgia almost all the aforesaid herbaceous species are garden and vineyard weeds (Kvavadze, Chichinadze, 2007). The fact that pollen grains are perfectly preserved, the presence of grape pollen and its accompanying weeds suggests that the pots contained wine. Good preservation of pollen grains and low pollen concentration are characteristic precisely for a wine pollen spectrum (Rösch, 2005; Kvavadze, Chichinadze, 2007; Kvavadze et al., 2007).

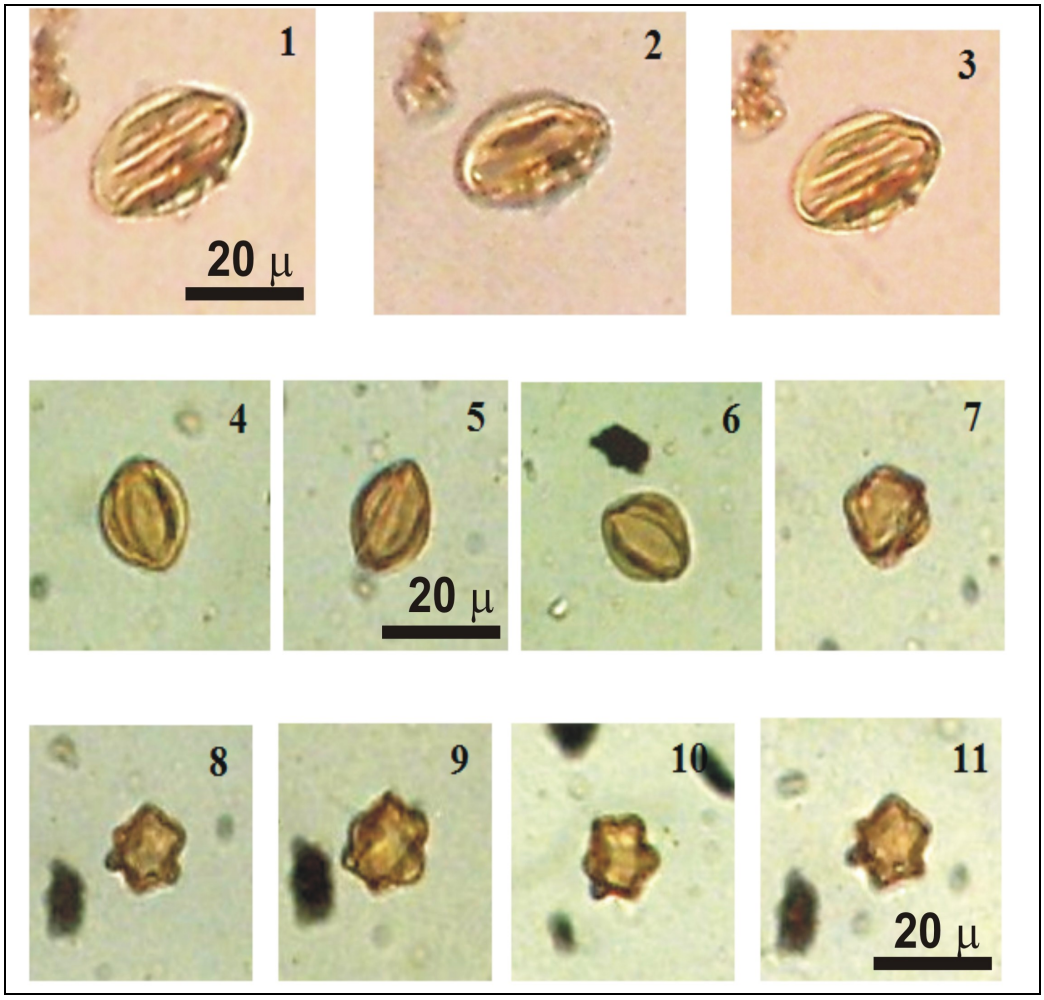

Fig.1. Pollen grains of *Vitis vinifera* from: 1,2,3 –Neolithic pot; 4-11 – fossil soil

To confirm our argument for the presence of wine in the studied pots, ceramics itself was analyzed using chemical methods in the laboratory. Contact of wine with ceramic remains on its walls tartaric acid or calcium tartrate, those are precipitated from wine and are slightly soluble in water. In aged ceramics dominates calcium tartrate. Fragments of investigated ceramic are characterized by rough, coarsely porous structure. If wine stays in pots with such structure, liquid is penetrating deeply in pores, and that's way calcium tartrate settles not only on the surface, but in pores too. Therefore, amount of settled tartrate will be enhanced in scraped powder of ceramics and can be analyzed by instrumental methods. Scraped powder was extracted with 0,5 ml of sulfuric acid for 45 min in ultrasonic bath. Extract was centrifuged at 9 000 rpm for 15 min. Liquid layer was transferred to sample vial and analyzed by HPLC. As shown from zoomed chromatogram in Fig. 2, at 10.9 min we can observe small peak belonging to tartaric acid.

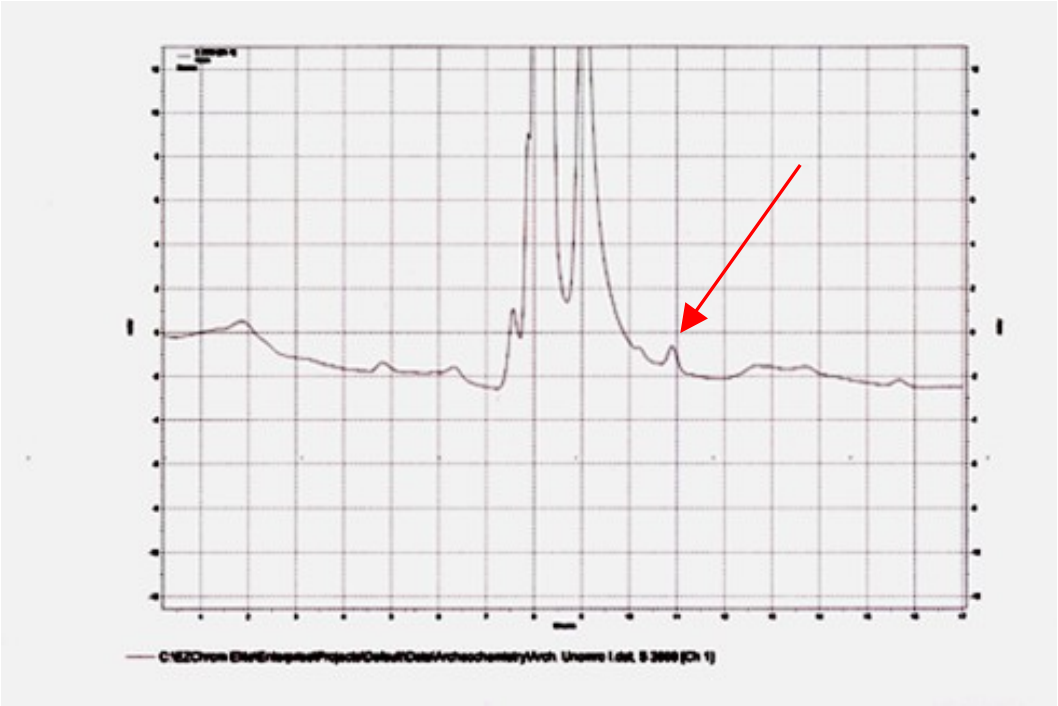

Fig. 2. Zoomed chromatogram of extract of scraped powder from Neolithic pot fragments

It should also be mentioned that pollen grains of *Vitis Vinifera* were found in the samples of soil formations of **Gadachrili Gora** in the sections of square CB-3, CB-12, DB-5. Soil pollen grains differ from wine pollen by worse preservation and low sizes (Fig.1). We also found pollen of *Vitis vinifera* in the soil and ash layers of Neolithic layers of the monument **Arukhlo** dated to 60 cent.BC and 54 cent.BC (Hansen et al. 2007).

CONCLUSIONS

The palynological analysis of organic remains in Neolithic pots allowed us to reveal the diet used by the population of that time. Plant food was essentially used since in the pots no bone crystals were revealed.

The presence of pollen of *Vitis vinifera* in the pots and in the soil formations of the settlements of **Gadachrili Gora, Shulaveri** and **Arukhlo** points to the existence of vine-growing in the Neolithic Age. The presence of pollen of many vineyard weeds found in the palynological spectrum of honey is an indirect evidence of existence of vine-growing.

Well preserved pollen of grape and its accompanying woods found in organic remains of the pots indicates existence of wine in the Neolithic epoch, which was also confirmed by chemical analyses.

Investigating fragments of Neolithic ceramic using HPLC-method trace amount of tartrate was found. This indicates contact of abovementioned pot with wine.

BIBLIOGRAPHY

[1] Hansen S., Mirtskhulava G., Bastert-Lamprichs K. 2007. Aruchlo: A Neolithic Settlement mound in the Caucasus. *Neo-Lithics,* 1: 13-19.

[2] Kvavadze E., Chichinadze M. 2007. Preliminary results of pollen deposition in vineyard of Kvemo-Magaro village (Kakheti, Georgia): comparison between pollen traps and surface soil samples. *Proceedings of the 6-th International Meeting of PollenMonitoring Programme*, Riga. : 45-47.

[3] Kvavadze E., Connor S., Narimanishvili G. 2007. Late Pleistocene and Holocene history of landscapes on the Tsalka plateu (southern Georgia), based on palynological analysis of lake and soil formation. *In. G.Mchedlidze (ed), Problems of Palaeobiology, Proceedings of Institute of Palaeobiology, Tbilisi.* :34-47.

[4] Moor P.D., Webb J.A., Collinson M.E. 1991. Pollen Analysis. Second Edition. Blackwell Scientific Publications, Oxford.

[5] Rösch M. 2005. Pollen analysis of the contents of excavated vessels – direct archaeobotanical evidence of beverages. *Vegetation History and Archaeobotany,* 14: 179-188.

METHOD ELABORATION FOR THE DETERMINATION OF ORGANOCHLORINE PESTICIDES IN WINE USING GAS-CHROMATOGRAPHY WITH ELECTRON CAPTURE DETECTOR

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ABSTRACT

Determination of organochlorine pesticides like DDT and its metabolites, as well as lindane, aldrin, endrin, etc. in wine was not the aim from nutritional point of view till last time. One of two possible methods to determine organochlorine pesticides in liquids is gas-chromatography with Electron Capture Detector. Therefore, for detection of their trace amounts in wine, extraction and preconcentration procedure is needed. Because of high volatility of organochlorine pesticides Solid-Phase extraction and preconcentration by evaporation are not acceptable. Therefore, Liquid-Liquid extraction technique seems to be appropriate. In our investigation we reached 10-fold preconcentration that is enough to determine trace amounts of organochlorine pesticides in wine. Selected chromatographic conditions allow good separation and resolution of compounds of interest and other components being in wine matrix. Elaborated method has been validated.

Bestimmung von chlororganischen Pestiziden wie z.B. DDT und seiner Metabolite, Lindan, Aldrin, Endrin und s.w. im Wein aus der Standpunkt der Nahrungsmittel war kein Thema bis letzter Zeit. Eine von zwei moglichen Methoden fuer die Bestimmung von chlororganischen Pestiziden in der Fluessigkeit ist gas-chromatographysche Methode mit ECD-Detektierung. Um Spurenmengen im Wein festzustellen, sind Extraktion und Vorkonzentrierung notwendig. Aufgrund der starken Fluechtigkeit der o.g. Pestizide, sind SPE und Vorkonzentrierung durch Verdampfung nicht anwendbar. Dadurch ist die fluessig-fluessige Extraktion am besten geeignet. Unsere Untersuchungen weisen 10-fache Aufkonzentrierung auf. Es ist genug um Spuren-mengen festzustellen. Sorgfaeltig angepasste chromatographysche Bedingungen zeigen gute Auftrennung zwischen den untersuchenden Verbindungen und anderen durch Weinmatrix vorkommenden Substanzen. Die neu entwickelte Methode wurde validiert.

INTRODUCTION

Despite of the objective that organochlorine pesticides aren't used in agriculture now, organochlorine pesticides and their metabolites can stay in soil for a long time over 30 years. They are weakly soluble in water. The vinification technique and maceration practice reduce significantly amount of organochlorine pesticides in wine in case of their presence on grapes. So, they can come in wine only in trace amount.

Like database of Codex Alimentarius [1] for MRLs of pesticides in food and feed, there is no information about MRLs of organochlorine pesticides in wine. There is a lot of information about various food and feed, including table grape, but nothing about wine. This can mean only the fact, that the possibility of presence of organochlorine pesticides in wine are negligible. Thereby, we can explain absence of analytical methods for the determination of organochlorine pesticides in wine. On the other hand, there are a lot of articles and information about different determination methods of organochlorine pesticides in solid and liquid food, drinking and other kind of water, soil, etc. [2-7]. Among them are EPA [8], ISO [9] methods and different methods elaborated by several manufacturers of analytical equipment like Varian [10-12], Agilent Technologies [13], etc.

This study looks into the possibility of elaboration of original method for the determination of organochlorine pesticides in wine samples.

MATERIALS AND METHODS

Apparatus

GC equipment - Varian 3900, with Electron Capture Detector; Autosampler - Varian CP-8400; Hydrogen generator - DBS, PGH Series 2; Air filtering unit - Anmetec, GR 3004 Software – Galaxie Chromatography Workstation Version 1.7

Reagents

Standarts solutions of next 11 organochlorine pesticides - α-HCH, β-HCH, γ-HCH (Lindane), δ-HCH, Heptachlor, Aldrin, Chlorpyrifos, DDE, Endrin, DDD, DDT with concentration 10 ng/µl were purchased from Dr.Ehrenstorfer GmbH (Germany). Cyclohexane (GC-grade) used as solvent, purchased from Merck (Germany).

Chromatographic conditions

Column:	-	CP 8754, Varian
Type	-	WOOT FUSED SILICA
Coating	-	CP SIL 8 CB
ID	-	0.32 mm
Coating thickness	-	0.25 µm
Length	-	60 m

Temperature:

Injector	-	260 °C		
Detector	-	300 °C		
Oven (Isotherm)		Initial temperature	-	90°C
		Duration	-	1 min
		Temperature step	-	25°C/min
		End temperature	-	120°C
		Duration	-	0.5 min
		Temperature step	-	3°C/min
		End temperature	-	300°C
		Duration	-	1 min

Carrier gas:

Type	-	Nitrogen
Pressure	-	3.5 kPa
Flow	-	29 ml/min
Split	-	50 ml/min at 0.5 min

Burning gas:

Hydrogen	-	30 ml/min
Air	-	300 ml/min

Injection:

Injection volume	-	5 µl
Volume transferred from injector to column	-	0,1 µl

Analysis time - 64 min

Retention time

Compound		minutes (approx.)
1.	α-HCH	24.35
2.	β-HCH	26.00
3.	γ-HCH (Lindane)	26.45
4.	δ-HCH	27.90
5.	Heptachlor	31.10
6.	Aldrin	33.26
7.	Chlorpyrifos	33.78
8.	DDE	39.50
9.	Endrin	40.81
10.	DDD	41.98
11.	DDT	44.08

Wine samples

The used test material were commercially available bottled wines.

DISCUSSION

Significant step for the analysis is the preconcentration step. It's necessary, because if organochlorine pesticides are presented in wine, they are only in trace amount. In fact besides preconcentration of compounds sought for, extraction of them is also significant, because many substances from wine matrix must be removed, which helps to achieve clear-cut chromatogram of pesticides' separation and makes quantitative analysis easier. Application of Solid-Phase-Extraction (SPE) for wine samples isn't effective due to the presence of concrete amount of alcohol in wine. Owing to presence of alcohol organochlorine pesticides don't absorb on the sorbent. Usage of Rotatory evaporator for vaporization of alcohol from wine is not effective too - organochlorine pesticides are easily volatile and melt together with alcohol.

Solely possible issue for sample preparing is liquid-liquid extraction mode. Applied extraction-method is further described. 10 ml wine were filtered trough the membrane filter (MERCK eurolab, 0.45 μm). The membrane filter is washed with 10 ml methanol and dried before. The sample was collected in 15 ml glass vial with cap. 1 ml cyclohexane was added. Vial was shacked violently during 40 min at electro shaker. After this step vial was placed vertically during 40 min until the fully exfoliation of liquid layers. The upper organic layer was gently transfered with the automatic pipette to the eppendorf vial and centrifuged it for 15 min at 8 000 rpm. The upper layer of sample was gently transferred to vial for further injection into the GC-system. In such a way 10-fold enrichment have been achieved which is enough to determine trace amounts of organochlorine pesticides in wine.

The typical chromatogram for standard solution with pesticide concentration – 2 μg/l of each, is shown on the **Fig. 1**.

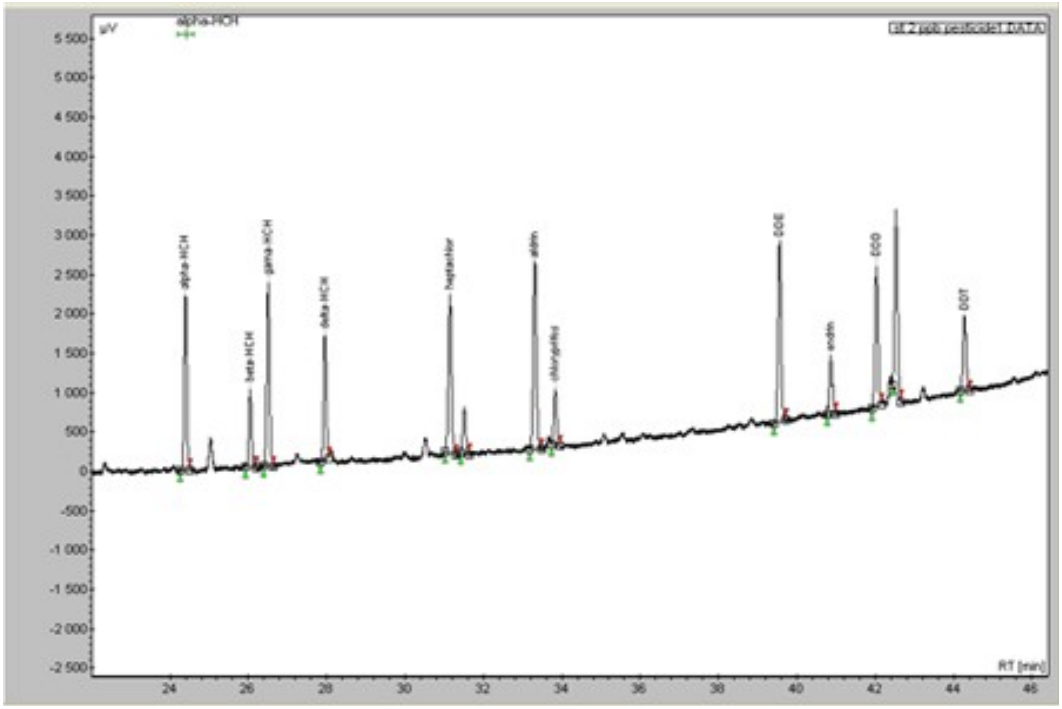

Fig. 1 chromatogram of standard solution of 11 organochlorine pesticides

Recovery after extraction step expressed in percents for each pesticide compound is listed in **Table 1.** To achieve these percent values wine sample was spiked with known amount of pesticides. 10 repeats were performed.

Tab. 1. Recovery percent for each pesticide compound

Compound		Recovery (%)
1.	α-HCH	94
2.	β-HCH	93
3.	γ-HCH (Lindane)	91
4.	δ-HCH	92
5.	Heptachlor	84
6.	Aldrin	85
7.	Chlorpyrifos	63
8.	DDE	54
9.	Endrin	61
10.	DDD	59
11.	DDT	57

On the **Fig. 2** chromatogram of extracted wine sample is shown. Trace amount of aldrin, DDE and DDD can be observed. Presence of DDE and DDD and absence of DDT means, that a long time ago in land area where grape cultivar has been build, DDT was presented, but it destructed completely. This objective is proved by presence of trace amount of its metabolites – DDE and DDD.

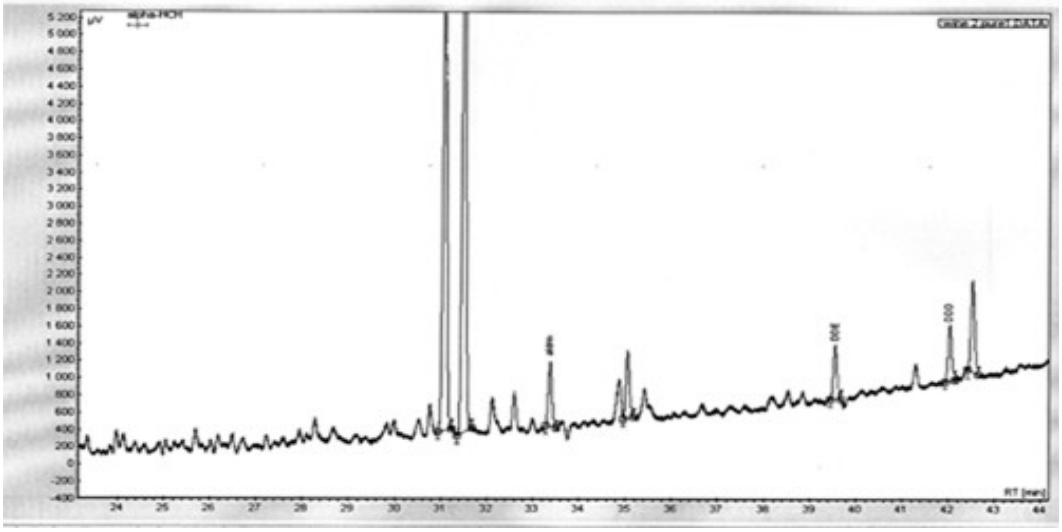

Fig. 2 chromatogram of extracted wine sample

The organochlorine pesticides found in wine are in fully negligible amount and cannot influence on human health. Concentrations of found pesticides are listed in **Table 2.**

Tab. 2. Concentration of pesticides in wine sample

N	Compound	Amount (µg/l)
1	Aldrin	0.077
2	DDE	0.126
3	DDD	0.161

CONCLUSIONS

Original and effective method for extraction and determination of organochlorine pesticides in wine has been elaborated. Achieved results allow to detect organochlorine pesticides in wine in sub-ppb level (in ppt range). This method was validated and tested in laboratory for a long period.

BIBLIOGRAPHY

[1] http://www.codexalimentarius.net/pestres/data/index.html

[2] Chai Lian Kuet A., Seng l., 2004. Solid Phase Extraction Cleanup for the Determination of Organochlorine Pesticides in vegitables. *Malaysian Journal of Chemistry,* 6(1): 39-47.

[3] Chai Mee Kin, Tan Guan Huat, Kumari A., 2006. Method Development for the Determination of Pesticide Residues Vegetables and Fruits by using Solid-Phase Microextraction. *Malaysian Journal of Chemistry* 8(1): 67-71.

[4] Tsipi D., Triantafyllou M., Hiskia A., 1998. *Analyst* 124 : 473-475.

[5] Bordet F., Inthavong D., Fremy J., 2002. *J.AOAC Int*. 85: 1398 – 1409.

[6] Tomkins B., Barnard A., 2002. Determination of organochlorine pesticides in ground water using solid-phase microextraction followed by dual-column gas chromatography with electron-capture detection. *J. of Chromatography A*, **964 :** 21-33.

[7] ASTM D5812-96 Standard Test Method for Determination of Organochlorine Pesticides in Water by Capillary Column Gas Chromatography http://www.astm.org/DATABASE.CART/HISTORICAL/D5812-96.htm

[8] EPA Method 508 http://www.caslab.com/EPA-Methods/PDF/508.pdf

[9] ISO 10382:2002; http://www.iso.org/iso/iso_catalogue/catalogue_tc/catalogue_detail.htm?csnumber=32422&commid=54328

[10] Application 2283 GC http://www.varianinc.com/media/sci/tech_scan/A02283.pdf

[11] Application 2336 GC http://www.varianinc.com/media/sci/tech_scan/A02336.pdf

[12] Application 2092 GC http://www.varianinc.com/media/sci/tech_scan/A02092.pdf

[13] http://www.chem.agilent.com/Library/applications/5988-4057EN300.pdf

INFLUENCE OF IRRIGATION AND COVER CROPPING ON THE PHENOLIC FRACTION OF *AGLIANICO* AND *NERO DI TROIA*

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ABSTRACT

The purpose of the present work was to assess the influence of water stress and cover cropping on the phenolic fraction of *Aglianico* and *Nero di Troia* grapes and wines, produced in 2007 and 2008 in Apulia region, Italy. Two parcels with soil tillage and cover crop, using *Festuca rubra*, *Festuca ovina* and *Trifolium subterraneum,* were considered. Each of the two parcels was divided into three sub parcels, and each one was subjected to different irrigation volumes corresponding to 36%, 24% and 0% of crop evapotranspiration, respectively. Grapes were submitted to traditional red winemaking using 5 days maceration, without any other oenological treatments. Results showed that the content of some phenols is strongly influenced by the season and water deficit, and partly by soil management. Water stress together with soil tillage led to an increased of phenolic compounds in grapes and, consequently, in wines, suggesting that the use of these agronomic practices could improve the oenological potential of the two cultivars grown in Apulia.

RIASSUNTO

Lo scopo del presente lavoro è stato quello di valutare l'influenza dello stress idrico e dell'inerbimento sulla frazione fenolica di uve e vini *Aglianico* e *Nero di Troia* prodotti in Puglia nel 2007 e 2008. Sono state considerate due parcelle, la prima con lavorazione del suolo e la seconda inerbita con *Festuca rubra*, *Festuca ovina* e *Trifolium subterraneum*. Ogni parcella è stata sottoposta a tre differenti volumi di irrigazione: restituzione del 36% di evapotraspirazione della coltura, 24% e 0%. Le uve sono state vinificate in maniera tradizionale con 5 giorni di macerazione e senza altri trattamenti enologici. I risultati hanno mostrato che il contenuto di alcuni composti fenolici è influenzato dall'annata e dallo stress idrico e parzialmente dalla gestione del suolo. Lo stress idrico in combinazione con la lavorazione del suolo ha comportato un accumulo di composti fenolici nelle uve e, conseguentemente, nei vini suggerendo l'utilizzo di queste pratiche agronomiche per aumentare le potenzialità enologiche delle due cultivar coltivate in Puglia.

INTRODUCTION

The quality of red wine is strongly affected by the phenolic fraction, which is responsible for sensory characteristics such as colour, texture and taste (Kosir *et al.*, 2004). Nevertheless, phenolic composition depends on the grape used, which is greatly affected by ground, agronomic techniques and climate, and on winemaking technology. Among the agronomic techniques, water management is fundamental in order to obtain the grape quality necessary for wine production. In fact, excess of water could lead both to a luxuriant vegetative development, resulting in poor grape maturation and to high risk of fungal attacks, whereas a strong water stress could lead to a block of maturation resulting in reduced yield and worse grape quality. Vineyard cover cropping is another agronomic strategy used for regulating the water intake by vine due to strong competition for water.

The purpose of the present work was the evaluation of water stress and cover cropping effects on the phenolic fraction of *Aglianico* and *Nero di Troia* grapes and wines, produced in 2007 and 2008 in Apulia region, Italy.

MATERIALS AND METHODS

Field trials.

The study was carried out in 2007 and 2008 seasons on *Aglianico* and *Nero di Troia* from vines planted in 2002 in the area around Minervino Murge in Apulia region, Southern Italy. The vines were trained on espalier trellis, grafted on SO4, planted 0.8m apart in rows and spaced at 2.0m (6,250 plants ha^{-1}). For each cv, 2 parcels with different soil management were considered: soil tillage (ST), and cover crop (CC) using a mixture of essences (*Festuca rubra*, *Festuca ovina*, *Trifolium subterraneum)*. Each of the two parcels was divided into three sub parcels, and each one was subjected to 3 different irrigation volumes supplied via drippers: volume equivalent to 36% of crop evapotranspiration (ETc), minus an allowance for effective rainfall (full irrigation, FI), 24% (standard irrigation, SI, which is usually employed) and 0% ETc (no irrigation, NI). ETc was estimated by potential ETp, assessed using a "A class" evaporimeter, corrected through Ep conversion coefficient and Kc cultural coefficient (Doorenbos and Pruitt, 1977). For each seasons and for each cv, the following 6 field trials were carried out: soil tillage and full irrigation (STFI), soil tillage and standard irrigation (STSI), soil tillage and no irrigation (STNI), cover crop and full irrigation (CCFI), cover crop and standard irrigation (CCSI) and cover crop and no irrigation (CCNI).

Sampling, winemaking and analyses.

At vintage, 300 berries of each trial were taken according to Di Stefano and Cravero (1991) for chemical analyses, and 100 kg grapes of each trial were submitted to traditional red winemaking (5 days of maceration, with 2 punching-down per day and without other oenological treatments). At the end of fermentation and after static decantation, wines were racked into dark green Bordeaux bottles. Chemical analyses of grapes were made according to EEC 2676 standard procedure (1990), whereas malic acid analysis was performed by HPLC according to Cane (1990). The extraction of phenolic substances from grape skins was carried out according to Di Stefano *et al.* (1989). Skin extracts and wines were analysed for flavans, anthocyans, total polyphenols, proanthocyanidins and flavans reacting with vanillin according to Di Stefano *et al.* (1989). Grapes and wines anthocyanin composition was assessed by HPLC according to Revilla *et al.* (2000). All analyses were made in triplicate and the values reported as average. All data were analyzed using ANOVA by means of the Statistica 6.0 software (StatSoft, Inc. 1984-2001), and Duncan's test was used to establish differences between averages.

RESULTS AND DISCUSSION

The investigated viticultural parameters showed to affect the main chemical and phenolic characteristics of *Aglianico* and *Nero di Troia* grapes both in 2007 and 2008 seasons (Tab.1).

Tab. 1: Chemical and phenolic characteristics of grapes at vintage.

Sample	TSS (°Brix)	pH	TA (g L^{-1})	MA (g L^{-1})	F (mg L^{-1})	A (mg L^{-1})	TP (mg L^{-1})	FRV (mg L^{-1})	P (mg L^{-1})
Aglianico 2007									
STNI	25.9a	3.63b	4.61e	2.04a	4783bc	2536c	5071a	657d	2257hi
STSI	24.8bc	3.54c	4.50e	2.06a	3793fg	2132de	5028ab	698d	2512ghi
STFI	23.8d	3.73a	5.10d	2.07a	3265h	2171de	3471e	578de	2195hi
CCNI	25.2b	3.72a	5.25d	1.93a	3444gh	2393cd	4519bc	332e	2874fg
CCSI	25.0bc	3.45d	5.44cd	2.11a	3282h	2262cde	4596abc	322e	2650gh
CCFI	25.0bc	3.54c	4.57e	1.97a	3922ef	1974e	3810de	478de	1986i
Aglianico 2008									
STNI	24.6c	3.43d	6.00b	2.06a	5430a	3781a	4277cd	1914a	5291a
STSI	24.8bc	3.24f	7.35a	2.07a	4700c	3865a	3987de	1408c	4015cd
STFI	25.0bc	3.39de	5.40cd	2.04a	5164ab	4041a	3955de	1380c	4731b
CCNI	24.9bc	3.33e	6.10b	1.93a	3434gh	2879b	2961f	1818ab	3263ef
CCSI	25.3b	3.41de	5.70bc	2.11a	4276de	2972b	3516e	1598bc	4366bc
CCFI	25.1bc	3.34e	5.16d	1.97a	4411cd	2949b	3584e	1566bc	3689de
Nero di Troia 2007									
STNI	21.2f	3.85ab	3.40cd	2.40a	3688b	1985a	3664c	639d	2740i
STSI	24.4a	3.86ab	3.30cd	1.00e	3883a	2006a	3199e	492e	3847f
STFI	23.9b	3.90ab	3.30cd	1.60d	3389c	1823c	2584g	1189b	5045a
CCNI	23.5bc	3.80ab	4.00ab	1.70c	3130de	1985a	4775a	927c	4549d
CCSI	21.8de	3.90ab	3.55bc	2.00b	3300c	1900b	3900b	1000c	2300m
CCFI	23.3c	3.90ab	3.00d	2.00b	3154d	1896b	3618d	478de	1932n
Nero di Troia 2008									
STNI	23.1c	3.84ab	3.10cd	0.12l	3378c	1536e	3194e	1620a	4646c
STSI	23.0c	3.93a	3.33cd	0.91f	2651f	1308g	2496h	1288b	3983e
STFI	23.0c	3.80b	4.26a	0.48h	2354g	849i	2161l	1266b	3197h
CCNI	23.1c	4.03a	2.94d	0.78g	3045e	1682d	2880f	1290b	4788b
CCSI	22.0d	4.04a	3.24cd	0.40hi	2579e	1380f	2185l	956c	3274g
CCFI	21.5ef	3.64b	4.30a	0.37i	2411g	1090h	2231i	1194b	2587l

In columns, different letters indicate significant differences at $P < 0.05$ for each variety in the two seasons. TSS, total soluble solids; TA, total acidity; MA, malic acid; F, flavonoids; A, anthocyans; TP, total polyphenols; FRV, flavans reagent with vanillin; P, proanthocyanidins.

Among the variables investigated, the season seems to influence on some phenolic classes such as flavonoids and anthocyans, which were in grapes of 2008 higher in *Aglianico* and lower in *Nero di Troia*. The results regarding soil tillage and irrigation practices were ambiguous, and showed enrichment of anthocyans only for soil tillage in the *Aglianico* sample 2008.

Some differences in the anthocyanin composition between *Aglianico* grapes of the two seasons were observed (Fig. 1). In particular, all grapes of 2007 showed higher concentration

of peonidin forms (glycosylate and acetilate) than those of 2008. As expected, soil management caused only small differences. In particular, grapes from cover crop trials showed slight higher amounts than those from soil tillage. Finally, water stress caused enrichment in anthocyanins in the 2008 not-irrigated trials.

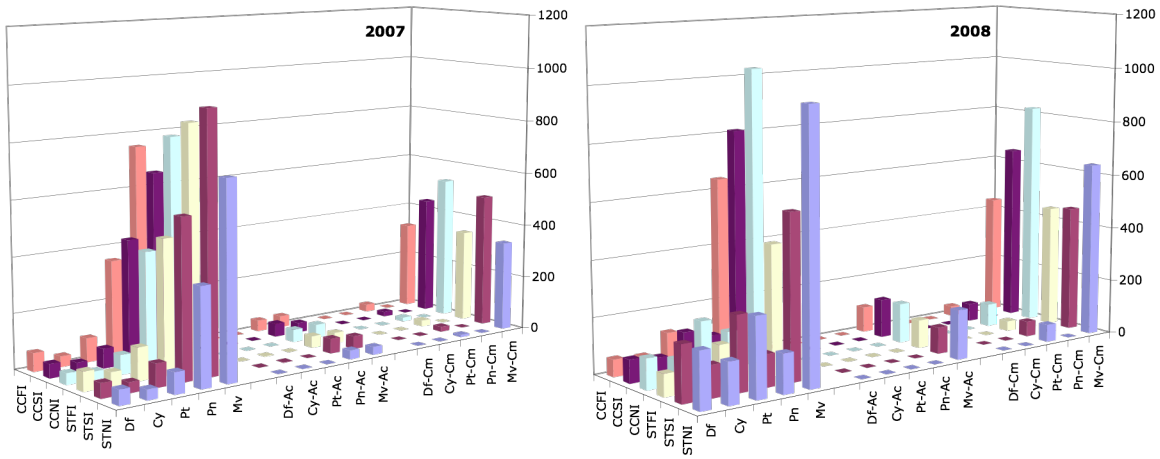

Fig. 1: Anthocyanin composition of *Aglianico* grapes (mg kg^{-1} berries).

Concerning *Nero di Troia*, great differences in anthocyanin composition between the two seasons were found (Fig. 2). In particular, grapes of 2007 were particularly rich in malvidin-coumarate. Not significant was the influence of soil management on anthocyanin amounts, whereas the water stress led to enrichment, except for grapes from cover crop trials in 2007.

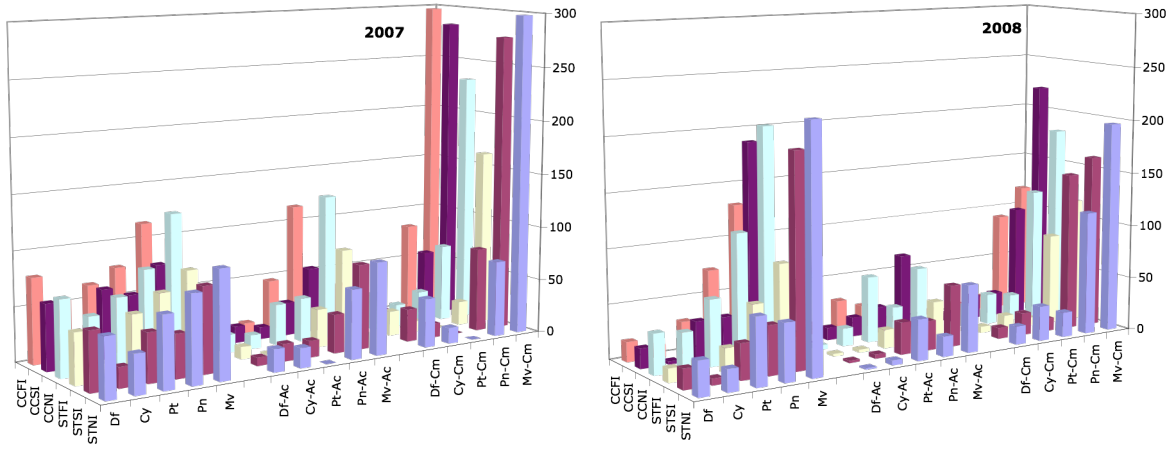

Fig. 2: Anthocyanins composition of *Nero di Troia* grapes (mg kg^{-1} berries).

The phenolic composition and colour indices of wines are reported in Tab. 2. *Aglianico* wines produced in 2008 were found richer in flavonoids, anthocyans and proanthocyanidins than in 2007, showing an influence of season on these parameters. Minor differences were observed in phenol compounds between wines obtained by grapes from soil tillage and cover

crop, showing that soil management applied had little influence on this fraction. Wines obtained from grapes subjected to water stress were found rich in phenol compounds and in colour intensity. Concerning *Nero di Troia*, wines of 2007 were found richer in flavonoids and total polyphenols and poor in proathocyanidins with respect to those of 2008. Also for this variety the water stress caused the enrichment in phenol compounds, whereas the influence of soil management was less evident.

Tab. 2: Phenolic composition and colour indices of wines.

Sample	F (mg L^{-1})	A (mg L^{-1})	TP (mg L^{-1})	FRV (mg L^{-1})	P (mg L^{-1})	CI	T
			Aglianico 2007				
STNI	1862cd	602cd	3281a	664ab	3008c	30.0a	0.52cd
STSI	1961bc	664c	2816b	704ab	2441ef	21.4b	0.50f
STFI	1479ef	539de	2242ef	656ab	2216f	12.0d	0.54b
CCNI	1866cd	539de	2518d	663ab	2645de	17.7c	0.51de
CCSI	1702de	630c	2645c	545b	2884cd	23.2b	0.49f
CCFI	1800cde	519e	2231ef	589b	2478ef	11.3d	0.53c
			Aglianico 2008				
STNI	2102ab	817a	2847b	772a	3747a	30.4a	0.50ef
STSI	2178a	744b	2604cd	762a	3633a	24.2b	0.51de
STFI	1648f	503e	2183f	803a	2735cde	17.0c	0.56a
CCNI	2132ab	816a	2700c	787a	3684a	22.4b	0.52cde
CCSI	2086ab	820a	2302ef	698ab	3294b	21.5b	0.50ef
CCFI	1854cd	543de	2314e	666ab	2729cde	18.0c	0.54b
			Nero di Troia 2007				
STNI	2340bc	593c	3536a	763def	2151ef	14.6a	0.73d
STSI	1788fg	708b	2201ef	590h	2325ef	9.3d	0.74cd
STFI	1965ef	431g	2792c	766def	2507e	9.3d	0.79b
CCNI	2534b	767a	3154b	820cde	1577g	13.7b	0.65f
CCSI	2987a	777a	3103b	1214a	3569ab	12.8c	0.70e
CCFI	2134de	536d	3126b	580h	2332ef	9.1d	0.72cd
			Nero di Troia 2008				
STNI	2105e	599c	2544d	1091b	3755a	8.4e	0.75c
STSI	1637gh	274h	2115f	680g	3046cd	8.3e	0.88a
STFI	1559hi	431g	2141f	736fg	2932cd	6.7g	0.81b
CCNI	1793fg	548de	2313e	864c	3782a	8.4e	0.79b
CCSI	1676gh	500f	2072f	811cde	3263bc	7.7f	0.80b
CCFI	1448i	317h	1744g	838cd	2850d	6.0h	0.79b

In columns, different letters indicate significant differences at $P < 0.05$ for each variety in the two seasons. F, flavonoids; A, anthocyans; TP, total polyphenols; FRV, flavans reagent with vanillin; P, proanthocyanidins; CI, colour intensity; T, tonality.

Concerning *Aglianico* wines, as expected, the malvidin-3-monoglucoside was the predominant compound in all samples (Fig. 3). Little differences in anthocyanin composition were observed due to season and soil management, whereas the water stress caused highest values in anthocyanins, more marked in 2008. As far as *Nero di Troia* is concerned, the malvidin forms were the most abundant, in the following order: Mv > Mv-Ac > Mv-Cm (Fig.

4). Certain differences in anthocyanins concentration were observed between the two seasons (2007 > 2008). As for viticultural practices, highest values in malvidin-3-monoglucoside were found in wines of 2007 obtained from grapes with moderate water stress (CCSI and STSI) and of 2008 from not-irrigated (CCNI and STNI) grapes.

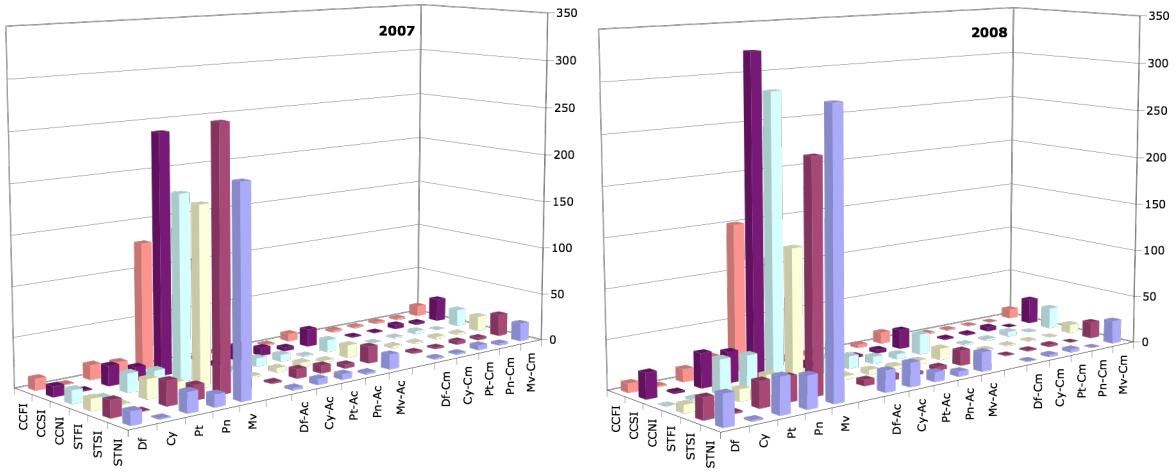

Fig. 3: Anthocyanin composition of *Aglianico* wines (mg L^{-1}).

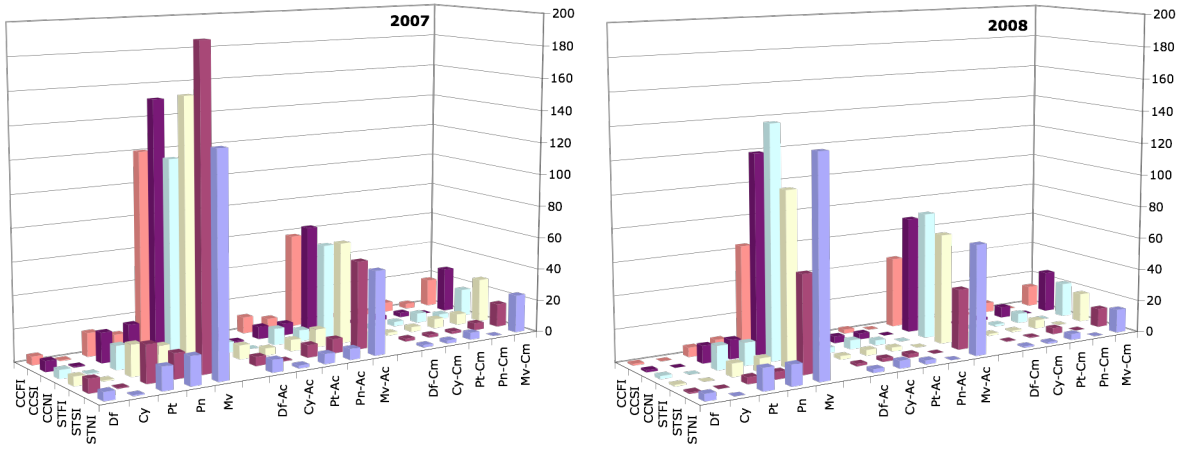

Fig. 4: Anthocyanins composition of Nero di Troia wines (mg L^{-1}).

CONCLUSIONS

The results obtained showed that season has a major influence on grapes and wines phenolic composition of *Aglianico* and *Nero di Troia* cultivars grown in Apulia. *Aglianico* grapes and wines of 2008 were richer in phenol substances than those of 2007; the contrary was observed for *Nero di Troia*. For both varieties, the water stress together with soil tillage caused accumulation of the phenolic compounds in wines. In conclusion, the application of these viticultural practices allows the obtaining of wines particularly rich in phenolic substances, improving the wine quality, even if it seems also strictly dependent on season.

ACKNOWLEDGEMENTS

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REFERENCES

Cane N., 1990. Il controllo di qualità dei vini mediante HPLC: determinazione degli acidi organici. *L'Enotecnico*, 26:69-72.

Di Stefano R., Cravero M.C. 1991. Metodi per lo studio dei polifenoli dell'Uva. *Riv. Vitic. Enol.,* 44:37-45.

Di Stefano R., Cravero M.C., Genfilini N. 1989. Metodi per lo studio dei polifenoli dei vini. *L'Enotecnico*, 25:83-89.

Doorenbos J., Pruitt W.O. 1977. Crop water requirements. *Irrigation and Drainage Paper No. 24*, (rev.) FAO, Rome, Italy, 144p.

EEC. 1990. European Communities. Commission Regulation No 2676/90 on "Community Analysis Methods to Use in Wine Sector". *Official J. European Communities*. No. L272/3.10.90.

Kosir I.J., Lapornik B., Andrensek S., Wondra A.G., Vrhovsek U., Kidri J. 2004. Identification of anthocyanins in wines by liquid chromatography, liquid chromatography-mass spectrometry and nuclear magnetic resonance. *Anal. Chim. Acta*, 513:277-282.

Revilla E., Ryan J.M. 2000. Analysis of several phenolic compounds with potential antioxidant properties in grape extracts and wines by high-performance liquid chromatography-photodiode array detection without sample preparation. *J. Chrom. A*, 881:461-469.

INFLUENCE OF IRRIGATION AND CLUSTER THINNING ON THE PHENOLIC FRACTION OF *SANGIOVESE* GRAPE AND WINE

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ABSTRACT

The aim of the present work has been to evaluate the effects of water stress and cluster thinning on the phenolic fraction of *Sangiovese* grapes and wines, produced in 2007 and 2008 in Apulia region, Italy. Two parcels with two levels of crop load were considered: standard yield and cluster thinning (removal of 30% of the clusters at the beginning of the véraison). Each parcel was submitted to three different irrigation volumes: volume equivalent to 36% of crop evapotranspiration, 24% and 0%. Grapes were submitted to a traditional red winemaking using 5 days of maceration and without other oenological treatments. Results have shown that total polyphenols content of grapes was mainly influenced by season (2007 > 2008) and that slight deficit irrigation in combination with cluster thinning allowed a more accumulation of phenolic compounds, but only in grapes harvested in 2007. Concerning wines, minor differences were observed in phenolic fraction between the two years. Samples deriving by standard yield and full irrigation trials showed the lowest total polyphenols content.

RIASSUNTO

Lo scopo del presente lavoro è stato quello di valutare l'influenza dello stress idrico e del diradamento sulla frazione fenolica di uve e vini *Sangiovese* prodotti in Puglia nel 2007 e 2008. Sono state considerate due parcelle con due differenti livelli di produzione: produzione standard e diradata (rimozione del 30% dei grappoli all'inizio dell'invaiatura). Ogni parcella è stata sottoposta a tre differenti volumi di irrigazione: restituzione del 36% di evapotraspirazione della coltura, 24% e 0%. Le uve sono state sottoposte a vinificazione tradizionale in rosso con 5 giorni di macerazione e senza altri trattamenti enologici. I risultati hanno mostrato che il contenuto in polifenoli totali delle uve è stato principalmente influenzato dalla stagione (2007 > 2008) e che un leggero deficit irriguo in combinazione con il diradamento ha comportato un maggior accumulo di composti fenolici, ma solo nelle uve raccolte nel 2007. Per quanto riguarda i vini, sono state osservate minori differenze tra le due annate. I vini delle uve diradate ed irrigate hanno mostrato il più basso contenuto fenolico.

INTRODUCTION

Phenolic compounds are grape metabolites extracted from skin and seeds during winemaking and are known for being responsible for bitterness, astringency, and colour intensity of wine, thus playing a major role on wine sensory quality (Ribereau-Gayon et al., 1998; Kosir et al., 2004). Moreover, phenolic composition of a wine depends on the phenolic profile of the grape used, which is greatly affected by ground, agronomic techniques and climate, and on the winemaking technologies applied (Katalinic, 1997, 1999). Water management is a fundamental tool in order to improve the grape quality especially when vine is cultivated in arid environment. In fact, a strong water stress could lead to a block of maturation resulting in reduced yield and grape quality, while an excess of water could lead to a luxuriant vegetative development resulting in a poor grape maturation. Also crop load adjustment is an important vineyard management tool for premium-quality wine production. In fact, some studies report that clusters reduction causes advantageous changes in the characteristics of grapes and wines (Jackson and Lombard, 1993; Naor and Gal, 2002; Kliewer and Dokoozlian, 2005). Therefore, the identification of vineyard cultural practices that could positively affect chemical composition of the grapes and the relative wines is desirable.

The purpose of the present work was the evaluation of water stress and cluster thinning effects on the phenolic fraction of *Sangiovese* grapes and wines, produced in 2007 and 2008 in Apulia region, Italy.

MATERIALS AND METHODS

Field trials. The study was focused in 2007 and 2008 seasons on *Sangiovese* from vine planted in 1998 in the area around San Severo in Apulia region, Southern Italy. The vine is trained on a tendone trellis, grafted on 140 Ru, planted 2.5 m apart in rows and spaced at 2.5 m (1,600 plants ha^{-1}). Two parcels were set up with two different yield levels. The first was characterized by the standard yield (SY) whereas the second one was subjected to cluster thinning removing 30% of the cluster from each plant at the beginning of the véraison (CT). Each parcel was divided into three lots with different irrigation volumes supplied via drippers. Crop evapotranspiration (ETcrop) was estimated by potential ETp, assessed using an "A class" evaporimeter, corrected through Ep conversion coefficient and Kc cultural coefficient (Doorenbos and Pruitt, 1977). Lots from the control treatment were irrigated with enough water to 36% compensate for crop evapotranspiration losses, minus an allowance for effective rainfall (FI: full irrigation). Other two regulated deficit irrigation treatments were implemented to deliver different levels of irrigation including 24% (PI: partial irrigation) and 0% (NI: no irrigation) of the control. For each season a total of 6 field trials were carried out: standard yield and full irrigation (SYFI), standard yield and partial irrigation (SYPI), standard yield and no irrigation (SYNI), cluster thinning and full irrigation (CTFI), cluster thinning and partial irrigation (CTPI) and cluster thinning and no irrigation (SYNI).

Sampling, winemaking and analyses. At the vintage, 300 berries of each trial were sampled according to Di Stefano and Cravero (1991), whereas 100 kg of grapes of each trial were submitted to a traditional red winemaking (5 days of maceration, with 2 punching-down per day and without other oenological treatments). At the end of fermentation and after static decantation, wines were racked into dark green Bordeaux bottles. Basic analyses of grapes were made according to EEC 2676 standard procedure (1990). Malic acid analysis was performed by HPLC according to Cane (1990). The extraction of phenolic substances from

grape skins was carried out according to Di Stefano *et al.* (1989). Skin extracts and wines were analysed for flavans, anthocyanins, total polyphenols, proanthocyanidins and flavans reacting with vanillin according to Di Stefano *et al.* (1989). Grapes and wines anthocyanin composition was assessed by HPLC according to Revilla *et al.* (2000). Analyses were made in triplicate and the values reported as average ± standard deviation. All data were analyzed using ANOVA by means of the Statistica 6.0 software (StatSoft, Inc. 1984-2001), and Duncan's test was used to establish differences between averages.

RESULTS AND DISCUSSION

Different chemical and phenolic characteristics of grapes in relation with the season were observed (Tab. 1). Grapes harvested at 2008 were found with higher degree of ripening than 2007, showing the influence of season on fruit maturation. Little differences in the ripening of fruits in relation with cluster thinning and irrigation were observed. This probably could be due to the great load of fruits in the vines that have limited the efficacy of cluster thinning (little difference between standard yield and cluster thinning trials) and of the irrigation. Concerning phenol fraction, 2007 grapes were characterized by amounts in total polyphenols (TP) and flavans reagent with vanillin (FRV) higher than 2008 grapes. As far as agronomic variables are concerned, in the both seasons the cluster thinning practice led to enrichment of phenols, especially in that of 2007 submitted to moderate water stress (CTSI).

Tab. 1: Chemical and phenolic characteristics of grapes at vintage (average ± SD).

Sample	TSS (°Brix)	pH	TA (g L^{-1})	F (mg L^{-1})	A (mg L^{-1})	TP (mg L^{-1})	FRV (mg L^{-1})	P (mg L^{-1})	Estimated yield (t ha^{-1})
2007									
CTNI	18.2±0.7f	3.64±0.02cd	4.65±0.15d	1535±191c	993±20de	3126±94b	1139±116b	1169±138d	28.4±c
CTSI	20.2±0.4d	3.62±0.01d	5.27±0.08b	2401±293a	1453±99a	4177±288a	1357±164a	2000±183a	34.4±b
CTFI	18.5±0.6ef	3.77±0.03b	4.59±0.17de	1558±114c	1048±81de	2952±220bc	657±39e	1049±73d	36.3±b
SYNI	18.2±0.3f	3.78±0.02b	4.67±0.13cd	1462±94c	815±49f	2697±116c	820±20d	825±86e	33.2±bc
SYSI	19.2±0.5e	3.62±0.02d	4.82±0.18cd	1546±57c	927±61ef	2198±146d	891±10cd	1095±78d	41.2±a
SYFI	18.4±0.6ef	3.62±0.02d	4.92±0.09c	2312±38a	1082±53cd	3121±23b	992±84c	1142±95d	40.3±a
2008									
CTNI	24.4±0.3a	3.87±0.03a	4.36±0.16ef	1481±190c	1220±19b	1303±93e	478±49fg	1473±122b	27.9±c
CTSI	21.8±0.2c	3.78±0.02b	4.59±0.18de	1475±248c	985±97de	1311±288e	342±30gh	974±188de	30.8±bc
CTFI	23.5±0.4b	3.52±0.01e	6.00±0.20a	1837±104b	1046±82de	1448±217e	550±36ef	1376±71bc	35.0±b
SYNI	19.0±0.6ef	3.53±0.02e	5.34±0.08b	1726±96bc	1097±46cd	1495±114e	370±23gh	1885±89a	31.5±c
SYSI	22.5±0.4c	3.66±0.01c	4.83±0.17cd	1722±58bc	1181±64bc	1353±142e	347±14gh	1563±76b	35.3±b
SYFI	22.0±0.7c	3.75±0.03b	4.29±0.11f	1203±34d	835±53f	1600±23e	269±88h	1186±95cd	35.7±b

In columns, different letters indicate significant differences at $P < 0.05$.
TSS, total soluble solids; TA, total acidity; F, flavonoids; A, anthocyans; TP, total polyphenols; FRV, flavans reagent with vanillin; P, proanthocyanidins.

In both seasons the anthocyanin composition of grapes was characterized almost completely by the not-acylated forms, showing that these compounds could be considered as a distinctive character for the Sangiovese cv (Fig. 1). Samples deriving from 2007 season showed a major amount of anthocyanins with respect of those from 2008. Minor influence was noted for the

viticultural practices applied. Nevertheless, the grapes deriving from the not-irrigated and thinned vines showed a slight enrichment in anthocyanins. Therefore, the coupling of these practices could be favourably used to assure a greater accumulation of anthocyanins in Sangiovese grape.

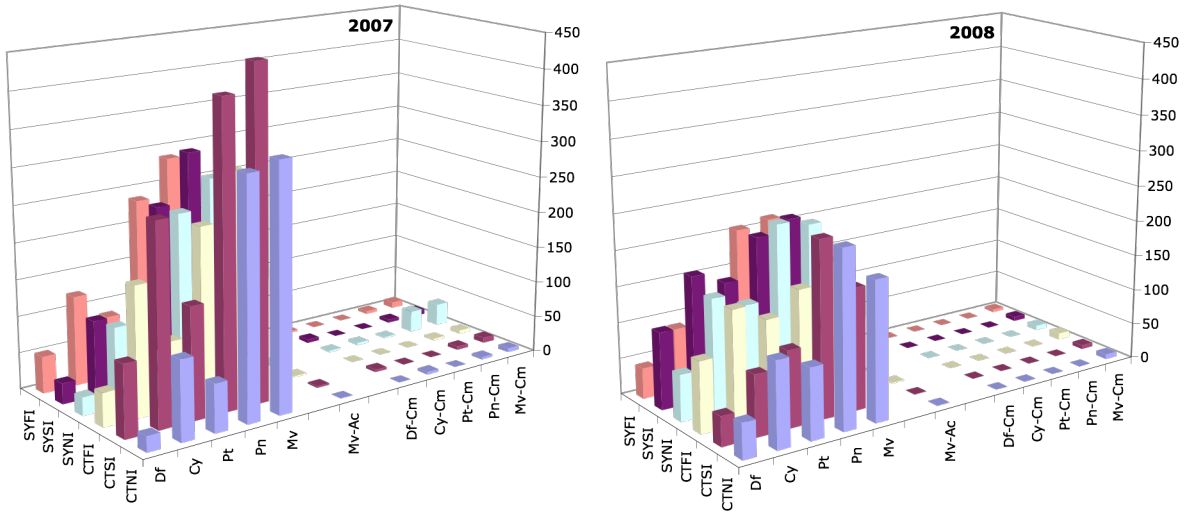

Fig. 1: Anthocyanin composition of *Sangiovese* grapes (mg kg^{-1} berries).

Tab. 2 shows the chemical characteristics of wines. As expected, 2008 wines showed higher values in ethanol than 2007 ones, because obtained from grapes more rich in total soluble solids. Little differences in the other chemical parameters between 2007 and 2008 wines were observed. Also the cluster thinning and the irrigation led to little differences among wines.

Tab. 2: Chemical characteristics of wines (average ± SD).

Sample	E (% v/v)	pH	TA ($g\ L^{-1}$)	VA ($g\ L^{-1}$)	MA ($g\ L^{-1}$)	LA ($g\ L^{-1}$)	DRE ($g\ L^{-1}$)	ASH ($g\ L^{-1}$)
2007								
STNI	10.8±0.1l	4.00±0.06ab	3.94±0.01a	0.26±0.03bc	0.67±0.03a	2.72±0.03a	24.3±0.06c	3.79±0.12cd
STSI	11.6±0.1g	4.00±0.07ab	3.38±0.03fg	0.28±0.05bc	0.65±0.04a	1.94±0.08e	23.9±0.14c	3.79±0.08cd
STFI	11.9±0.1f	4.10±0.04a	3.43±0.03ef	0.34±0.04ab	0.67±0.02a	2.16±0.03d	25.1±0.06b	4.11±0.11a
SYNI	10.5±0.1m	3.93±0.05ab	3.36±0.02g	0.33±0.07ab	0.43±0.02c	1.90±0.04e	23.1±0.13d	3.60±0.12def
SYSI	10.9±0.1i	3.96±0.09ab	3.69±0.04c	0.32±0.04abc	0.44±0.03c	2.39±0.13c	22.2±0.33e	3.47±0.14ef
SYFI	11.5±0.1h	4.10±0.15a	3.77±0.02b	0.29±0.05bc	0.51±0.03b	2.53±0.06b	25.1±0.39b	4.03±0.10ab
2008								
STNI	12.6±0.1a	4.07±0.06ab	3.44±0.05e	0.23±0.04c	0.31±0.02e	1.86±0.06ef	26.1±0.29a	3.78±0.12cd
STSI	12.4±0.1b	4.06±0.13ab	3.16±0.03i	0.31±0.07abc	0.19±0.02g	1.67±0.04h	23.9±0.22c	3.80±0.11cd
STFI	12.3±0.1c	4.08±0.04ab	3.42±0.02ef	0.39±0.03a	0.38±0.01d	1.79±0.04fg	24.1±0.16c	3.85±0.10bc
SYNI	12.2±0.1d	3.98±0.12ab	3.42±0.01ef	0.26±0.02bc	0.35±0.02d	1.55±0.05i	23.0±0.08d	3.41±0.10f
SYSI	12.5±0.1ab	4.02±0.14ab	3.26±0.04h	0.34±0.04ab	0.27±0.02f	1.72±0.03gh	23.2±0.48d	3.63±0.11de
SYFI	12.0±0.1e	3.91±0.04b	3.55±0.04d	0.29±0.07bc	0.28±0.01ef	1.91±0.03e	23.3±0.55d	3.68±0.10cd

In columns, different letters indicate significant differences at $P < 0.05$.
E, ethanol; TA, total acidity; VA, volatile acidity; MA, malic acid; LA, lactic acid; DRE, dry reduced extract; ASH, ashes.

The phenolic composition and colour indices of wines are reported in Tab. 3. In both seasons all wines showed a poor phenolic composition probably due to the excessive fruit load of vines. Wine obtained from 2007 grapes and deriving from standard yield and not-irrigated was richer in phenols than other, whereas little differences in these compounds among 2008 wines were noted. Finally, wines of both seasons deriving from standard yield and full irrigated grapes showed the lowest content in total polyphenols.

Tab. 3: Phenolic composition and colour indices of wines.

Sample	F (mg L^{-1})	A (mg L^{-1})	TP (mg L^{-1})	FRV (mg L^{-1})	P (mg L^{-1})	CI	T
2007							
CTNI	667±18e	298±16bcd	878±8g	96±4h	189±9i	4.63±0.08f	0.92±0.01g
CTSI	915±27b	334±14b	1082±11c	150±11e	429±13fg	6.66±0.08a	0.85±0.01h
CTFI	886±39b	327±13bc	1292±30a	168±4bcd	407±10g	5.06±0.18e	0.96±0.01f
SYNI	960±37a	379±41a	1300±26a	209±6a	479±22f	6.16±0.10bc	0.80±0.01i
SYSI	737±26d	285±20d	903±12fg	116±9fg	153±12i	4.57±0.21f	0.91±0.01g
SYFI	754±21d	261±28d	912±8f	155±8cde	327±25h	6.74±0.53a	0.95±0.01f
2008							
CTNI	624±16f	293±24cd	1065±11cd	113±5g	581±13e	5.07±0.34e	1.28±0.01a
CTSI	668±24e	290±24cd	1132±29b	154±8de	1081±23a	6.49±0.20ab	1.18±0.02b
CTFI	802±20c	208±8e	1059±19cd	166±13bcd	942±68c	5.74±0.10d	1.10±0.01d
SYNI	628±16ef	287±12d	1041±11de	169±7bc	1011±60b	6.73±0.22a	1.03±0.01e
SYSI	579±17g	271±18d	1013±8e	177±6b	814±24d	6.00±0.10cd	1.16±0.01c
SYFI	757±19d	266±13d	931±13f	128±3f	849±27d	5.82±0.10cd	1.16±0.01c

In columns, different letters indicate significant differences at $P < 0.05$.
F, flavonoids; A, anthocyans; TP, total polyphenols; FRV, flavans reagent with vanillin;
P, proanthocyanidins; CI, color intensity; T, tonality.

Anthocyanin composition of all wines was characterized by the absence of acylate forms, as already observed for grapes, and confirming that these compounds could be used as a distinctive character also for the Sangiovese wine (Fig. 2). Concerning the effect of viticultural practices, the cluster thinning did not influence the anthocyanin amount, whereas water stress led to highest amount, especially for wine obtained from 2007 grapes of standard yield and not irrigated trial.

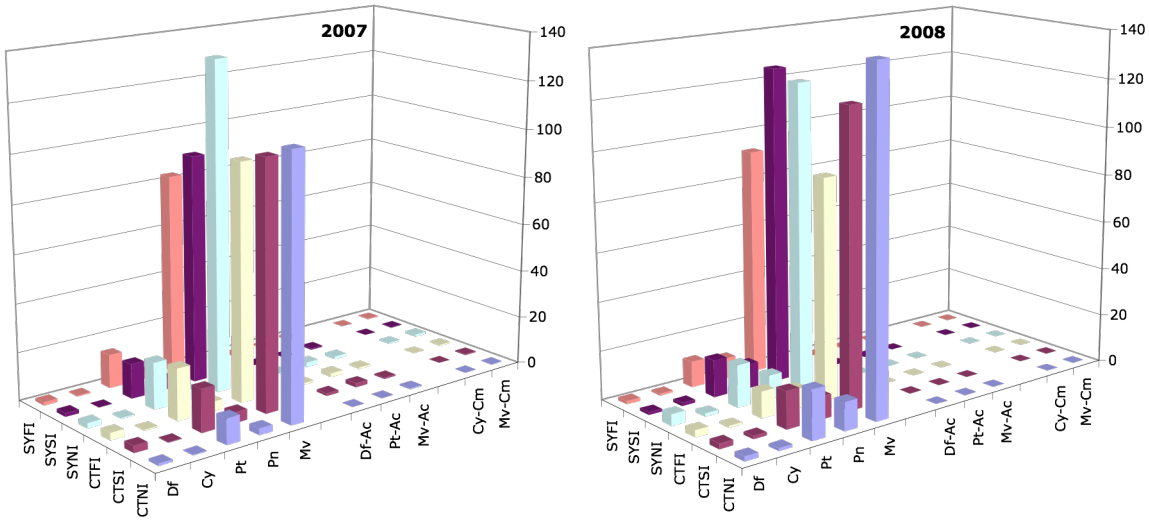

Fig. 2: Anthocyanin composition of *Sangiovese* wines (mg L^{-1}).

CONCLUSIONS

Cluster thinning and irrigation did not significantly affect chemical and phenolic composition of Sangiovese grown in Apulia, Southern Italy, whereas the season showed a moderate effect. This is probably due to the high load of crop that characterized the experimental vines (from 27.9 to 41.2 t ha^{-1}), very high quantities that have drastically decreased the efficacy of the effect of the viticultural practices applied. Therefore the influence of irrigation and cluster thinning on phenol fraction of Sangiovese grown in Apulia region should be studied on vines with a moderate crop load.

ACKNOWLEDGEMENTS

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REFERENCES

Cane N., 1990. Il controllo di qualità dei vini mediante HPLC: determinazione degli acidi organici. *L'Enotecnico*, 26:69-72.

Di Stefano R., Cravero M.C. 1991. Metodi per lo studio dei polifenoli dell'Uva. *Riv. Vitic. Enol.,* 44:37-45.

Di Stefano R., Cravero M.C., Genfilini N. 1989. Metodi per lo studio dei polifenoli dei vini. *L'Enotecnico*, 25:83-89.

Doorenbos J., Pruitt W.O. 1977. Crop water requirements. *Irrigation and Drainage Paper No. 24*, (rev.) FAO, Rome, Italy, 144p.

EEC. 1990. European Communities. Commission Regulation No 2676/90 on "Community Analysis Methods to Use in Wine Sector". *Official J. European Communities*. No. L272/3.10.90.

Jackson D.I., Lombard P.B. 1993. Environmental and management practices affecting grape composition and wine quality: A review. *Am. J. Enol. Vitic*., 44:409-430.

Katalinic V. 1997. High-performance liquid chromatographic determination of flavan fingerprint in plant extracts. J. Chromatogr A, 775:359-367.
Katalinic V. 1997. Grape catechins-natural antioxidant. J. Wine Res., 10:15-23.
Kliever W.M., Dokoozlian N.K. 2005. Leaf area/crop weight ratios of grapevines: Influence on fruit composition and wine quality. *Am. J. Enol. Vitic*., 56:170-181.
Kosir I.J., Lapornik B., Andrensek S., Wondra A.G., Vrhovsek U., Kidri J. 2004. Identification of anthocyanins in wines by liquid chromatography, liquid chromatography-mass spectrometry and nuclear magnetic resonance. *Anal. Chim. Acta*, 513:277-282.
Naor A., Gal Y. 2002. Shoot and cluster thinning influence vegetative growth, fruit yield, and wine quality of "Sauvignon blanc" grapevines. *J. Am. Soc. Hortic. Sci*., 127:628-634.
Revilla E., Ryan J.M. 2000. Analysis of several phenolic compounds with potential antioxidant properties in grape extracts and wines by high-performance liquid chromatography-photodiode array detection without sample preparation. *J. Chrom. A*, 881:461-469.
Ribereau_Gayon P., Maujean A., Dubordieu D. 1998. *In*: Trattato di Enologia II. Chimica del vino, Stabilizzazione, Trattamenti. Bologna, Italia: EdAgricole.

PHENOLIC FRACTION OF APULIAN RED WINES AS AFFECTED BY WINEMAKING TECHNIQUES

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ABSTRACT

The aim of the present work was to assess the influence of different winemaking technologies on the basic chemical characteristics in general and phenolic fraction in particular of *Aglianico*, *Montepulciano*, *Nero di Troia* and *Sangiovese* wines produced in Apulia region, Southern Italy. Four different winemaking technologies were considered: traditional (5 days of maceration at 25°C with three daily punching-down), prolonged maceration (10 days), addition of tannins, and cryo-maceration (24 h at 5°C using solid CO_2), without any other oenological treatments. Results showed that the different winemaking techniques did not influence the phenolic fraction of *Aglianico* cultivar, which is known to be naturally rich of phenols, whereas prolonged maceration and addition of tannins led to an increase of phenols concentration in *Montepulciano* and *Nero di Troia* and to a decrease in *Sangiovese*. Phenols extraction from grapes was found to be mostly dependent on the grape variety rather then on the winemaking technology applied.

RIASSUNTO

Lo scopo del presente lavoro è stato quello di valutare l'influenza delle tecnologie di vinificazione sulle caratteristiche chimiche di base e sulla composizione della frazione fenolica dei vini *Aglianico*, *Montepulciano*, *Nero di Troia* e *Sangiovese* prodotti in Puglia, Italia. Sono state prese in esame 4 differenti tecnologie di vinificazione: tradizionale (5 giorni di macerazione a 25°C con tre follature giornaliere), macerazione prolungata (10 giorni), aggiunta di tannini e criomacerazione (5°C per 24 ore utilizzando CO_2 solida), senza altri trattamenti enologici. I risultati hanno mostrato che le tecniche di vinificazione non hanno influenzato la frazione fenolica dell'*Aglianico*, cultivar notoriamente ricca, mentre la macerazione prolungata e l'aggiunta di tannini hanno portato ad un aumento delle sostanze fenoliche nel *Montepulciano* e nel *Nero di Troia* e ad una diminuzione nel *Sangiovese*. In conclusione, l'estrazione dei composti fenolici dalle uve sembra dipendere principalmente dalla varietà e meno dalla tecnologia di vinificazione applicata.

INTRODUCTION

Grapes contain a large amount of different phenolic compounds in skins, pulp and seeds, that are partially extracted during winemaking (Jackson, 1994). These compounds together with those deriving from the chemical reactions occurring during winemaking and aging play a fundamental role in some sensory properties of grapes and wines, such as colour, astringency and taste (Ribereau-Gayon *et al*., 1998; Kosir *et al.*, 2004). The phenolic composition in wines depends on grape variety, ripening, vine agronomic practices, and winemaking techniques. Among these techniques, prolonged maceration, tannins addition and pre-fermentative cold maceration could improve the extraction of phenol compounds.

The aim of the present study was the assessment of the influence of four different winemaking technologies on the phenolic fraction of *Aglianico*, *Montepulciano*, *Nero di Troia* and *Sangiovese* wines produced in Apulia region, Southern Italy.

MATERIALS AND METHODS

Samples and vinifications.

Aglianico and *Nero di Troia* are grown in Minervino Murge, *Montepulciano* and *Sangiovese* are grown in San Severo. The *Aglianico* and *Nero di Troia* vines are trained on espalier trellis, grafted on SO4, planted 0.8m apart in rows and spaced at 2.0m (6,250 plants ha^{-1}), whereas *Montepulciano* and *Sangiovese* vines are trained on tendone trellis, grafted on 140 Ru and 1130 Paulsen, respectively, planted 2.5m apart in rows and spaced at 2.5m (1,600 plants ha^{-1}). For each cv, about 100 kg of grapes, previously de-stemmed, were used for each of the winemaking technologies described in Tab. 1. At the end of fermentation and after static decantation, wines were racked into dark green Bordeaux bottles.

Tab.1 – Winemaking technologies tested.

Winemaking technologies	Action
Traditional (T)	5 day of maceration at 25°C with 2 punching-down per day. Addition of: yeast (*Saccharomyces cerevisiae*, Zymasil, 15 g/100 kg, AEB, Brescia, Italy); yeast activator (preparation based on ammonium phosphate bibasic, thiamine chlorohydrate, yeast cell walls, cellulose, Bioact Plus, 25 g/100 kg, Oliver Ogar, Montebello Vicentino, Italy); potassium metabisulphite (15 g/100 kg), without any other oenological treatments.
Prolonged maceration (PM)	10 days.
Addition of tannins (AT)	10 g/100 kg (Ellagitan, AEB, Brescia, Italy).
Cryo-maceration (CM)	Cooling of de-stemmed grapes with solid CO_2 (about 12 kg/100 kg of pellets) and maintenance of the sample at 5°C for 24 h.

Chemical analyses.

At the vintage, 300 berries of each cv were sampled according to Di Stefano and Cravero (1991). Chemical analyses of grapes were made according to EEC 2676 standard procedure (1990). Malic and lactic acids were determined by HPLC according to Cane (1990). The extraction of phenolic compounds from grape skins was carried out according to Di Stefano *et al.* (1989). Basic analyses of wines were performed by means of WineScan Auto instrument (FOSS, Padova, Italy). Skin extracts and wines were analysed for flavans, anthocyans, total polyphenols, proanthocyanidins and flavans reacting with vanillin according to Di Stefano *et al.* (1989). Anthocyanin composition in grapes and wines was assessed by HPLC according to Revilla *et al.* (2000). All determinations were made in triplicate and values were reported as average ± standard deviation. Data were analyzed using ANOVA by means of the Statistica

6.0 software (StatSoft, Inc. 1984-2001), and Duncan's test was used to establish differences between averages.

RESULTS AND DISCUSSION

In Tab. 1 the technological ripening indices and the phenolic composition of each cv of grapes at vintage are reported. As expected, results were related to the different grapes variety. *Aglianico* and *Montepulciano* showed the highest total soluble solids and total acidity contents showing an optimal ripening status. The highest phenolic fraction was found in *Aglianico,* the lowest in *Sangiovese*.

Tab. 1: Chemical and phenolic characteristics of grapes at vintage (means ± SD).

Parameters	*Aglianico*	*Montepulciano*	*Nero di Troia*	*Sangiovese*
Total soluble solids (°Brix)	25.3±0.4	24.0±0.1	22.0±0.2	22.5±0.3
pH	3.41±0.04	3.49±0.04	4.04±0.04	3.66±0.01
Total acidity (g L^{-1})	5.70±0.12	6.75±0.10	3.24±0.16	4.83±0.17
Malic acid (g L^{-1})	0.89±0.08	2.12±0.05	0.90±0.04	1.38±0.15
Flavonoids (mg L^{-1})	4276±210	2980±37	2579±39	1722±58
Anthocyans (mg L^{-1})	2972±225	1630±219	1380±19	1181±64
Total polyphenols (mg L^{-1})	3516±118	1814±15	2185±33	1353±142
Flavans reagent with vanillin (mg L^{-1})	1598±90	797±49	956±25	347±14
Proanthocyanidins (mg L^{-1})	4366±212	2469±52	3274±29	1563±76

In Fig. 1 the anthocyanin composition for all grapes is illustrated. Results obtained were very different among the cultivars. *Aglianico* was richest in anthocyanins, especially in malvidin forms (Mv > Mv-Cm > Mv-Ac), *Montepulciano* was characterized by the highest amount of peonidin forms, *Nero di Troia* by the prevalence of Mv-Cm and *Sangiovese* exclusively by not-acylated forms. Similar results were obtained by La Notte *et al.*, (2008).

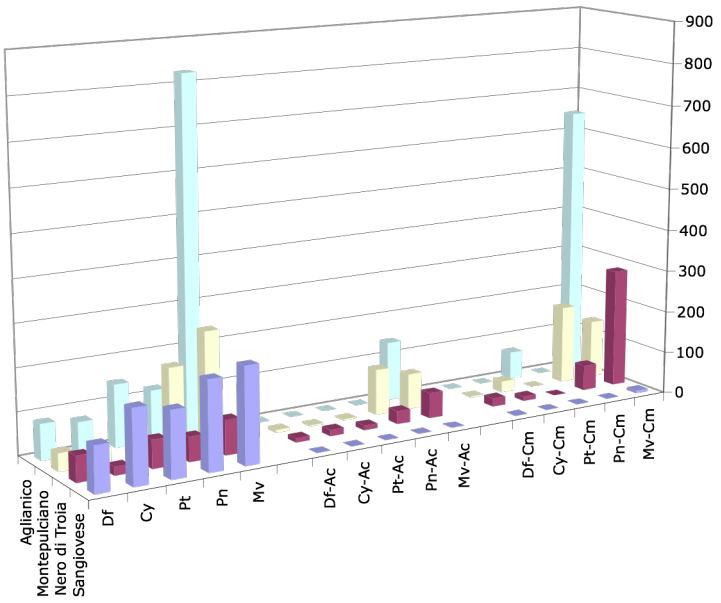

Fig. 1 – Anthocyanin composition of the skin extracts (mg kg^{-1} grape).

Chemical characteristics of wines were in agreement with grapes ripening (Tab. 2). *Aglianico* and *Montepulciano* showed higher values of ethanol and total acidity than *Nero di Troia* and *Sangiovese*. Winemaking technologies did not affect the basic characteristics of *Aglianico* and *Montepulciano*, whereas cryo-maceration led to a minor extraction of substances from grape marc, as demonstrated by the lower values of dry reduced extract and ashes, probably due to the lower temperatures during the maceration.

Tab. 2: Chemical characteristics of wines (means ± SD).

Sample	E (% v/v)	pH	TA (g L^{-1})	VA (g L^{-1})	MA (g L^{-1})	LA (g L^{-1})	DRE (g L^{-1})	ASH (g L^{-1})
Aglianico								
T	13.8±0.1a	3.40±0.09a	7.00±0.24a	0.33±0.04a	0.86±0.06b	0.04±0.02b	26.0±0.32a	2.76±0.14a
PM	13.4±0.1a	3.40±0.11a	6.85±0.08a	0.29±0.11a	0.97±0.08a	0.35±0.17a	26.3±0.60a	2.83±0.14a
AT	13.6±0.5a	3.38±0.25a	6.72±0.08a	0.30±0.03a	0.75±0.07b	0.29±0.07a	27.0±0.81a	2.68±0.15a
CM	13.6±0.1a	3.34±0.24a	6.92±0.10a	0.26±0.04a	0.82±0.07b	0.46±0.11a	26.2±0.20a	2.62±0.06a
Montepulciano								
T	13.0±0.1a	3.61±0.05b	5.61±0.16a	0.25±0.07a	1.79±0.07b	0.31±0.01c	23.0±0.09a	1.28±0.16a
PM	12.8±0.1b	3.78±0.04a	4.35±0.45c	0.34±0.04a	0.23±0.04c	1.53±0.05a	22.3±0.40a	1.43±0.07a
AT	13.1±0.1a	3.70±0.03ab	5.26±0.03b	0.24±0.08a	1.90±0.03a	0.34±0.02c	23.1±1.33a	1.34±0.07a
CM	13.0±0.4a	3.63±0.07b	5.65±0.04a	0.28±0.03a	1.72±0.03b	0.41±0.03b	23.3±0.19a	1.28±0.03a
Nero di Troia								
T	12.3±0.1c	3.93±0.05a	3.84±0.15a	0.23±0.07b	0.39±0.08a	1.38±0.01a	25.3±0.09a	3.88±0.15a
PM	12.9±0.1b	3.82±0.03b	3.39±0.02c	0.39±0.08a	0.21±0.02b	1.11±0.02b	24.2±1.44ab	3.45±0.07b
AT	13.1±0.1a	3.77±0.08b	3.46±0.04c	0.35±0.03a	0.04±0.02c	0.93±0.03c	22.0±0.21c	3.27±0.04c
CM	13.2±0.1a	3.72±0.04b	3.65±0.04b	0.31±0.04ab	0.06±0.04c	0.94±0.05c	23.2±0.45bc	3.06±0.06d
Sangiovese								
T	12.4±0.1a	4.02±0.04a	3.26±0.15b	0.34±0.02a	0.27±0.02a	1.72±0.07a	21.2±0.10b	3.63±0.18a
PM	12.0±0.2b	3.95±0.02ab	3.50±0.14a	0.25±0.02b	0.25±0.03a	1.75±0.19a	26.8±0.04a	3.38±0.06b
AT	12.0±0.1b	3.94±0.08ab	3.28±0.04ab	0.23±0.03b	0.21±0.02b	1.70±0.07a	21.5±0.28b	3.37±0.08b
CM	12.1±0.1b	3.92±0.04b	3.19±0.11b	0.18±0.3c	0.27±0.01a	1.69±0.10a	20.2±0.15c	3.18±0.12b

In columns and for each variety, different letters indicate significant differences at $P < 0.05$. E, ethanol; TA, total acidity; VA, volatile acidity; MA, malic acid; LA, lactic acid; DRE, dry reduced extract; ASH, ashes.

As expected, *Aglianico* wine showed the highest phenols content and colour intensity (CI) regardless the winemaking techniques used (Tab. 3). Winemaking techniques exerted a different influence on the extraction of phenolic substances and results were related to the grapes variety. With respect to traditional winemaking, the following results were obtained. As for *Aglianico*, prolonged maceration led to a slight decrease of flavans (F) and anthocyans (A), the addition of tannins led to a slight decrease of A, and cryo-maceration determined lower values of F, A and CI. Concerning *Montepulciano*, prolonged maceration led to a decrease in A and CI, the addition of tannins led to an increase of total polyphenols (TP) and CI, and cryomaceration caused a decrease in F and A, and an increase in TP and CI. The winemaking techniques applied to *Nero di Troia* caused enrichment in phenols, in particular

for A that play an important role on sensory characteristics of wine, whereas to *Sangiovese* determined impoverishment in phenolic substances, probably due to the poor characteristics of grapes used (Tab. 1).

Tab. 3: Phenolic composition and colour indices of wines.

Sample	F (mg/L)	A (mg/L)	TP (mg/L)	FRV (mg/L)	P (mg/L)	CI	T
Aglianico							
T	2086±204a	820±53a	2302±175a	698±26a	3294±94a	23.8±1.9a	0.50±0.05a
PM	1629±56b	697±38b	2314±63a	712±59a	3087±305a	21.7±0.2ab	0.54±0.01a
AT	2003±73a	704±19b	2379±88a	775±67a	3236±119a	21.0±0.1a	0.53±0.01a
CM	1626±21b	647±21b	2194±67a	800±75a	3023±86a	19.2±1.4b	0.54±0.1a
Montepulciano							
T	1058±55a	423±17a	1216±20b	189±24a	1179±145a	8.1±0.4b	0.90±0.04b
PM	974±50ab	327±39b	1220±67b	270±56a	1182±313a	7.0±0.1c	0.99±0.01a
AT	1002±73ab	415±21a	1358±89a	279±60a	1100±125a	9.0±0.1a	0.89±0.04b
CM	946±23b	354±23b	1358±62a	253±70a	1244±85a	9.4±0.3a	0.85±0.01b
Nero di Troia							
T	1637±57b	274±16b	2115±19c	680±23b	3046±147b	8.3±0.1a	0.88±0.02a
PM	2004±75a	335±20a	2365±89b	1000±66a	4057±121a	7.5±0.2b	0.76±0.01b
AT	1704±23b	372±23a	2592±64a	1076±73a	4249±85a	7.6±0.3b	0.76±0.05b
CM	1637±52b	372±40a	2152±62c	992±63a	3231±313b	7.0±0.2c	0.76±0.02b
Sangiovese							
T	579±22a	271±14a	1013±13a	177±21a	814±12a	6.0±0.4a	1.16±0.03c
PM	512±36b	221±16b	869±20b	119±7b	534±9c	5.9±0.3a	1.12±0.01c
AT	534±24ab	263±12a	916±41b	116±7b	597±20b	4.9±0.2b	1.29±0.01b
CM	423±35b	213±20b	767±29c	122±15b	346±12d	3.8±0.2c	1.39±0.03a

In columns and for each variety, different letters indicate significant differences ($P < 0.05$).
F, flavonoids; A, anthocyans; TP, total polyphenols; FRV, flavans reagent with vanillin; P, proanthocyanidins; CI, color intensity; T, tonality.

In Fig. 2 the anthocyanin composition for each wine is illustrated. Little differences were observed for both *Aglianico* and *Montepulciano*, whereas different results were obtained for *Nero di Troia* and *Sangiovese*. With respect traditional winemaking, addition of tannins and cryomaceration led to highest amount in malvidin forms in *Nero di Troia*, whereas prolonged maceration and cryomaceration led to a decrease of anthocyanins in *Sangiovese*.

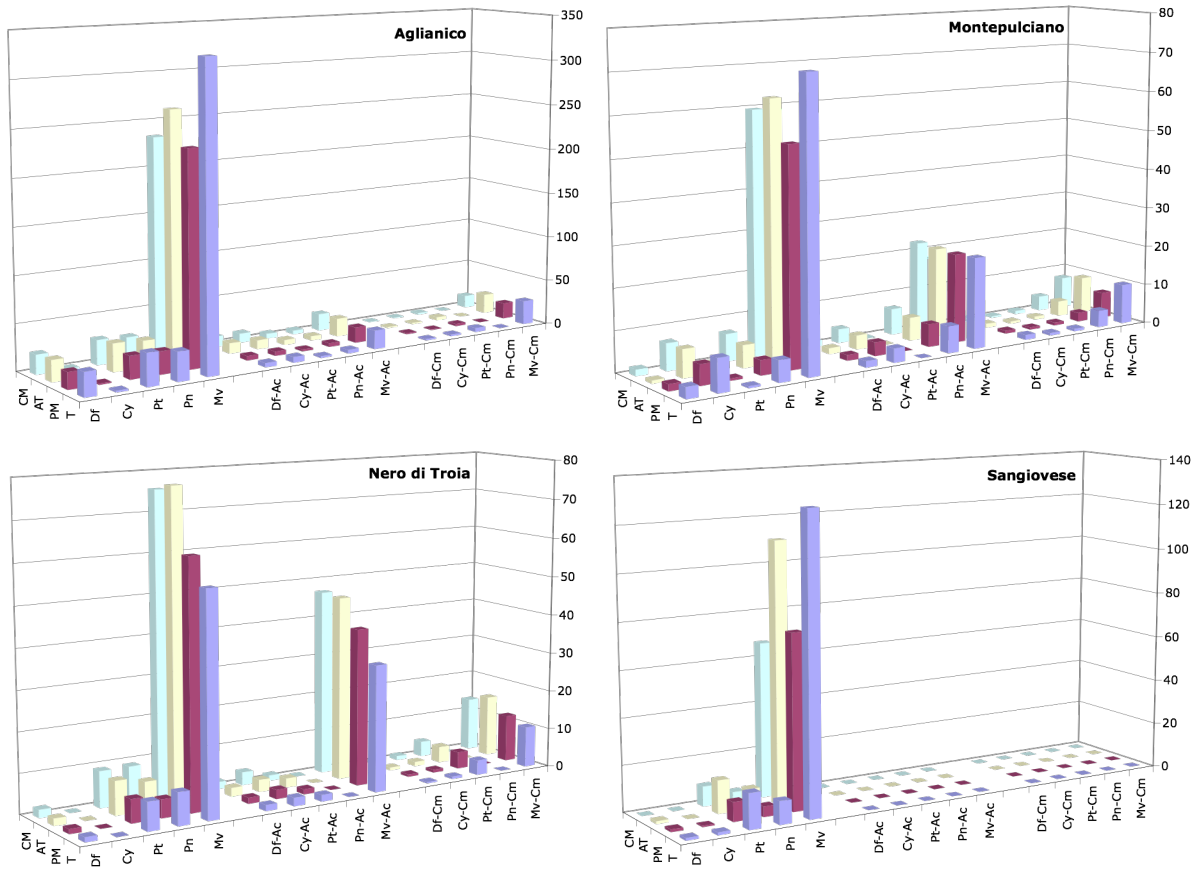

Fig. 2 – Anthocyanin composition of wines (mg L^{-1}).

CONCLUSIONS

The grape cultivars investigated in this work, *Aglianico*, *Montepulciano*, *Nero di Troia* and *Sangiovese,* showed different phenol contents and composition. The four winemaking techniques applied exerted a different impact on the phenol fraction of wines, probably due to intrinsic characteristics of grapes in terms of ripening and richness in phenols. Phenol composition of *Aglianico* was not influenced by winemaking techniques due to the natural richness in phenols of this variety, whereas some differences were observed for the other varieties. Prolonged maceration and addition of tannins led to enrichment in total phenols of *Montepulciano* and *Nero di Troia*, whereas all techniques applied led to a decrease of phenols in *Sangiovese*. Results obtained in this work support the conclusion that the phenol structure of wines seems to be mostly dependent on the grape variety rather then on the winemaking technology applied.

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REFERENCES

Cane N., 1990. Il controllo di qualità dei vini mediante HPLC: determinazione degli acidi organici. *L'Enotecnico*, 26:69-72.

Di Stefano R., Cravero M.C. 1991. Metodi per lo studio dei polifenoli dell'Uva. *Riv. Vitic. Enol.,* 44:37-45.

Di Stefano R., Cravero M.C., Genfilini N. 1989. Metodi per lo studio dei polifenoli dei vini. *L'Enotecnico*, 25:83-89.

EEC. 1990. European Communities. Commission Regulation No 2676/90 on "Community Analysis Methods to Use in Wine Sector". *Official J. European Communities*. No. L272/3.10.90.

Jackson R.S. 1994. Wine science: principles and applications. New York: Academic Press.

Kosir I.J., Lapornik B., Andrensek S., Wondra A.G., Vrhovsek U., Kidri J. 2004. Identification of anthocyanins in wines by liquid chromatography, liquid chromatography-mass spectrometry and nuclear magnetic resonance. *Anal. Chim. Acta*, 513:277-282.

La Notte E., Antonacci D., la Gatta M., Pati S., Coletta A., Gambacorta G. Influence of irrigation and grape production on the phenolic and volatile fraction of black wine grape Sangiovese. *In*: XXXIth World Congress of Vine and Wine. Verona, 15-20 June 2008.

Revilla E., Ryan J.M. 2000. Analysis of several phenolic compounds with potential antioxidant properties in grape extracts and wines by high-performance liquid chromatography-photodiode array detection without sample preparation. *J. Chrom. A*, 881:461-469.

Ribereau_Gayon P., Maujean A., Dubordieu D. 1998. Trattato di Enologia II. Chimica del vino, Stabilizzazione, Trattamenti. Bologna, Italia: EdAgricole.

THE POLLEN PRODUCTION AND YELD IN VINEYARD OF KAKHETI ACCORDING TO THE RESULTS OF POLLEN MONITORING

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ABSTRACT

Three pollen traps have been placed on the territory of the vineyard in Kvemo Magaro village where the Rkatsiteli variety was planted. Seven last years of monitoring have shown that the grape pollen influx in the traps is high and varies rather significantly from year to year. The pollen production and yield exhibit maximum values in humid 2003. A strong decrease in the grape pollen influx and yield is observed in 2004 when climate drying took place. Pollen production of cultural grape is high in the natural conditions of Kakheti. In the peak years the grape pollen influx under the shrubs is 114 000 gr/cm^2/year. For comparison it should be noted that the pollen influx of *Hedera helix* which is also the liana in maximum years reaches no more than 18 000 gr/cm^2/year.

In the conditions of the study region a specific complex of grape weeds consists of different species of *Chenopodiaceae, Convonvulus, Brasicaceae, Lathyrus.* In humid years there is a lot of *Polygonaceae, Ranunculaceae, Malva.*

Es wurden 3 Blütenstaubnetze auf dem Weinberg im Dorf Kvemo Magharo aufgestellt, wo die Rebsorte Rkatsiteli angebaut ist. Das Monitring von letzten 7 Jahre hat bewiesen, dass sich der Andrang des Blütenstaubs bei den Weintrauben in diesen Netzen deutlich variert. Die Produktivität des Blütenstaubs war in dem feuchten 2003 maximal ausgeprägt. Die starke Senkung des Blütenstaubandranges hat man im trockenen Jahr 2004 beobachtet. Die Fruchtbarkeit des Blütenstaubs ist für den Weinanbau in Kakhetien hoch. In den Spitzenjahren hat die Fruchtbarkeit auf jede Rebe 114 00 gr/cm^2/year ausgemacht. Für den Vergleich muss man anmerken, dass während des Blütenstaubandranges von Liane *Hedera helix*, die Fruchtbarkeit auch in diesen Jahren nicht Ihrer maximalen Leistung 18000 Quadratkorn überstiegen hat. Bei den Bedingungen der untersuchten Region besteht der Komplex der Rebe aus verschiedenen Sorten, wie z. B: *Chenopodiacae, Convonvulus*, *Brasicaceae, Lathyrus*. In den feuchten Jahren machen sich *Polygonaceae*, *Ranunculaceae* und *Malva* bemerkbar.

INTRODUCTION

Pollen monitoring in Georgia started in 1996 under the framework of the International Pollen Monitoring Programme (Hicks et al., 1996). This year 10 Tauber pollen traps have been placed on the territory of the Lagodekhi Reserve in different altitudinal belts (Kvavadze, 1999, 2001). Later, three pollen traps were placed in the vineyard of the Kvemo Magaro village occupied in 45 km to the south of Lagodekhi (Fig.1). The purpose of the pollen monitoring, besides clarification of taphonomy problems (pollen preservation, dispersion, production, etc.), is to establish a relationship between the pollen production of separate taxa and the change in climatic conditions. It was necessary to find out how one or another plant responds to climate worsening or improving, i.e. to reveal signals of climatic changes. In palaeopalynology such kind of investigations can contribute to more correct reconstructions of climates of past epochs.

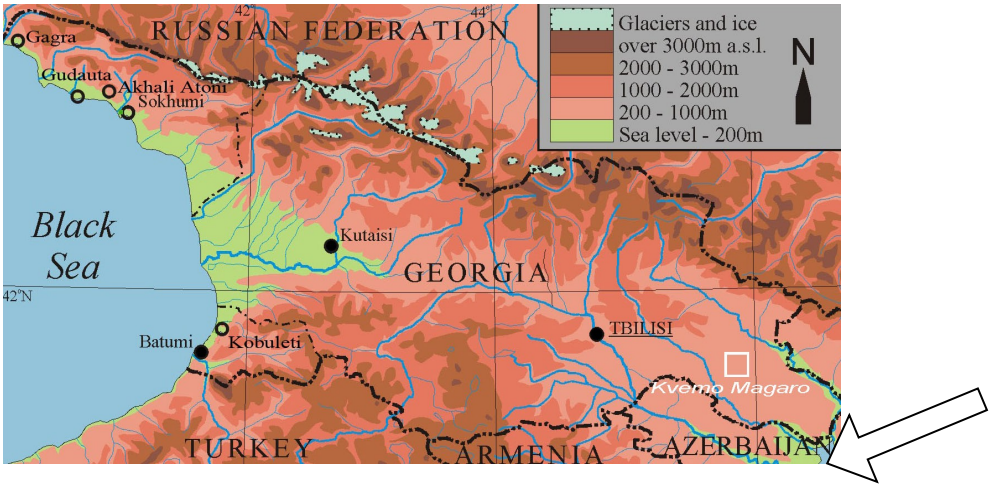

Fig.1. Map of Georgia and location of Kvemo Magaro village

Fig.2. Vineyard with pollen trap

Since from time immemorial Georgia is a vine-growing country, which is indicated by the palaeobotanic material collected here (Lisitsina, Prischepenko, 1976; Ramishvili, 2000; Constantini et al., 2006, Rusishvili 2007; Rukhadze, Bokeria, 2007; Kvavadze, Chichinadze, 2007), in 2002 a decision was taken to launch pollen monitoring in Kakheti, one of the centres of development of modern vine-growing. In the Kvemo Magaro village of Signakhi region, in a peasant's vineyard located at an altitude of 611 m above sea level (coordinates: N 41° 33' 30.3" ; E 45° 50' 42.7") three Tauber pollen traps were placed (Fig.2). The vineyard area is 1000 m^2 where in 10 rows there grow 360 vine bushes of Rkatsiteli variety. The vineyard was planted in 1991 and thus it is 19 years old. The preliminary results of pollen monitoring have partially been published and covered essentially taphonomy problems such as grape pollen grain preservation and its dispersion (Kvavadze, 2005; Kvavadze, Chichinadze, 2007). However, the main purpose of the present investigation is: 1) to establish peculiarities of grape pollen production under conditions of the study region; 2) to reveal changes in the pollen production caused by climate variations for the last 7 years; 3) to compare the pollen production data with those of grape yield.

MATERIALS AND METODS

Three Tauber traps (No.11-13) were placed in the vineyard. Trap No.11 was placed in the very centre of the vineyard, and traps No.12, 13 – in its periphery. A modified Tauber pollen trap is a nearly 5 liter vessel with opening with 5 cm in diameter. The trap is buried into the soil so that the opening is at the soil level. For better preservation of the accumulated pollen a liquid containing glycerin, thymol and formalin is placed into the trap (Hicks et al. 1996, 1999). One-year accumulated pollen together with rain water is precipitated in laboratory conditions. At the next stage, the organic content from the precipitate is collected by centrifuging. In case of the presence of large quantities of clay or other soil formations, mineral particles were separated from the pollen by centrifuging in cadmium liquid. The further treatment is carried out using standards methods. To count the pollen influx (amount of accumulated pollen per each square centimeter), three *Lycopodium* tablets with barch No.124961 were added (Stockmar, 1971,1973).

RESULTS AND DISCUSSION

A seven-year series of observations showed that the grape pollen production slightly varies in different parts of the vineyard. As a whole, in the periphery it appeared to be somewhat lower than in its central part. For example, in 2008 and 2009 in trap No.12 located in the north periphery of the rows the pollen influx was 28-30 thousand gr/cm^2/year. In trap No.11 placed in the very central internal part of the vineyard this index reaches up to 42-50 thousand gr/cm^2/year (Fig.3). As for the averaged indices of pollen influx for all three traps, its maximum quantity was recorded in the first year of observations (2003) and was 114 293 pollen gr/cm^2/year. In 2004 and 2006 the pollen influx decreased substantially and was only 24 253 and 24 922 gr/cm^2, respectively. 2005 and 2007 were distinguished by average pollen production indices when the pollen influx reached 64666 - 65698 gr/cm^2, respectively. The given indices indicate high grape pollen production. However, beyond the natural vine habitat grape pollen production is not so high (Turner, Brown 2004). If we compare the grape pollen production with that of other lianas, for example, of ivy (*Hedera helix*), it will be found out that even in the peak years the ivy pollen influx is only 18 000 gr/cm^2/year, i.e. nearly 7 times lower that the grape pollen production (Kvavadze, Chichinadze, 2007).

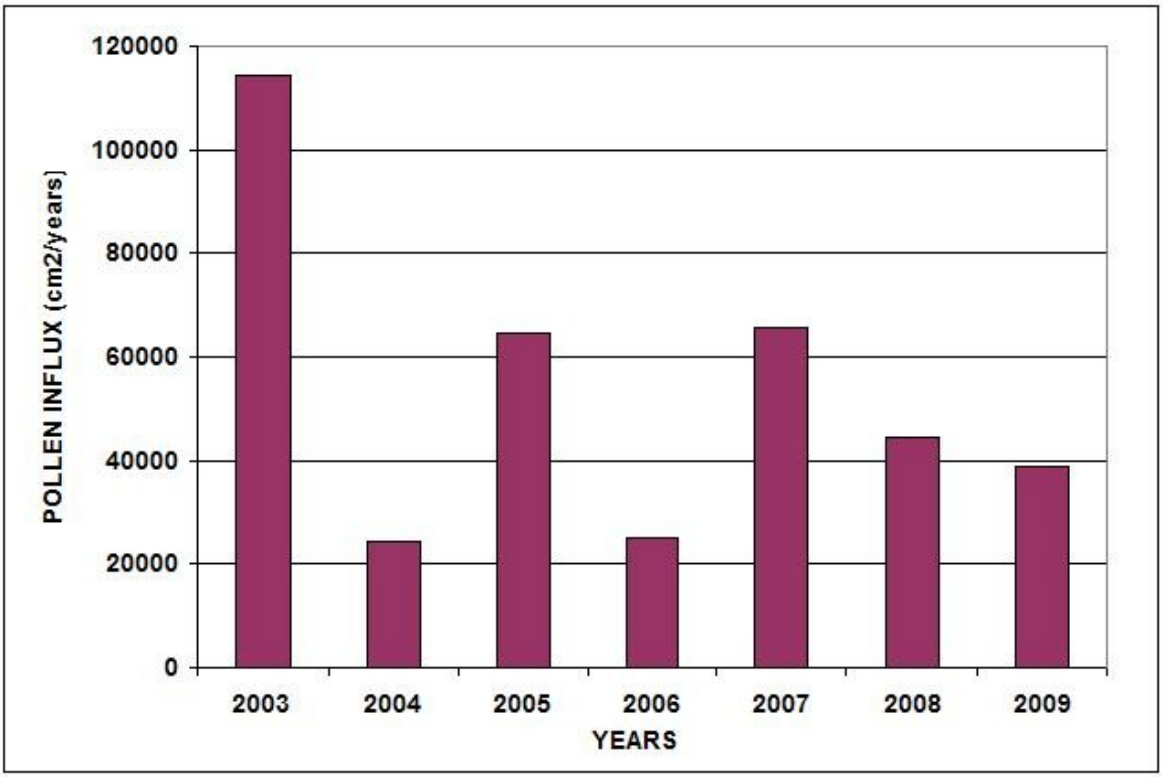

Fig.3. Pollen production (=influx) of *Vitis vinifera* in the 2003-2009 years.

It should also be mentioned that in favourable years accompanied by high pollen production the size of grape pollen grains is big and reaches 40-45 μ. In the low pollen production years the size of pollen grains is 20-25 μ. and smaller (Fig.4).

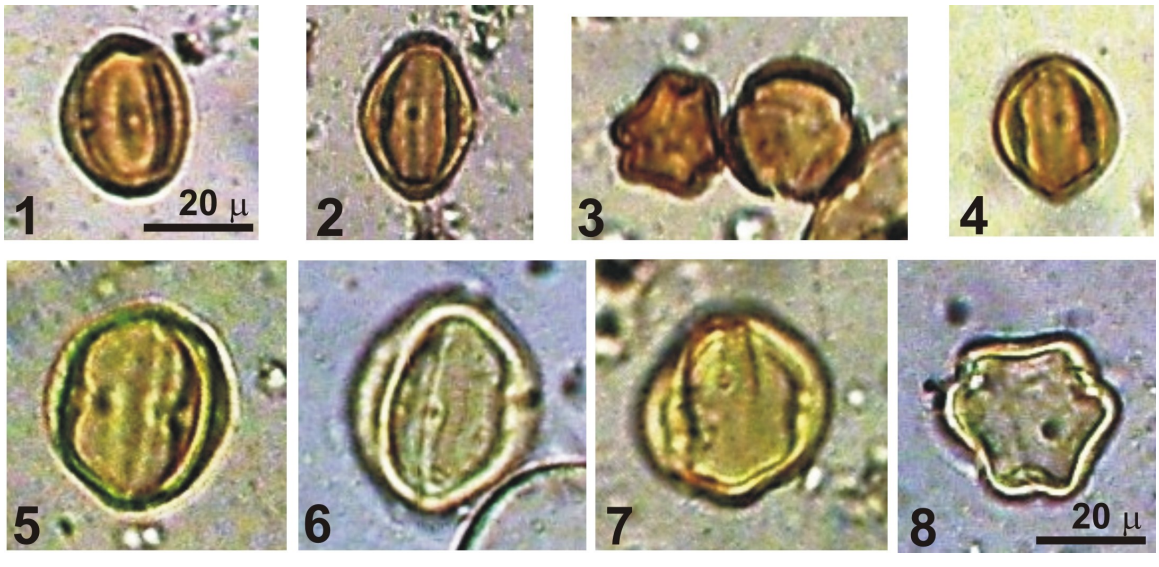

Fig. 4. The size of *Vitis* pollen grains in dry (1-4) and in humid (5-8) years.

Rather interesting is also the fact that grape pollen production and yield are in direct relationship, which is quite natural. Precisely in 2003 the biggest amount of grape reaching

1250 kg. The pollen production was also maximal in this year. In the low grape pollen production years the quantity of the gathered grape did not exceed 500 kg. In 2005 and 2007 characterized by average pollen production indices 800 kg per year was gathered in the vineyard. However in 2009, here only 350 kg was gathered. This is partially explained by poor care of the vineyard caused by the disease of the vine-grower and by summer hail.

A comparison of pollen production and yield with climatic indices in the study region allowed us to establish the following. Climate drying (Fig.5) exerts stronger influence on the grape yield than climate cooling, as was believed earlier (Kvavadze et al., 2007). For example, in the arid year of 2004 the pollen influx decreased nearly 5 times as compared with humid 2003. In the arid year of 2006 the pollen production decreased about 4 times (Fig.3). As shown in the diagram, a similar relationship between the pollen influx and humidity is recorded during the whole series of observations.

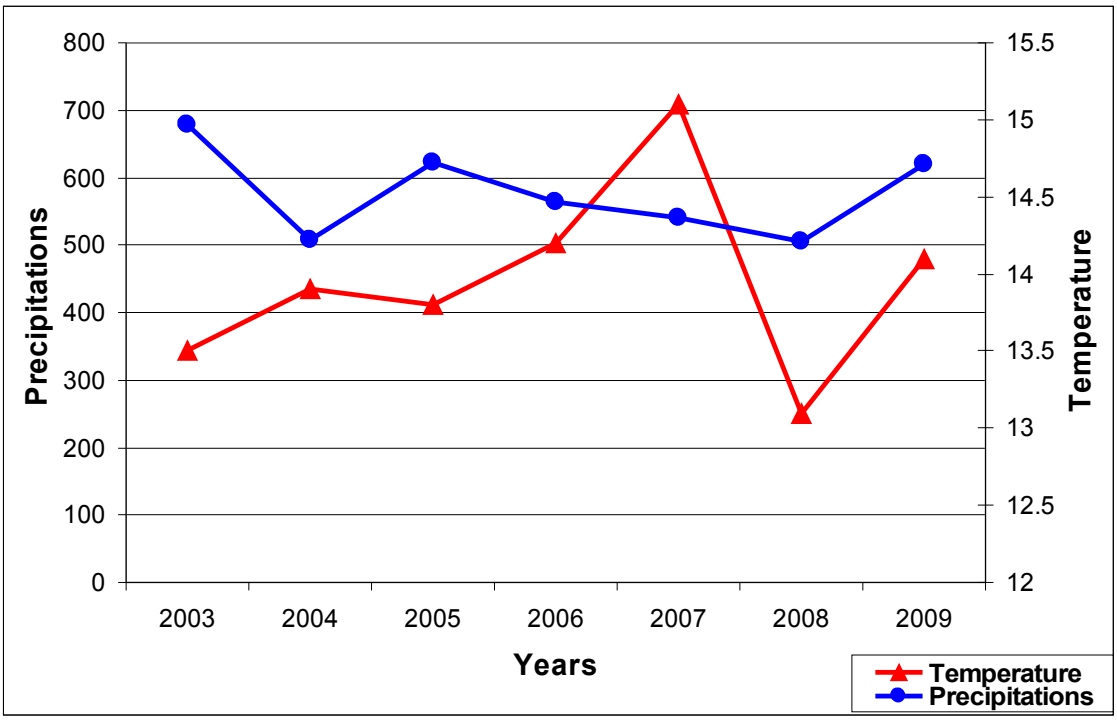

Fig.5. The climatic indices in the investigate region in 2003-2009 years.

We performed a palynological analysis of the wine made of the yield of the study vineyard. One liter of wine in 2006 that was the least productive year contained 9 thousand grape pollen grains, and in 2007 when both pollen production and yield increased – more that 11 thousand pollen grains. In the group of arboreal species *Juglans regia, Corylus* grown in the farm house fruit gardens adjoining the vineyard are present in the wine spectrum. Among herbs the pollen of such weeds as *Chenopodiaceae* and *Artemisia* are predominant. *Brassica, Urtica, Rumex, Plantago, Papaver, Chenopodium alba* pollen grains are recorded. As we see, the wine pollen spectrum reflected all main peculiarities of vineyard cenosis. The same conclusion was made by M.Rösch (Rösch, 2005). As for the degree of preservation of pollen grains, they were in perfect condition. Good preservation of pollen found in alcoholic drinks and especially in the vessel content is an important indicator during archaeopalynological investigations.

CONCLUSION

Pollen production of cultural grape is high in the natural conditions of Kakheti. In the peak years the grape pollen influx under the shrubs is 114 000 gr/cm^2/year. For comparison, it should be noted that the pollen influx of *Hedera helix* which is also the liana in maximum years reaches no more than 18 000 gr/cm^2/year.

In the most productive years 1200 kg of grapes was gathered in the territory of the vineyard, while in the years of low productivity caused either by climatic conditions or by poor care no more that 500-350 kg of grapes was gathered.

Climate drying has stronger influence on the grape pollen production and yield than climate cooling. In arid years there is nearly a 4-5 time decrease in the yield than in humid years.

Unfavorable climatic conditions have a strong effect on the size of vine pollen grains, which should be taken into consideration when studying the archaeological material.

Investigation of modern wine has shown that by pollen grain size and quantity of counted grape pollen one can judge of productivity and climatic conditions, which is also important for ethnopalynological studies.

BIBLIOGRAPHY

[1] Constantini L., Kvavadze E., Rusishvili N. 2006. The antiquiity of grapevine cultivation in Georgia. *Jurnal of Grapevine and Wine*. Tbilisi, 1-2 : 62-81.

[2] Hicks Sh., Ammann B., Latalowa M. Pardoe H.1996. European Pollen Monitoring Programme. Project description and guidelines.University of Oulu, Finland.

[3] Hicks Sh., Tinsley H., Pardoe H., Peter.C.l.1999. European Pollen Monitoring Programme. Supplement to the guidelines.University of Oulu, Finland.

[4] Kvavadze E. 1999. The first results of the Pollen Monitoringe Programme in the Caucasus Mountains (Georgia). *Acta Palaeobotanica, 39(1*):171-177.

[5] Kvavadze E. 2001. Annual modern pollen deposition in the foothills of the Lagodekhi Reservation (Caucasus, East Georgia) related to vegetation and climate). *Acta palaeobotanica,* 41(2):355-364.

[6] Kvavadze E. 2005. Modern arboreal pollen deposition in pollen traps, moss polster and soils in Lagodekhi and Kvemo Magaro (Eastern Georgia). Abstracts of 5-th International Meeting of PMP, Varna (Bulagaria), pp.14.

[7] Kvavadze E., Chichinadze M. 2007. Preliminary results of pollen deposition in vineyard of Kvemo-Magaro village (Kakheti, Georgia): comparison between pollen traps and surface soil samples. *Proceedings of the 6-th International Meeting of PollenMonitoring Programme*, Riga, 2007, pp.45-47.

[8] Kvavadze E., Connor S., Narimanishvili G. 2007. Late Pleistocene and Holocene history of landscapes on the Tsalka plateu (southern Georgia), based on palynological analysis of lake and soil formation. In. G.Mchedlidze (ed), Problems of Palaeobiology, Proceedings of Institute of Palaeobiology, Tbilisi, pp.34-47.

[9] Lisitsina G.N., Prischepenko L.V. 1977. Paleoetnobotanicheskie nakhodki Kavkaza i Blizhnego Vostoka (The palaeoetnobotanocal founds of Caucasus and Near East). Nauka, Moskow.

[10] Ramishvili R. 2000. History of Georgian grapevine and vine. Historical. archaeological and ampeolographical investigation. Tbilisi.

[11] Rukhadze L., Bokeria M. 2007. To the question of investigation of fossil and recent species of europian vine on the territory of Georgia. In.: In. G.Mchedlidze (ed), Problems of Palaeobiology, Proceedings of Institute of Palaeobiology, Tbilisi, pp.56-60.

[12] Rösch M. 2005. Pollen analysis of the contents of excavated vessels – direct archaeobotanical evidence of beverages. *Vegetation History and Archaeobotany, 14*:179-188.

[13] Rusishvili N. 2007. New information about archaeobotanical *Vitis* from Georgia. Abstracts of the 14-th Symposium of the IWGP, Krakow, pp.104.

[14] Stockmarr J.1971. Tables with spores used in absolute pollen analysis. *Pollen et Spores,* 13*:* 612-621.

[15] Stockmarr J. 1973. Determination of spore concentration with an electric particle counter. *Dan.Geol.Unders.Arbog.* 1972: 87-89.

[16] Turner S.D., Brown A.G. 2004. Vitis pollen dispersal in and from organic vineyards I. Pollen trap and soil pollen data. *Review of Palaeobotany &Palynology* 129: 117-132.

Composition phénolique de raisins de Cabernet-Sauvignon et de Merlot de la région de Bordeaux (millésime 2009): comparaison aux millésimes 2006, 2007 et 2008

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RESUME

Cette étude vise à étudier la nature phénolique de pellicules et de pépins de raisins des cépages Cabernet-Sauvignon et Merlot pour déterminer l'influence du cépage et du millésime sur la composition phénolique des raisins bordelais. Les teneurs en polyphénols, tanins et anthocyanes totaux sont déterminées à partir d'extraits de pellicules et de pépins de raisins CS et M du bordelais. Les proanthocyanidines monomères et oligomères sont identifiées/quantifiées par CLHP-UV-Fluo. Les pourcentages de galloylation, de prodelphinidines et le degré moyen de polymérisation des tanins oligomères sont déterminés par CLHP-UV-SM. La composition anthocyanique des extraits de pellicules est déterminée par CLHP-SM. Les influences « millésime » et « cépage » sont étudiées sur ces paramètres. Dans les extraits de pépin, les teneurs en polyphénols et en tanins totaux sont plus importantes dans les raisins M que dans ceux CS, permettant de discriminer ces cépages. Dans les extraits de pellicules, aucune différence dans les concentrations en polyphénols totaux n'est observée. Les analyses CLHP-UV-fluo confirment ces résultats ; elles soulignent des différences de concentration et de composition des flavanols libres et proanthocyanidiques entre les deux cépages. Le cépage influence principalement le DPm et le %G. L'effet du millésime sur la composition en polyphénols des raisins est confirmé. En comparaison aux précédents millésimes, des concentrations faibles en proanthocyanidines et élevées en anthocyanes sont trouvées dans les extraits de pépins et de pellicules. Ces observations reflètent le millésime 2009, « exceptionnel » pour le bordelais, en raison des conditions climatiques favorables à la maturité des raisins.

INTRODUCTION

Cépages mondialement reconnus, le Cabernet Sauvignon (CS) et le merlot (M), représentent la majorité de l'encépagement du vignoble bordelais. Leur composition phénolique et son suivi au cours des années constituent des paramètres qualitatifs d'intérêt particulièrement dans cette région viticole. Effectivement, les proanthocyanidines ou tanins condensés du raisin sont des composés phénoliques de grande importance pour la qualité du vin en raison de leurs propriétés d'astringence et d'amertume et de leur rôle dans la stabilité de la couleur du vin (interaction tanins/anthocyanes) (Peleg *et al.*, 1999). La taille moléculaire des proanthocyanidines affecte leur amertume et astringence, les monomères étant plus amers qu'astringents tandis que l'inverse est observé pour les dérivés de plus haut poids moléculaire. De plus les proanthocyanidines de la pellicule de raisin diffèrent de celles des pépins : les tanins des pellicules comprennent en plus des procyanidines (polymère de catéchines et d'épicatéchine) contenus dans le pépin, des prodelphinidines (polymère de gallocatéchines et d'épigallocatéchine). De plus ils possèdent un degré de polymérisation (dP) plus élevé et une plus faible proportion de sous-unités galloylées (estérification avec l'acide gallique) que les tanins des pépins (Somers, 1971).

Dans la littérature, peu d'études s'attachent à comparer la composition phénolique des divers cépages. L'objectif de cette étude est d'étudier l'impact du cépage sur la composition en

proanthocyanidines et en anthocyanes de variétés de raisins CS et M du bordelais. Il s'agit de déterminer si la composition phénolique d'extraits de pellicule et de pépins permet de discriminer les deux cépages. Faisant suite à une étude de 3 ans (Chira *et al*. 2009), l'intérêt est également de constituer une base de données de la composition phénolique des raisins du vignoble bordelais la plus exhaustive possible (années 2006, 2007, 2008, 2009) et d'étudier le facteur « millésime » sur la composition phénolique des pépins et pellicules des deux cépages.

MATERIELS ET METHODES

L'étude est menée sur sept parcelles localisées dans la région bordelaise et encépagées par Vitis Vinifera L. Cabernet Sauvignon (CS) et Merlot (M). Ces parcelles sont situées dans les appellations Médoc (Pauillac, Margaux, P1 et P2 respectivement), Saint Emilion (P3 et P4), Côtes de Bourg (P5), Graves (P6) et Entre Deux Mers (P7). Un échantillon de baies a été collecté dans chaque parcelle à maturité.

Les protocoles d'extraction utilisés sont ceux élaborés par Chira *et al*. (Chira *et al*. 2009). Les pépins et les pellicules séparés des raisins sont d'abord lyophilisés puis broyés pour obtenir des poudres. Puis celles-ci sont extraites par un mélange acétone/eau (80 : 20, v/v) et par un mélange méthanol/eau (60 : 40, v/v). Les surnageants obtenus après centrifugation sont évaporés et repris dans l'eau distillée avant d'être lyophilisés. Une partie des extraits bruts de pépin et de pellicule ainsi obtenus est dissous dans une solution eau/éthanol (90 :10 v/v, ajusté à pH 3.5 avec de l'acide tartrique) pour déterminer les teneurs globales en polyphénols totaux (Folin Ciocalteu), tanins totaux (Bate-Smith) et anthocyanes (décoloration au bisulfite de sodium).

L'autre partie de l'extrait brut est purifiée par une extraction liquide/liquide en deux étapes. L'extrait brut solubilisé est d'abord extrait trois fois par le chloroforme pour éliminer les composés lipophiles. La phase aqueuse est alors extraite trois fois par de l'acétate d'éthyle pour obtenir un extrait enrichi en tanins de faibles poids moléculaire (monomères, oligomère) en phase organique et un extrait enrichi en tanins de plus hauts poids moléculaires en phase aqueuse. Ces deux types d'extraits purifiés sont concentrés et lyophilisés pour obtenir une poudre sèche. Ils sont caractérisés par CLHP-UV-fluorimétrie et CLHP-SM. Le degré moyen de polymérisation et la composition des unités monomères (pourcentages de galloylation (%G), de prodelphinidines (%P)) sont étudiés à l'aide de la phloroglucinolyse (Kennedy et Jones, 2001). La composition en anthocyanes des glucosides, glucosides acétylées et glucosides coumarylées des extraits de pellicules de raisins est déterminée par CLHP-SM. Les influences du millésime (2006, 2007 et 2008, résultats d'une étude précédente (Chira *et al*. 2009) et 2009) et du cépage sont étudiées sur l'ensemble de ces paramètres.

RESULTATS ET DISCUSSION

Dans les extraits de pépin, les teneurs moyennes en polyphénols totaux et en tanins totaux étaient 1,4 fois plus importantes dans les raisins de Merlot que dans ceux de Cabernet-Sauvignon. Ces données permettent donc de discriminer efficacement les deux cépages. En revanche, dans les extraits de pellicules, aucune différence significative dans les concentrations moyennes en polyphénols totaux, tanins totaux ou anthocyanes totales n'a été observée (Figure1).

Les analyses en CLHP ont confirmé ces premiers résultats ; elles ont souligné des différences importantes de concentration absolue et de composition des flavanols libres et proanthocyanidiques entre les deux cépages comme Chira *et al*. avaient déjà pu l'observer (Chira *et al*. 2009). Ainsi pour l'ensemble des proanthocyanidines quantifiées, les concentrations dans le cépage Merlot sont toujours supérieures à celles du cépage Cabernet-Sauvignon. Par exemple, pour la parcelle p2 (Médoc), dans les pépins de raisins issus de cépage Merlot, la concentration en (+)-catéchine était de 1,8 mg/g (poids sec) alors qu'elle

était de 0,6 mg/g (poids sec) pour les raisins issus de cépage Cabernet-Sauvignon. Les variations sont aussi importantes pour l'épicatéchine, que ce soit dans les pépins ou les pellicules puisque les concentrations obtenues pour le cépage Merlot peuvent être jusqu'à 8 fois supérieures à celles obtenues pour le cépage Cabernet-Sauvignon.

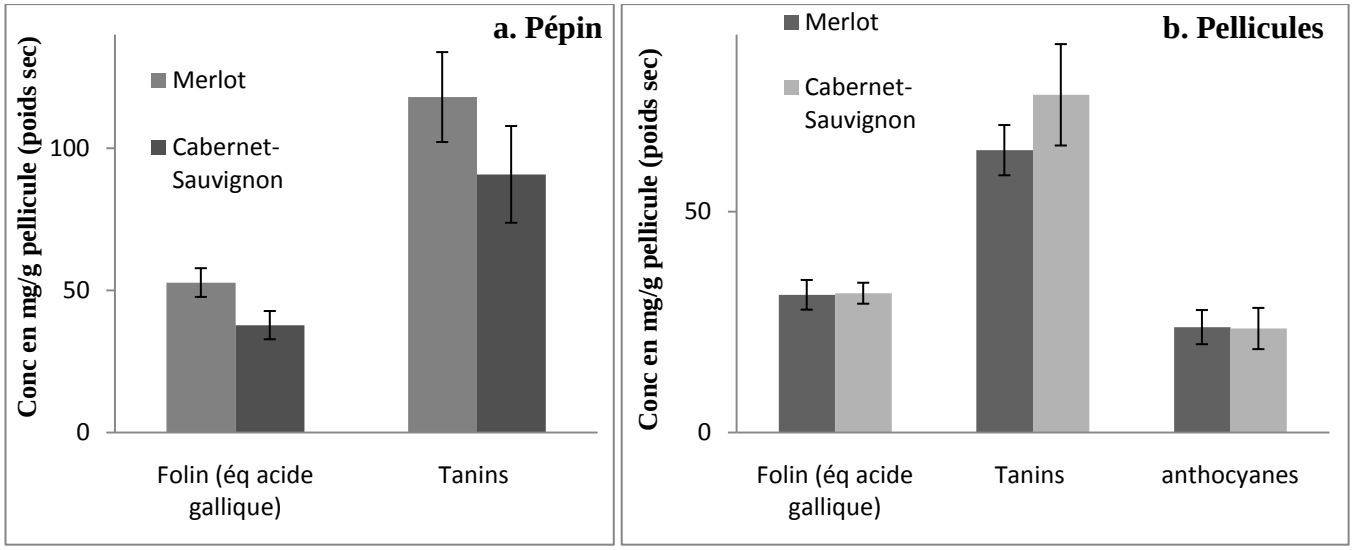

Figure 1 : a. Teneurs en polyphénols totaux (Folin) et en tanins totaux des pépins ; b. Teneurs en polyphénols totaux (Folin), en tanins totaux et en anthocyanes des pellicules

Quelque soit le millésime étudié, l'influence du cépage est aussi observée sur les paramètres tels que le DPm, le % G et le % P (pellicule) pour les extraits de pépin comme pour ceux de pellicules. De façon générale, pour les extraits issus de CS, ces paramètres sont supérieurs à ceux obtenus pour les extraits issus de cépage M.

L'effet significatif du millésime sur le la composition en polyphénols des raisins a été confirmé pour 2009 (Chira *et al*. 2009). En comparaison aux millésimes 2006, 2007 et 2008, des concentrations particulièrement faibles en proanthocyanidines et particulièrement élevées en anthocyanes ont été trouvées dans les extraits de pépins et de pellicules (Tableau 1). Ces observations reflètent le millésime 2009 qualifié « d'exceptionnel » pour le vignoble bordelais en raison des conditions climatiques favorables à la maturité des raisins (fort ensoleillement, hautes températures durant l'été et le mois de septembre).

	Millésime	Moyenne pépin			Moyenne pellicule		
(+)-catéchine	2006	8,47	+/-	2,95	0,13	+/-	0,06
	2007	17,42	+/-	13,50	0,56	+/-	0,27
	2008	3,81	+/-	0,64	0,05	+/-	0,03
	2009	**1,95**	**+/-**	**0,96**	**0,02**	**+/-**	**0,01**
(-)-épicatéchine	2006	5,17	+/-	2,59	0,10	+/-	0,04
	2007	8,54	+/-	7,56	0,623	+/-	0,73
	2008	2,07	+/-	0,59	0,04	+/-	0,03
	2009	**1,59**	**+/-**	**0,61**	**0,01**	**+/-**	**0,01**

Tableau 1 : Effet du millésime sur les teneurs moyennes en (+)-catéchine et épicatéchine des pépins et des pellicules de raisins de cépage CS (mg/g matière sèche).

CONCLUSIONS

Dans cette étude, des raisins de deux cépages bordelais (Cabernet Sauvignon, Merlot ; millésime 2009) ont été analysés pour leur contenu phénolique et les résultats ont été comparés à ceux des trois millésimes précédents.

L'utilisation d'indicateurs globaux tels que le dosage des polyphénols totaux (Folin Ciocalteu) et des tanins totaux (Bate Smith) a permis de discriminer les deux cépages pour les extraits de pépins ; les pépins issus de cépage Merlot apparaissent plus concentrés en polyphénols et en tanins totaux que les raisins issus du cépage Cabernet-Sauvignon.

Les analyses plus fines en CLHP confirment que les teneurs en composés monomères et oligomères proanthocyanidiques ((+)-catéchine, (-)-épicatéchine, (-)-épicatéchine-*O*-gallate et oligomères) permettent de discriminer les cépages ($concentrations_M$ > $Concentrations_{CS}$). Elles soulignent aussi l'influence du cépage sur la composition des unités proanthocyanidiques (mDP_{CS} > mDP_M) et %G ($\%G_{CS}$ > $\%G_M$). Ces résultats confortent donc ceux obtenus par Chira *et al.* pour les trois millésimes précédents.

L'effet millésime est également vérifié pour le millésime 2009. Ses effets sont accentués (faibles teneurs en proanthocyanidines) en raison du millésime « exceptionnel 2009 » (fort ensoleillement et fortes chaleurs). Ces résultats permettent donc de compléter la base de données de la composition phénolique des raisins du vignoble bordelais. A terme, ils permettront de comprendre les divers mécanismes de la maturation des raisins, fonction de l'implantation et de l'état du vignoble, des conditions climatiques et des caractéristiques du sol.

BIBLIOGRAPHIE

Chira K., Schmauch G., Saucier C., Fabre S., Teissedre P. L., 2009. Grape Variety Effect on Proanthocyanidin Composition and Sensory Perception of Skin and Seed Tannin Extracts from Bordeaux Wine Grapes (Cabernet Sauvignon and Merlot) for Two Consecutive Vintages (2006 and 2007). *Journal of Agricultural and Food Chemistry*, 57: 545-553.

Kennedy J.A et Jones G.P., 2001. Analysis of proanthocyanidin cleavage products following acid-catalysis in the presence of excess phloroglucinol. Journal of Agricultural and Food Chemistry, 49: 1740–1746.

Peleg H., Gacon K., Schlich P., Noble A. C., 1999. Bitterness and astringency of flavan-3-ol monomers, dimers and trimers. Journal of the Science of Food and Agriculture. 79: 1123–1128.

Somers T. C., 1971. The polymeric nature of wine pigments. *Phytochemistry*, 10 : 2175–2186.

SELECTED YEAST STRAINS (*SACCHAROMYCES CEREVISIAE*) WITH GLYCOLYTIC INEFFICIENCY AND METABOLIC INHIBITORS TO REDUCE ALCOHOLIC DEGREE IN VINES FROM WARM REGIONS

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ABSTRACT

During alcoholic fermentation of grape must, yeast transform glucose and fructose in ethanol and CO_2 as major compounds, and other metabolites as minor compounds but with very relevant sensorial repercussion because of its role in aroma, acidity and structure. Not all the *Saccharomyces cerevisiae* strains are able to produce the same amount of ethanol from the must even when all the sugars are metabolized. The selection of yeast with glycolytic inefficiencies let us to find strains with an ethanol production below of the media and deviate metabolism to the production of other metabolic compounds with suitable sensorial repercussion. This can be a good biotechnology to apply in warm regions or affected by climatic change with problems of high alcoholic degree. This work has studied 30 yeast strains belonging to *Saccharomyces cerevisiae* specie, isolated in different Spanish regions, to evaluate its glycolytic efficiency, and its capacity to ferment musts reaching ethanol contents below of the medium value. On the other hand, the production of other fermentative metabolites was evaluated. Finally metabolic inhibitors were studied to evaluate their capacity to modify the fermentative metabolism working on some glycolysis enzymes in order to reduce alcoholic degree.

***Keywords*:** *Saccharomyces cerevisiae*, glycolytic inefficiency, alcoholic degree reduction, enology of warm regions.

RESUMEN

Durante la fermentación alcohólica del mosto de uva, las levaduras transforman la glucosa y fructosa en etanol y CO_2 mayoritariamente, y de forma minoritaria en otros compuestos que en muchos casos tienen una repercusión sensorial interesante ya que participan en la fracción aromática, en algunos casos en la acidez y también en la estructura de los vinos. No todas las cepas de *Saccharomyces cerevisiae* producen la misma cantidad de etanol a partir de un mosto de la misma concentración inicial. La selección de cepas ineficientes glicolíticamente permite seleccionar levaduras con una producción de etanol inferior al promedio y desviar el metabolismo hacia la producción de otros metabolitos cuya repercusión sensorial puede ser positiva. Puede constituir una biotecnología interesante para aplicar en regiones cálidas o afectadas por cambio climático con problemas de grado alcohólico elevado. El presente trabajo ha estudiado 30 cepas de levadura de la especie *Saccharomyces cerevisiae* asiladas en distintas regiones españolas para evaluar su eficiencia glicolítica y su capacidad para fermentar mostos alcanzando un contenido de etanol inferior al normal. Por otra parte, se ha

evaluado la producción de otros metabolitos fermentativos. Finalmente se han estudiado bloqueadores metabólicos para evaluar su capacidad de modificar el metabolismo fermentativo actuando sobre algunas enzimas de la glicólisis y favoreciendo la reducción de grado alcohólico.

INTRODUCCIÓN

In warm regions potential alcoholic degree and unequilibrated must, especially in acidity are real problems to be resolved. Strains of *Saccharomyces cerevisiae* have different yields to produce ethanol from the same content of sugars. These peculiarities can be named glycolytic inefficiencies. We can select yeast strains with these properties in order to reduce the final alcoholic degree together with the production of some metabolic intermediates that can have repercussion in the sensorial profile like polyalcohols or organic acids.

The aim of this work was select *Saccharomyces cerevisiae* with low ethanol production and the detection and use of some metabolic inhibitors which are able to deviate the conventional glycolysis to the production of other molecules than ethanol.

MATERIALS AND METHODS

Glycolytic efficiency

Were fermented musts with a potential alcoholic degree 14.7 (250 g/L of sugar) using each yeast strain after a synchronization process. 1 mL of inocula was used to inoculate the fermentations over a volume of 60 mL. Al the fermentations were performed isotermically at 25 ºC and in triplicate.

Alcohol

Ethanol was measured by ebullometry.

Sugars

Residual sugars (glucose and fructose) were analyzed using enzymatic tests.

Metabolic inhibitors

Furfural was used as inhibitor at doses of 1, 5, 10, 25 and 50 mg/L. The addition was made at the beginning of fermentation by mean of an aqueous solution. The conditions of the fermentations were as in the study of glycolityc efficiency. The yeast strain used was 7VA, *Saccharomyces cerevisiae* isolated in Ribera del Duero.

RESULTS AND DISCUSSION

Table 1 show the alcoholic degree reached in each fermentation. The more interesting yeast were those which finalizing the fermentation with low alcoholic degree and without residual sugars. The highlighted strains in bold type had a low alcoholic degree compared with the value of reference and this was produced with a small amount of residual sugars. These yeast strains showed an alcoholic degree between 0.5-1 lower than the others. Some of them had better values but associated to high amounts of residual sugars, probably due to low fermentative power.

Table 1. Alcoholic degree and residual sugars produced by the strains studied

Yeast strain	Origin	º Alcohol	Glucose (g/L)	Fructose (g/L)
CTPL5		14.6 ± 0.1	0.12 ± 0.13	1.39 ± 0.53
CTPL14	Ribera del Duero 2005	13.8 ± 0.0	2.35 ± 0.27	15.66 ± 1.22
CTPL22		13.7 ± 0.1	1.52 ± 1.97	8.56 ± 7.52
G2T4		14.8 ± 0.1	0.12 ± 0.05	0.87 ± 0.15
S2(4)	Trujillo 2005	14.4 ± 0.5	0.17 ± 0.30	0.52 ± 0.40
S2(11)		15.0 ± 0.1	0.52 ± 0.09	0.23 ± 0.20
G237		14.5 ± 0.2	0.20 ± 0.10	4.03 ± 1.03
TT5(1)		13.1 ± 0.5	4.07 ± 2.93	12.47 ± 9.75
CS1(7)	Toro 2007	11.7 ± 0.1	7.17 ± 1.11	17.69 ± 2.32
CS2(11)		12.9 ± 0.1	0.43 ± 0.09	4.81 ± 1.25
TP2A(2)		14.1 ± 0.3	0.13 ± 0.08	0.53 ± 0.56
TP2A(4)		14.4 ± 0.0	0.23 ± 0.18	0.75 ± 0.74
TP2A(16)		**13.7 ± 0.1**	**0.13 ± 0.12**	**0.53 ± 0.15**
TP3A(1)		**13.6 ± 0.2**	**0.23 ± 0.05**	**0.52 ± 0.23**
TP3A(4)	Toro 2008	14.0 ± 0.2	0.34 ± 0.23	0.14 ± 0.13
TP3A(6)		14.4 ± 0.2	0.17 ± 0.10	0.38 ± 0.05
TP3A(7)		14.8 ± 0.1	0.14 ± 0.05	0.15 ± 0.10
TP4A(8)		14.9 ± 0.3	0.14 ± 0.11	0.35 ± 0.18
CB(3)		14.1 ± 0.6	0.27 ± 0.25	0.36 ± 0.38
CB(9)		14.4 ± 0.0	0.31 ± 0.33	0.26 ± 0.18
7VA		14.7 ± 0.0	0.28 ± 0.10	0.35 ± 0.23
1VA	Ribera del Duero 2001	15.0 ± 0.2	0.17 ± 0.00	0.50 ± 0.10
3VA		14.8 ± 0.1	0.32 ± 0.28	0.16 ± 0.10
4CV		14.2 ± 0.7	0.17 ± 0.09	0.16 ± 0.11
5CV	Rioja 2001	14.3 ± 0.1	0.29 ± 0.22	0.61 ± 0.09
9CV		14.3 ± 0.1	0.17 ± 0.00	0.37 ± 0.37
1EV		14.8 ± 0.1	0.15 ± 0.10	0.26 ± 0.09
2EV	Navarra 2001	14.7 ± 0.4	0.40 ± 0.27	0.40 ± 0.56
7EV		**13.9 ± 0.2**	**0.10 ± 0.06**	**0.20 ± 0.13**
CT007	**Comercial strain**	**13.7 ± 0.6**	**0.17 ± 0.09**	**0.67 ± 0.39**
S6U	Comercial strain	14.3 ± 0.1	0.20 ± 0.05	3.13 ± 2.49

Figure 1 show the effect of make fermentations with furfural. This molecule inhibits some enzymes from the glycolytic pathway reducing the transformation of sugar in ethanol. Reduction of alcoholic degree is proportional to furfural concentration from 1 to 10 mg/L higher amounts don't have a bigger influence in the reduction of alcohol. The observed reduction can be about 0.5 º of ethanol.

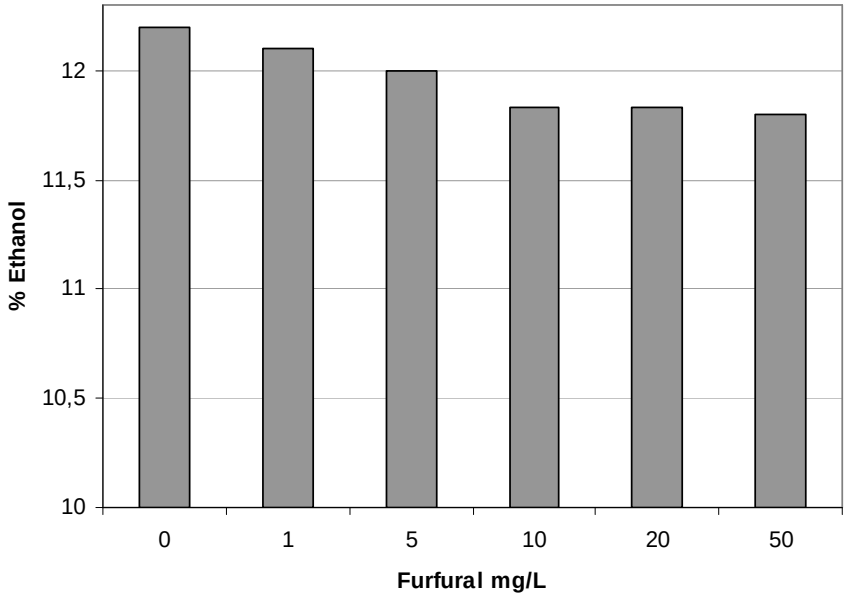

Figure 1. Effect of furfural concentration in the ethanol concentration at the end of fermentation

CONCLUSIONS

Use of selected yeast strains with glycolytic unefficiencies can be a natural way to control the alcoholic degree in warm regions. This phenomena can be increased using metabolic inhibitors like furfural. Must be verified the residual amount of furfural in the wines produced using this technique.

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Identification and quantification of *C*-glycosidic ellagitannins and their coupling derivatives in red wine aged in oak barrels or in stainless steel vats with oak chips

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ABSTRACT

C-glycosidic ellagitannins are found in wine as a result of its ageing in oak barrels or in stainless steel vats with oak chips, once dissolve in this slightly acidic solution vescalagin can react with grape-derived nucleophilic entities such as ethanol, catechin and epicatechin to generate condensed products with retention of configuration at the C-1 center. During this study, we first monitored the evolution of the native *C*-glycosidic ellagitannins in a red wines aged in oak barrels or in stainless steel vats with oak chips. Then, we were also able to estimate for the first time the formation and accumulation of vescalagin coupling derivative such as the flavano-ellagitannins (acutissimin A/B and epiacutissimin A/B) and β-1-*O*-ethylvescalagin during the red wine ageing in oak barrels.

Key word : *C*-glycosidic ellagitannins, flavano-ellagitannins, anthocyano-ellagitannin, oak barrel.

RESUME

Les ellagitannins *C*-glycosidique sont présent dans les vins élevés en fût de chêne, après avoir été extraits dans cette solution légèrement acide, la vescalagine réagie avec les espèces nucléophiles du vin tel que l'éthanol, les flavanols (catéchine et épicatéchine) ou les anthocyanes (oenine) pour générer des adduits présentant une rétention de configuration au niveau du centre C-1. Au cour de cette étude, nous avons dans un premier temps suivi l'évolution des ellagitannins *C*-glycosidique natif dans un vin rouges élevé en fût de chêne ou en cuve inox avec ajout de copeaux de chêne. Puis dans un second temps, nous avons pour la première fois estimées la formation et l'accumulation des dérivés de couplage de la vescalagine tels que les flavano-ellagitannins (acutissimine A/B et epiacutissimine A/B) ainsi que la β-1-*O*-ethylvescalagine au cours de l'élevage d'un vin rouges en fût de chêne.

Mots clef : ellagitannins *C*-glycosidique, flavano-ellagitannins, anthocyano-ellagitannin, fût de chêne.

INTRODUCTION

The *C*-glycosidic ellagitannins constitute a subclass of hydrolyzable tannins, whose present remarkable structural diversity, today over 500 members of this family of gallic acid-derived polyphenolic natural products have been isolated from various plants and fully characterized (Okuda et al. 1995; Okuda 2005; Quideau, S. and Feldman, K. S., 1996). Castalagin (**1**) and its C-1 epimer vescalagin (**2**) are the first *C*-glycosidic ellagitannins that have been isolated and characterized after their isolation thirty years ago in *Castanea* (chestnut) and *Quercus* (oak) woody species from the *Fagaceae* family (Mayer et al. 1976). Six other NHTP-containing *C*-glycosidic ellagitannins were later isolated from fagaceous *Quercus* and *Castanea* hardwood species, *i.e.*, the dimers roburins A (**5**) and D (**6**) and the lyxose/xylose-bearing monomers grandinin (**3**) and roburin E (**4**) and dimers roburins B (**7**) and C (**8**) (Hervé du Penhoat et al. 1991). Among these eight typical *C*-glycosidic ellagitannins, vescalagin and castalagin largely predominate in the fagaceous woody species containing them, representing for example between 40% and about 60% by weight of this group of *C*-glycosidic ellagitannins in *Quercus petraea* and *robur* heartwoods (Fernández de Simón et al. 1999). The presence of *C*-glycosidic ellagitannins in a large variety of beverages such as wines and sprits results from their presence in oak heartwood used to make barrels in which those beverages are stored or aged. During this storage or aging periods, the contact between the wood and theses hydro-alcoholic beverages (~12% of alcohol for wine) results in an extraction of the *C*-glycosidic ellagitannins presented in the wood as well as some others small phenols such as vanillin. Once in the wine, the *C*-glycosidic ellagitannins are slowly but continuously transformed through condensation, hydrolysis, and oxidation reactions.

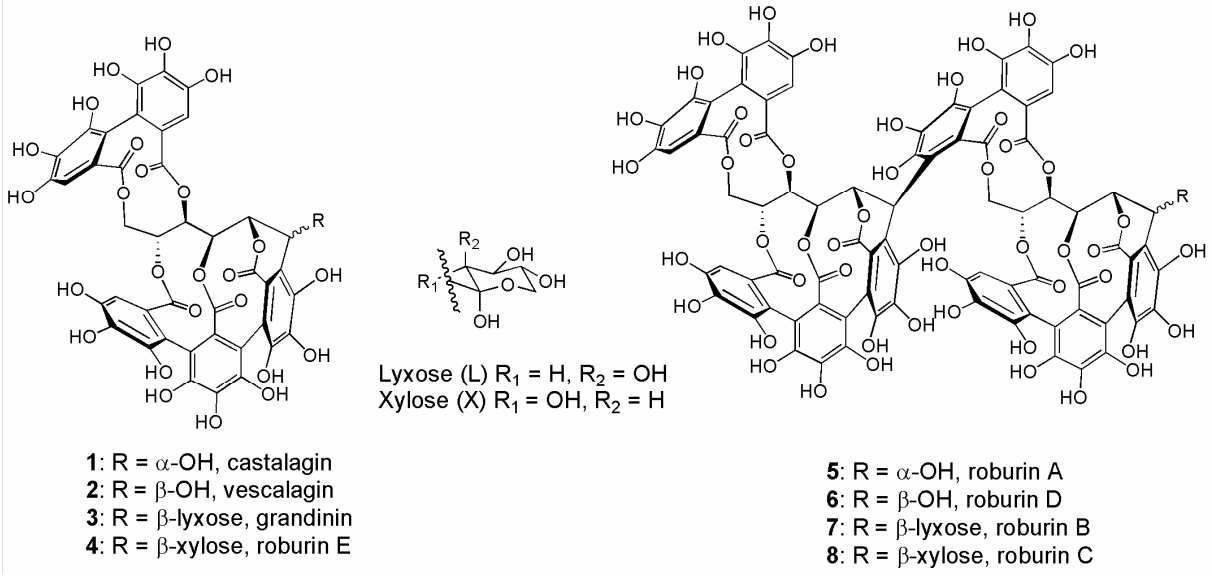

Figure 1: Structure of main monomeric *C*-glycosidic ellagitannins vescalagin (**2**), castalagin (**1**), as well as the grandinin (**3**) and roburine A-E (**4**-**8**) which are the main NHTP-containing *C*-glycosidic ellagitannins isolated form *Castanea* (chestnut) and *Quercus* (oak) species.

Moreover, the *C*-glycosidic ellagitannins sub-class also includes so-called complex tannins also known as flavano-ellagitannins which are hybrid tannins with a *C*-glycosidic ellagitannin moieties derived such as vescalagin (**2**), and a flavan-3-ol moieties such as catechin or epicatechin. In these complex tannins, both moieties are connected via *C–C* linkage between the carbon-1 of the *C*-glucosidic ellagitannin moieties and either the carbon-8 or carbon-6 of

the A ring of the flavan-3-ol unit. Acutissimin A (**14**) and B (**15**) are the first catechin/vescalagin-based complex tannins that have been isolated and characterized in the bark of *Quercus acutissima* (Ishimaru *et al.*, 1987, Nonaka *et al.*, 1990). Therefore, the formation flavano-ellagitannins in red wines aged in oak barrels could be expected since these red wines contained flavan-3-ol (deriving from grape seed and skim) and *C*-glycosidic ellagitannins (extracted from oak wood).

In order to answer this assumption, we first achieved the hemisynthesis of flavano-ellagitannins in a very high yield between vescalagin and flavan-3-ols (catechin/epicatechin) in organic acidic media as well as in a wine model solution. Then using the flavano-ellagitannins obtained by hemisynthesis as standard, we developed a new efficient detection and quantification procedure for the *C*-glycosidic ellagitannins as well as flavano-ellagitannins in order to monitor their extractions, evolutions and formations during different wine ageing conditions (oak barrels or stainless steel vats with oak chips)wine ageing.

RESULTS AND DISCUSSION

Hemisynthesis of Flavano-Ellagitannin

The hemisynthesis of the **11** and **12** was achieved in a very high yield (~87%) between vescalagin (**2**) and catechin (**9**) in acidic media (1.5% (v/v) TFA/THF) at 60°C over a period of 7 h (Quideau et al. 2003; Quideau et al. 2005). Interestingly, the mixture of **11** and **12** obtained by hemisynthesis present a similar ratio comparing to the two regioisomers isolated from *Quercus acutissima* (Ishimaru et al. 1987) 75:25 and 81:19, respectively. The formation of acutissimin A (**11**) as the main regioisomers *in vitro* and *in vivo*, in plants and by hemisynthesis, result of the more accessible and higher nucleophilic character of the carbon C-8' of the catechin (**9**) (Delcour *et al.* 1983; Okajima 2001). The hemisynthesis of the unknown epiacutissimin A (**13**) and B (**14**) was also achieved between vescalagin (**2**) and epicatechin (**10**) using the same procedure. Both epiacutissimins were obtained with similar yield (~78%) as the reaction leading to the acutissimin but with a ratio between both regioisomers slightly different (67:33).

The mechanistic description of those hemisyntheses is a classical SN1-type nucleophilic substitution, with first the protonation of the OH-1 group leading to the formation of a benzylic cation (Haslam 1998). This stable cation intermediate is then attacked by the carbon C-8 of the flavan-3-ol units and in a lesser extent by carbon C-6 due to a lower nucleophilic character and an important steric hindrance of this position (Delcour et al. 1983; Okajima 2001) which results in the ratio difference between the A and B regioisomer. This nucleophilic substitution proceeds with full diastereofacial differentiation resulting in the retention of the vescalagin configuration (i.e. β-orientation at C-1 center) in the newly formed carbon-carbon linkage between the flavan-3-ol units and the *C*-glycosidic ellagitannins moieties. This diastereoselectivity can appear surprising for an SN1-type mechanism, however this high specific stereochemical control of these nucleophilic substitutions attack on the benzylic cation intermediate was rationalized by computer modeling investigation (Quideau et al. 2005).

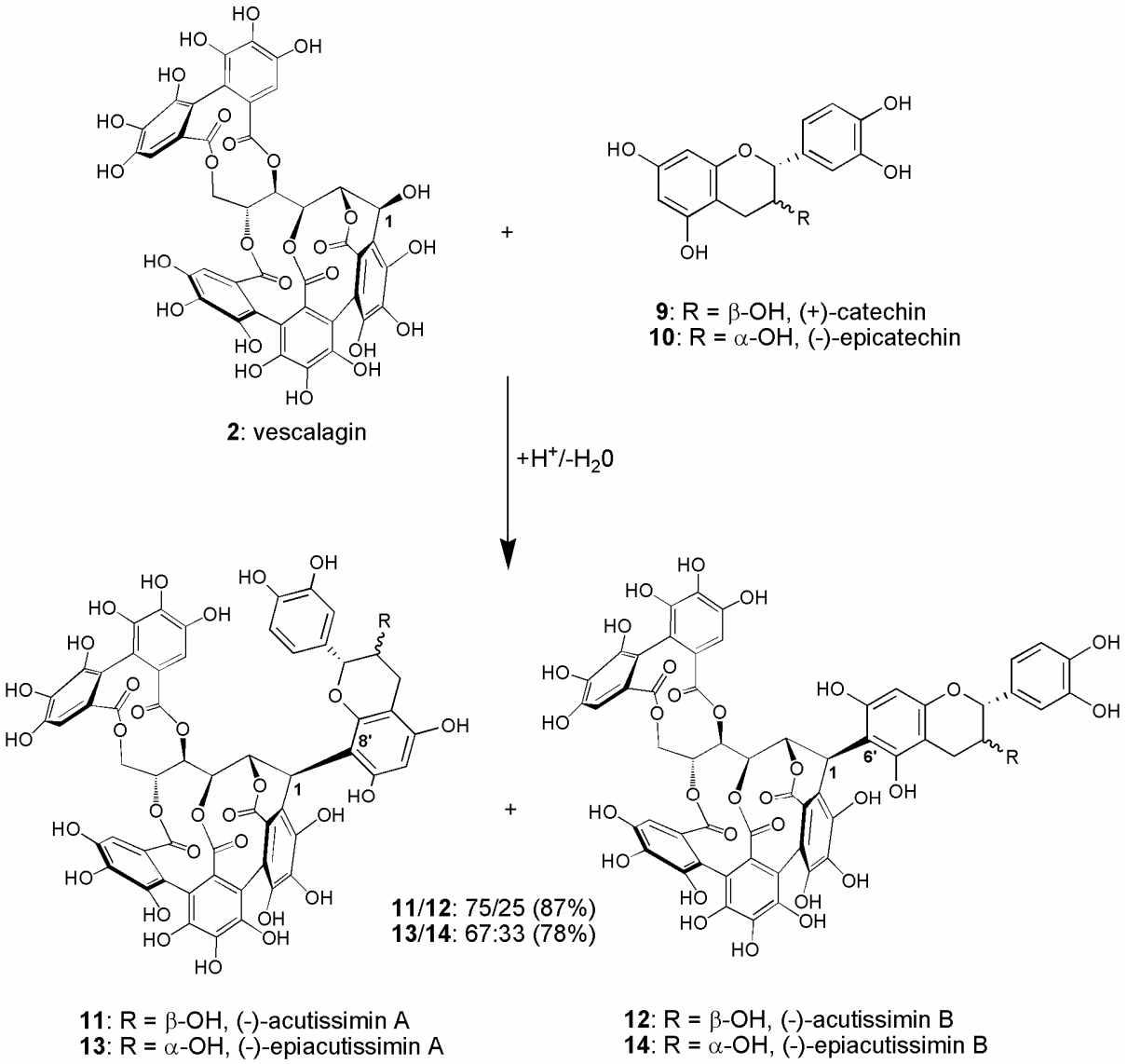

Figure 2: Hemisynthesis of acutissimins **11**, **12** and epiacutissimins **13**, **14** from vescalagin (**2**) and catechin (**9**) and epicatechin (**10**) respectively, in acidic media. (Isolated yield form organic media hemisynthesis).

Hemisynthesis of Flavano-Ellagitannin in wine model solution

The hemisyntheses of the flavano-ellagitannins **11**, **12**, **13** and **14** was also performed in wine model solution, consisting of a 12% (v/v) hydro-alcoholic solution with 5 g/L of tartaric acid at pH 3.2, in order to confirm there formation in wine. After several days at room temperature, the reaction mixture between **2** and **9** or **10** result in the formation of the corresponding flavano-ellagitannins along with the β-1-*O*-ethylvescalagin (**15**) (Figure 3). The formation β-1-*O*-ethylvescalagin (**15**) in the wine model solution media is the result of the nucleophilic attack of ethanol on the benzylic cation obtained by deshydroxylation of **2** in acidic condition.

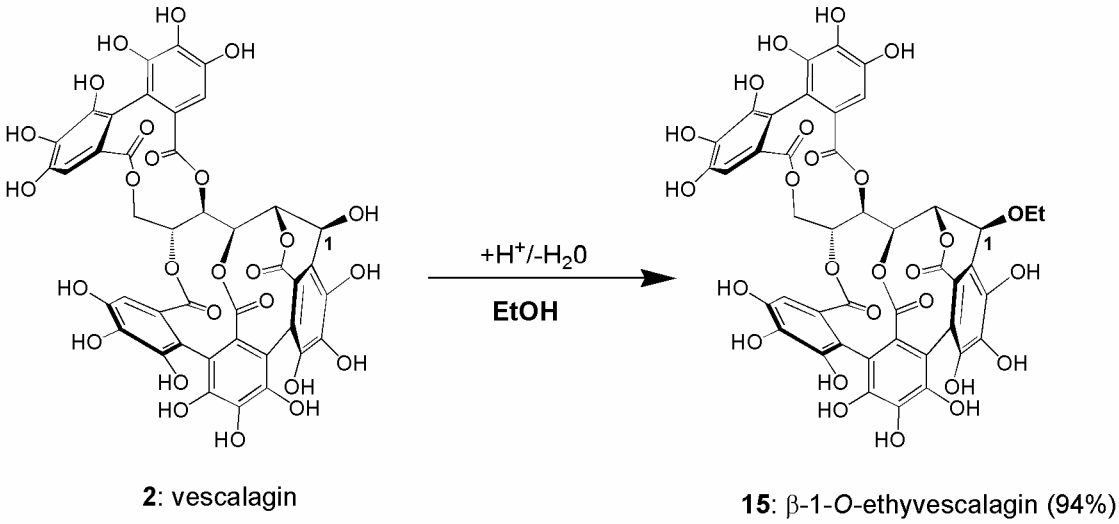

Figure 3: Hemisynthesis of β-1-*O*-ethylvescalagin (**15**) from vescalagin (**2**) and ethanol in acidic media. (Isolated yield form organic media hemisynthesis).

Identification and quantification of native C-glycosidic ellagitannins and Flavano-Ellagitannin in red wine aged in oak barrels.

Taken together the fact that wine aged in the oak barrels extracts *C*-glycosidic ellagitannins as well as the fact that wine (especially red wine) contains a large amount of flavan-3-ols such as catechin and epicatechin with a concentration about 115 to 190 mg/L and 80 mg/L, respectively (Cabanis et al. 1998; Carando and Teissedre 1999), thus, the presence in red wine aged in oak barrel of the acutissimins **11** and **12**, the epiacutissimins **13** and 1**4** as well as the β-1-*O*-ethylvescalagin (**15**) was investigated since from a chemical perspective wine can simply be considerate as a slightly acidic hydro-alcoholic solution (i.e., ~12% of alcohol and pH~3–4).

The detection and quantification of the flavano-ellagitannins **11-14**, the β-1-*O*-ethylvescalagin (**15**) and the native *C*-glycosidic ellagitannins **1-8** in the same red wines aged in oak barrels or in stainless steel vats with oak chips was perform by HPLC-UV-MS following the previously describe procedure (Saucier et al. 2006). In order to perform a proper quantification of these five vescalagin (**2**) derivative **11-15** and of the native *C*-glycosidic ellagitannins **1-8**, calibration curves were established using the pure flavano-ellagitannins **11-14** as well as β-1-*O*-ethylvescalagin (**15**) obtained by hemisynthesis as standard and with Chlorogenic acid as internal standard.

It appear that during wine ageing (Figure 4), the concentration in native *C*-glycosidic ellagitannins increased regularly to reach its maximum at 3 to 4 months if aged in oak barrels and 1.5 to 2 months if aged in stainless steel vats with oak chips (data not show). Then, in both cases, these concentrations slightly decreased with time. Moreover, during the first several months, the *C*-glycosidic ellagitannins composition (ratio between each native *C*-glycosidic ellagitannins) was similar to the *C*-glycosidic ellagitannins composition observed in oak wood used (barrels or chips). Thus, during these first months the composition of native *C*-glycosidic ellagitannins is influenced only by their extraction from oak by the red wine.

Then, after several months following the decreasing of the concentration native *C*-glycosidic ellagitannins, the composition of the native *C*-glycosidic ellagitannins change with time. For example the concentration of vescalagin (**2**) diminishes faster than the concentration of castalagin (**1**). This behavior differences is mainly due to the specific reactivity of vescalagin (**2**) in wine and especially to the formation of the flavano-ellagitannins **11-14** as well as β-1-*O*-ethylvescalagin (**15**) since the concentration of theses vescalagin (**2**) derivatives **11-15** were detectable even after 3-4 months of ageing. Then the concentration of theses vescalagin (**2**) derivative increased regularly during the studied wine ageing.

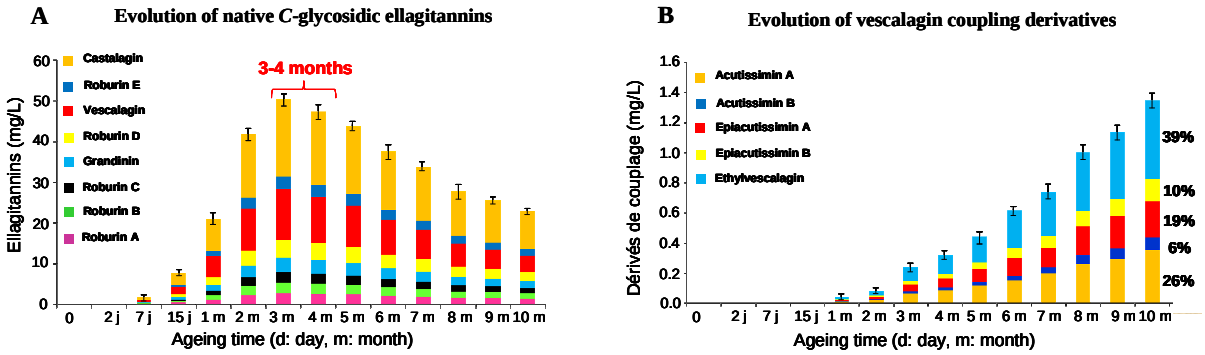

Figure 4: Evolution of native *C*-glycosidic ellagitannins concentration (A) and vescalagin (**2**) coupling derivative concentration in a red wine aged in oak barrels.

CONCLUSIONS

Using our acknowledge regarding the efficient hemisynthesis of the flavano-ellagitannins **11-14** in organic and in wine model solutions as well the hemisynthesis of the β-1-*O*-ethylvescalagin (**15**), we were able to monitor the evolution of the native *C*-glycosidic ellagitannins **1-8** in a red wines aged in oak barrels or in stainless steel vats with oak chips. In our wine ageing conditions, the concentration in native *C*-glycosidic ellagitannins increased regularly to reach its maximum at 3 to 4 months and then slowly decreased during the rest of the ageing in barrels. During this study we were also able to monitor for the first time the formation and accumulation of some vescalagin (**2**) coupling derivative such as the flavano-ellagitannins **11-14** and β-1-*O*-ethylvescalagin (**15**) during red wine ageing.

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BIBLIOGRAPHY

Bakker, J. and Tinmberlake, C. F. (1997). Isolation, identification, and characterisation of new color-stable anthocyanins occurring in some red wines. *J. Agric. Food Chem.*, 45, 35-43.

Cabanis, J. C., Cabanis, M. T., Cheynier, V. and Teissedre, P.-L. (1998). Oenologie-Fondements Scientifiques et Technologiques. in Oenologie-Fondements Scientifiques et Technologiques. C. Flanzy. Paris, TEC and DOC Lavoisier. pp. 315-336.

Carando, S. and Teissedre, P.-L. (1999). Plant Polyphenols 2: Chemistry, Biology, Pharmacology, Ecology. New York, Kluwer Academic/Plenum Publishers.

Chatonnet, P., Ricardo-Da-Silva, J.-M. and Dubourdieu, D. (1997). Influence de l'utilisation de barriques en chêne sessile européen (*Quercus petrae*) ou en chêne blanc américain (*Quercus alba*) sur la composition et la qualité des vins rouges. *Rev. Fr. Oenol.*, 165, 44-48.

Clifford, M. N. (2000). Review anthocyanins-nature, occurrence and dietary burden. *J. Agric. Food Chem.*, 80, 1063-1072.

Delcour, J. A., Ferreira, D. and Roux, D. G. (1983). Synthesis of condensed tannins. Part 9. The condensation sequence of leucocyanidin with (+)-catechin and with the resultant procyanidins. *J. Chem. Soc. Perkin Trans.*, 1711-1717.

Fernández de Simón, B., Cadahía, E., Conde, E. and García-Vallejo, M. C. (1999). Ellagitannins in woods of Spanish, French and American oaks, *Holzforschung*, 53, 147-150.

Fulcrand, H., Benadbeljalil, C., Rigaud, J., Cheynier, V. and Moutounet, M. (1998). A new class of wine pigments generated by reaction between pyruvic acid and grape anthocyanins. *Phytochemistry*, 47, 1401-1407.

Haslam, E. (1998). Practical Polyphenolics-From Structure to Molecular Recognition and Physiological Action. Cambridge, Cambridge University Press.

Hervé du Penhoat, C. L. M., Michon, V. M. F., Ohassan, A., Peng, S., Scalbert, A. and Gage, D. (1991). Roburin A, a dimeric ellagitannin from heartwood of *Quercus robur*, *Phytochemistry*, 30, 329–332.

Ishimaru, K., Nonaka, G. I. and Nishioka, I. (1987). Tannins and related compounds. LV. Isolation and characterisation of acutissimins A and B, novel tannins frorm *Quercus* and *Castanea* species. *Chem. Pharm. Bull.*, 35, 602-610.

Lefeuvre, D. (2006). Reactions de substitution nucleophile et d'oxydation des ellagitannins C-arylglycosidiques. Ph.D. Thesis, Sciences Chimiques, Universite Bordeaux 1, Order N 3271.

Liao, H., Cai, Y. and Haslam, E. (1992). Polyphenol interactions. Anthocyanins: Co-pigmentation and colour changes in red wines. *J. Sci. Food Agric.*, 59, 299-305.

Mayer, W., Gabler, W., Riester, A. and Korger, H. (1967). Die Isolierung von castalagin, vescalagin, castalin und vescalin. *Liebigs Ann. Chem.*, 707, 177-181.

Okajima, T. (2001). Ab initio MO investigation on the reactivity for electrophilic substitution of phenolic with oxirane and aziridine, as the model compounds of binding site of mutagen. *J. Mol. Struct. THEOCHEM*, 536, 73-82.

Okuda, T., Yoshida, T. and Hatano, T. (1995). Hydrolyzable tannins and related polyphenols, *Prog. Chem. Org. Nat. Prod.*, 66, 1–117.

Okuda, T. (2005). Systematic and health effects of chemically distinct tannins in medicinal plants, *Phytochemistry*, 66, 2012–2031.

Pissarra, J., Lourenco, S., Gonzales-Paramas, A. M., Nmateus, N., Santos-Buelga, C., Silva, A. M. S. and De Freitas, V. (2004). Structural characterization of new malvidin 3-glucoside-catechin aryl/alkyl-linked pigments. *J. Agric. Food Chem.*, 52, 5519-5526.

Quideau, S. and Feldman, K. S. (1996). Ellagitannin chemistry. *Chem. Rev.*, 96, 475-503.

Quideau, S., Jourdes, M., Saucier, C., Glories, Y., Pardon, P. and Baudry, C. (2003). DNA Topoisomerase inhibitor acutissimin A and other flavano-ellagitannins in red wine. *Angew Chem. Int. Ed.*, 42, 6012-6014.

Quideau, S., Jourdes, M., Lefeuvre, D., Montaudon, D., Saucier, C., Glories, Y., Pardon, P. and Pourquier, P. (2005). The chemistry of wine polyphenolic *C*-glycosidic ellagitannins targeting human topoisomerase II. *Chem. Eur. J.*, 11, pp.°6503-6513.

Salas, E., Atanasova, V., Poncet-Legrand, C., Meudec, E., P, M. J. and Cheynier, V. (2004). Demonstration of the occurence of flavanol-anthocyanin adducts in wine and wine model solutions. *Anal. Chim. Acta.*, 513, 325-332.

Saucier, C., Jourdes, M., Glories, Y. and Quideau, S. (2006). Extraction, detection, and quantification of flavano-ellagitannins and ethylvescalagin in a bordeaux red wine aged in oak barrels. *J. Agric. Food Chem.*, 54, 7349-7354.

Somers, T. C. (1971). The polymeric nature of wine pigments. *Phytochemistry*, 10, 2175-2186.

ANALYSE DE 19 PESTICIDES DANS LE VIN PAR SBSE (STIR BAR SORPTIVE EXTRACTION)

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RÉSUMÉ

La SBSE suivie d'une chromatographie gazeuse couplée à un détecteur de spectrométrie de masse (MS) a été optimisée (température, ajout de NaCl et temps) pour l'analyse de 19 pesticides les plus représentés dans les vins. Les conditions analytiques suivantes ont été retenues : 12,5 mL de vin sont agités à 400 tr/min pendant 45 minutes à température ambiante. L'addition de 2,5 g de NaCl, a considérablement augmenté le rapport signal/bruit probablement en améliorant l'affinité des pesticides pour le PDMS (polydimethysiloxane). La quantification est réalisée par ajout de trois différents standards internes variant par l'intensité du signal de détection. La validation de la méthode a été réalisée selon les recommandations fixées par la Guide de validation OIV (oeno 10/05) et le document SANCO/2007/3131. La méthode a été appliquée avec succès à une étude interlaboratoire, ainsi qu'à l'analyse de 30 vins. En conclusion, cette méthode sans solvants, est rapide et fiable et peut être utilisée par une vaste gamme de laboratoires œnologiques équipés de SBSE-CPG-SM.

Stir bar sorptive extraction (SBSE) followed by a thermal desorption-gas chromatography coupled to a mass spectrometry (MS) detector was evaluated for analyzing 19 of the most important pesticides in wines. After optimization of the SBSE method (temperature, NaCl addition and time), the optimal analytical conditions were: 12,5 mL of wine sample was stirred at 400 rpm for 45 min at room temperature. The addition of 2,5 g of NaCl significantly increased the ratio signal/noise probably by improving the affinity of pesticides for polydimethysiloxane (PDMS) sorbent. Experiments were carried out using samples spiked with three different internal standards varying by the detection signal in the MS detector. The method was validated according to the OIV validation Guide (oeno 10/05) and the document SANCO/2007/3131. The method has been successfully applied to an inter collaborative trial, and to the analysis of 30 wine samples. Eventually, this method is solvent free, fast and reliable and can be used by a large range of enological laboratories possessing SBSE-GC-MS.

INTRODUCTION

Depuis de nombreuses années, la qualité des produits alimentaires consommés par l'homme est devenue un sujet important tant pour les consommateurs que pour les filières les produisant. L'essor de l'agriculture biologique, le souci des consommateurs pour des produits sains ainsi que les enjeux commerciaux et réglementaires autour de ce sujet en sont autant de témoins.

Le vin n'échappe pas à cette tendance. En effet, depuis une quarantaine d'années, la consommation de vin en France a subi de profondes modifications. Désormais, les Français achètent moins mais mieux, la dépense moyenne par bouteille ne cessant d'augmenter. Ce mouvement n'est pas un phénomène isolé puisqu'il touche aussi d'autres pays de traditions viticoles comme l'Italie et l'Espagne.

La détection de molécules indésirables dans les vins est donc devenue un enjeu majeur pour les laboratoires de la filière. Parmi elles figurent les pesticides qu'il est devenu primordial de doser afin de rassurer consommateurs et professionnels sur la qualité des vins.

Dans cet objectif, INTER RHONE, interprofession des vins AOC Côtes du Rhône et Vallée du Rhône, cherche à développer une méthode de dosage multi-résidus des traces de pesticides dans les vins. Jusqu'à présent, les pesticides étaient dosés par des techniques mono-résidus. Les avantages d'une méthode multi-résidus résident dans une diminution des délais, des coûts et par la capacité à balayer une large gamme de pesticides en une seule analyse. La SBSE-GC/MS semble tout à fait appropriée à cet effet. Cette méthode analytique est simple à mettre en œuvre, rapide et plus sensible que des techniques classiques comme la GC-ECD, GC-FID et HPLC-DAD. L'utilisation de la SBSE pour extraire les contaminants permet, en outre, de s'affranchir complètement de l'utilisation de solvants. Cette méthode représente une alternative « sans solvant » à l'analyse de nombreux composés présents dans les vins. Les réductions de coût se font à double niveau : diminution des achats de solvant et diminution des coûts de retraitement des solvants assuré par des entreprises spécialisées. Ce dernier point est d'autant plus important pour les analyses faisant intervenir des solvants chlorés, dont le retraitement est encore plus onéreux. De plus, cette technique permet de prélever un faible volume d'échantillon et de diminuer les coûts de main d'œuvre.

L'objectif de ce travail consistait à mettre au point une méthode de dosage multi-résidus de pesticides dans les vins par SBSE-GC/MS et à valider cette méthode selon les spécifications émises par l'Organisation Internationale de la Vigne et du Vin (OIV).

MATERIELS ET METHODES

Les pesticides utilisés pour les analyses ont une pureté comprise entre 96 et 99,5%. Les analyses ont été effectuées sur un chromatographe gaz Agilent 7890A équipé d'un passeur d'échantillons Gerstel MPS 2 et d'une colonne HP-5MS (J&W Scientific) présentant les caractéristiques suivantes : longueur = 30m ; diamètre intérieur = 0.25 mm ; épaisseur du film de phase stationnaire = 0.25 µm ; phase stationnaire : 95% polydiméthylsiloxane greffée 5% diphényl.

Identification des molécules

L'identification des molécules (liste et caractéristiques Tab. 1) dans le vin a été réalisée selon les critères suivants :

- temps de rétention identique à celui de la molécule injectée seule dans le vin,
- reconnaissance des ions fragments caractéristiques de la molécule.

Tableau 1 : Temps de rétention (Tr), ions quantificateurs et qualificateurs (Q1, Q2, Q3), coefficients correcteurs en fonction de l'étalon interne choisi et rappel des principaux paramètres de validation.

			Ions				Coefficients correcteurs			Limites (µg/L)		Fidélité méthode	
	Abré-viation	Tr (min)	ion quan-tificateur	Q1	Q2	Q3	Hepta-chlor E.	Triphé-nyl	p-ter d14	LD	LQ	r (µg/L)	R (µg/L)
Benalaxyl	BA	19,49	148	91	206	204	0,10	1,09	2,98	1,7	5	0,52	7,81
Chlorpyriphos	CP	14,7	314	197	97		0,33	3,61	9,90	1,7	5	0,32	4,07
Cyproconazole	CZ	17,93	139	222	125		3,20	35,84	97,28	1,7	5	0,5	10,11
Cyprodinil	CD	15,32	224	225	210	226	0,33	3,66	10,04	1,7	5	0,33	3,43
Fenbuconazole	FB	27,5	129	198	125		1,34	15,29	39,66	1,7	5	0,45	18,15
Fenexhamid	FH	19,87	301	177	97		9,22	106,01	271,91	1,7	5	0,46	12,55
Fenitrothion	FE	14,23	277	260	125		0,34	3,93	10,22	3,4	10	0,6	13,95
Fludioxonil	FD	17,08	248	182	154		0,43	4,76	13,04	1,7	5	0,5	6,27
Flusilazole	FL	17,45	233	315	206		0,13	1,47	4,02	1,7	5	0,59	7,74
Iprodione	IP	18,47	187	244	189	246	2,11	24,05	62,33	3,4	10	1,19	6,61
Iprovalicarb	IC1 ; IC2	17,17 ; 17,45	134	116	58		0,69	7,91	20,82	3,4	10	0,98	9,26
Mepanipyrim	MP	16,46	222	223			0,09	1,03	2,71	3,4	10	0,89	7,06
Penconazole	PE	15,56	159	248	161		0,23	2,52	6,91	1,7	5	0,5	4,63
Procymidone	PR	15,99	283	285	96		0,24	2,62	7,19	1,7	5	0,45	6,2
Pyrimethanil	PL	12,48	198	199			0,68	7,48	20,49	3,4	10	0,68	2,61
Quinoxifen	QN	19,59	237	272	307		0,10	1,08	2,97	1,7	5	0,38	5,62
Tebuconazole	TE	20,3	250	125			0,48	5,43	14,40	3,4	10	0,88	11,08
Tebufenpyrad	TB	276	276	171	318		1,36	15,56	40,26	1,7	5	0,27	4,88
Vinchlozoline	VI	13,57	285	212	198		0,42	4,61	12,65	1,7	5	0,23	2,43

Quantification des molécules

Les pesticides ont été quantifiés selon la méthode de l'étalonnage interne.

La relation utilisée pour calculer la concentration en analyte dans l'échantillon ($C_{analyte}$) est la suivante :

$$C_{analyte} = \frac{Aire\ analyte}{Aire\ étalon} \times C_{étalon} \times \text{coefficient correcteur}$$

Avec :

Aire analyte : Aire du pic chromatographique de l'analyte

Aire étalon : Aire du pic chromatographique de l'étalon interne

$C_{étalon}$: Concentration de l'étalon interne dans l'échantillon.

Le coefficient correcteur rend compte du coefficient de réponse relatif du composé à doser vis-à-vis de l'étalon interne ainsi que de l'affinité de chaque composé pour le PDMS. Celui-ci est caractéristique de chaque couple molécule / étalon interne utilisé pour quantifier la molécule d'intérêt.

Il sera déterminé, pour chaque couple molécule / étalon interne, par injection d'une gamme permettant de tracer la droite de régression :

$$C_{analyte} = f(\frac{Aire\ analyte}{Aire\ étalon} \times C_{étalon})$$

Choix des étalons internes

Les trois étalons internes suivants ont été choisis :
- l'heptachlor epoxyde
- le triphénylphosphate
- le p-terphényl d14

La concentration finale des étalons internes dans l'échantillon est 5 µg/L à l'exception de l'heptachlor epoxyde (0,5 µg/L).

Mode opératoire

- Prélever 10 ml du vin à analyser à l'aide d'une pipette jaugée et les introduire dans un pilulier de 12.5 ml.
- Ajouter les étalons internes.
- Ajouter le twister (longueur = 10 mm, épaisseur du film de PDMS = 1 mm).
- Recouvrir le pilulier d'une feuille d'aluminium pour éviter toute contamination au contact de la cape puis refermer le pilulier avec la cape.
- Placer sur une table d'agitation pendant 45 mn à 400 tr/min.
- Récupérer le twister en vidant le pilulier au dessus d'une passoire métallique, le rincer à l'eau distillée pour éliminer d'éventuelles impuretés et l'essuyer sur du papier non pelucheux.
- Introduire le twister à l'aide d'une pince métallique dans un tube TDU.
- Lancer l'analyse.

RESULTATS ET DISCUSSION

Optimisation des conditions opératoires

La méthode a été préalablement optimisée afin d'obtenir les temps de rétention et les ions quantificateurs de chaque pesticide et des étalons internes (Tab. 1). D'autre part, des vins exempts de pesticides ont été choisis pour l'étude. Une méthode élaborée par le laboratoire SARCO a été testée pour la quantification des 19 molécules.

Cependant, les molécules suivantes : cyproconazole (CZ), fenbuconazole (FB), fenexhamid (FH), iprodione (IP), iprovalicarb (IC), metalaxyl-M (MF), tebuconazole (TE) sont difficiles à détecter à une concentration de 20 µg/L. Or, l'objectif était de disposer d'une méthode dont les limites de quantification pour chaque molécule est de l'ordre de 5 à 10 µg/L. Il est donc décidé de développer une méthode spécifique à ces sept pesticides ou une méthode globale permettant l'analyse simultanée des 19 pesticides. Ainsi, deux paramètres seront étudiés : les conditions chromatographiques et les conditions de la phase d'extraction des pesticides par SBSE.

Les conditions chromatographiques sont modifiées en fonction des températures d'ébullition des molécules extrapolées à partir de leurs temps de rétention avec la méthode de départ. De nombreuses modifications ont été réalisées concernant les gradients de température et les paramètres de l'unité thermique de désorption, néanmoins, aucun des essais n'apporte de variation significative de la réponse des molécules par rapport à la méthode de départ.

Une étude (Ochiai *et al.*, 2006) a montré l'influence de l'ajout de chlorure de sodium sur la modification des conditions d'extraction des pesticides par SBSE. En effet, l'ajout de chlorure de sodium dans la matrice à analyser permettrait d'augmenter les rendements d'extraction des composés les plus polaires c'est-à-dire ayant une faible affinité pour le PDMS du twister. Une

des explications serait que le chlorure de sodium permettrait de modifier les coefficients de partage Ko/w de chaque molécule et donc d'augmenter l'affinité des composés les plus polaires pour le PDMS. D'autre part, de nombreuses méthodes utilisant la technique « QuECHER » (Zhang *et al.*, 2009) ont aussi recours à l'ajout de chlorure de sodium pendant la phase d'extraction. L'influence de l'ajout de deux sels sur la réponse de ces 8 molécules a été étudiée : le chlorure de sodium et le sulfate de sodium.

L'ajout de sels permet d'augmenter significativement les réponses des pesticides (Figure 1). En effet, un ajout de NaCl permet d'augmenter les réponses d'un facteur 1.5 (FB) jusqu'à un facteur 15 (IC).

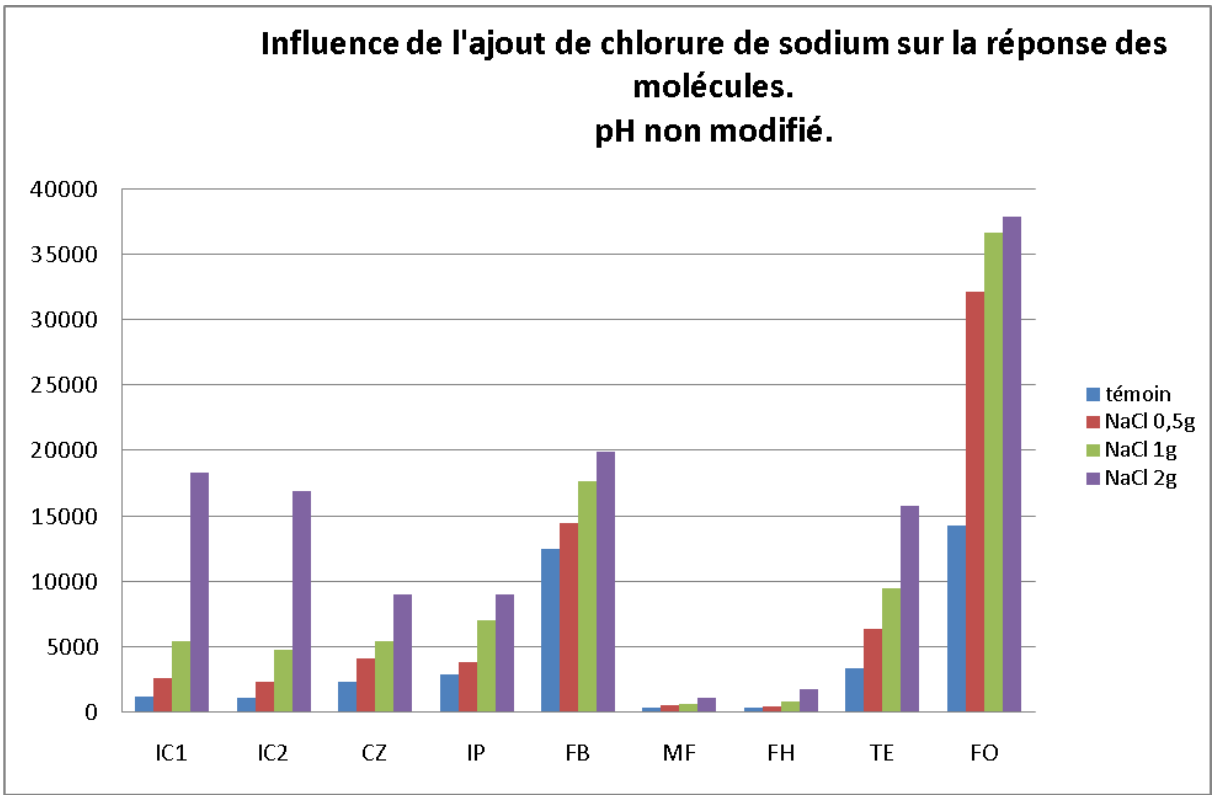

Figure 1 : réponses des molécules en fonction de la quantité de NaCl.

Pour Na_2SO4 (résultats non montrés), les facteurs de multiplication varient de 1.5 (FB) à 11 (IC).

Le chlorure de sodium étant un produit facilement disponible, économique, il sera préféré au sulfate de sodium. Des essais complémentaires ont montré que les réponses des molécules continuaient à augmenter jusqu'à un maximum de 2.5g de NaCl ajouté (réponses multipliées par 2 pour FB et jusqu'à 23 pour IC).

L'ajout de NaCl pendant la phase d'extraction permet d'augmenter suffisamment les réponses des 8 molécules pour qu'elles soient quantifiables à des concentrations de l'ordre de 5 à 10 µg/L. Toutefois, cela nécessite de réaliser deux analyses par échantillon. Cependant, afin de disposer d'une seule méthode pour l'analyse des 19 pesticides, la méthode a été transposée avec succès à l'ensemble des pesticides étudiés (Figure 2).

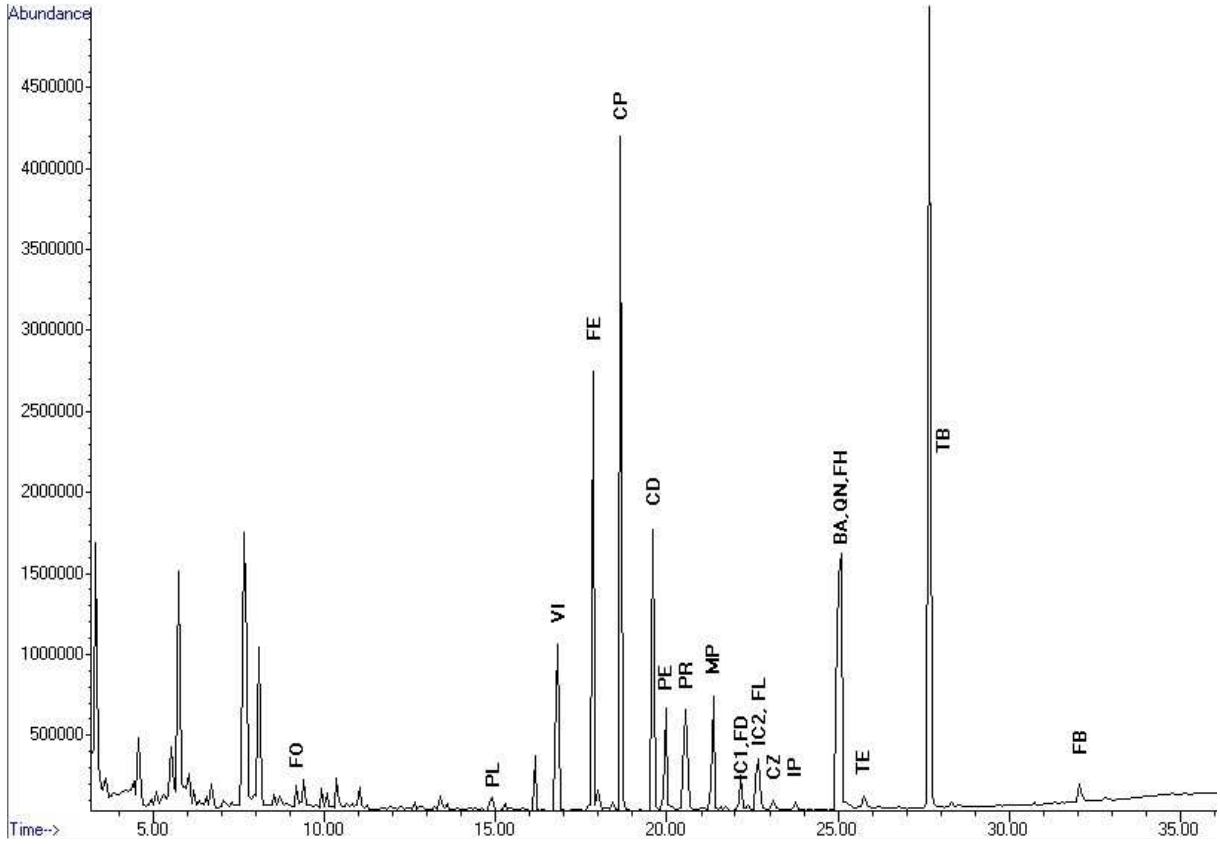

Figure 2 : Chromatogramme en mode SCAN des 19 molécules dans le vin avec la méthode optimisée.

Linéarité des étalons internes

La linéarité de l'heptachlor epoxyde, du triphénylphosphate et du p-terphényl d14 est contrôlée. Pour cela, chaque étalon est injecté seul dans le vin selon les gammes suivantes :

- Heptachlor epoxyde : 0.5 ; 1 ; 2.5 ; 5 ; 10 ; 20 µg/L
- Triphénylphosphate : 0.25 ; 0.5 ; 1.25 ; 2.5 ; 5 ; 10 µg/L
- p-terphényl d14: 0.25 ; 0.5 ; 1.25 ; 2.5 ; 5 ; 10 µg/L.

La linéarité est vérifiée pour chaque étalon interne par le tracé du graphique aire étalon = f (concentration étalon). Les trois molécules présentent une linéarité très satisfaisante avec un $R^2 > 0.998$. Ils seront utilisés comme étalons internes pour la suite de l'étude.

Les concentrations des trois étalons internes dans le vin ont été déterminées en fonction de leur réponse : faible, intermédiaire, grande, afin de couvrir l'ensemble des gammes de réponses des pesticides. Ils seront injectés à une concentration de 5 µg/L dans le vin :

- L'heptachlor epoxyde (aire attendue du pic = 5000 ua pour unité d'aire) servira à quantifier les molécules dont les aires de pics ne dépassent pas les 20 000 ua.
- Le triphénylphosphate (aire attendue du pic = 50 000 ua) permettra la quantification des molécules dont les aires de pics varient entre 20 000 et 100 000 ua.
- Le p-terphényl d14 (aire attendue du pic = 150 000 ua) permettra la quantification des molécules dont les aires de pics excédent les 100 000 ua.

Détermination des coefficients correcteurs

Les coefficients correcteurs ont été vérifiés par l'injection d'une gamme à 6 niveaux de concentration et 4 répliques par niveaux de concentration pour chaque molécule. Les répliques sont effectuées à des jours différents. Pour chaque couple pesticide / étalon interne, le coefficient correcteur a été déterminé de la façon suivante :

$$C_{analyte} = f(\frac{Aire\ analyte}{Aire\ étalon} \times C_{étalon})$$

Les coefficients correcteurs ont été calculés pour chaque pesticide (Tab. 1).

Le biais entre les mesures et les valeurs obtenues par la fonction de calibration n'excède pas les ± 20% de la courbe de calibration comme précisé dans le document N°SANCO/2007/3131 (2007).

Pour rappel, les conditions opératoires de la phase d'extraction des pesticides par SBSE sont les suivantes :

- ajout de 2.5 g de NaCl dans les piluliers avant introduction du vin,
- ajout de 30 µL de la solution de travail contenant les trois étalons internes.

La méthode est à présent optimisée. Pour chaque pesticide, le temps de rétention, l'ion quantificateur spécifique et les coefficients correcteurs ont été déterminés. La validation de la méthode peut désormais être réalisée selon les spécifications du Guide de validation OIV (oeno 10/05). Les paramètres de validation retenus sont les suivants : linéarité, vérification des coefficients correcteurs (à partir d'un vin provenant d'essais interlaboratoires), limites de détection et de quantification, répétabilité, reproductibilité intralaboratoire et spécificité. Les principaux résultats sont présentés dans le tableau 1.

CONCLUSION

Une méthode simple, rapide, peu coûteuse (absence de solvants) et permettant de doser 19 pesticides simultanément a été optimisée. L'ensemble des critères de validation ont été confrontés aux tests statistiques et se sont révélés positifs, permettant de conclure que la méthode est validée et qu'elle pourra être utilisée en routine pour l'analyse des pesticides dans les vins.

De plus, deux échantillons d'un même vin (vin rouge issu de l'agriculture biologique) provenant d'un essai interlaboratoire ont été analysés. L'essai portait sur 28 pesticides répartis de manière égale entre les deux échantillons (14 pesticides par échantillon).

Les deux échantillons ont été analysés par plusieurs opérateurs, et aucuns résultats faux-positifs n'ont été observés. Les écarts les plus importants avec la quantité de pesticide ajoutée aux échantillons se situent à ±40%, le biais moyen est de 18 % et l'écart médian de 16%. Ces résultats sont donc très satisfaisants et valident l'utilisation de la méthode en routine.

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BIBLIOGRAPHIE

Document N°SANCO/2007/3131. 2007. « Method validation and quality control procedures for pesticides residues analysis in food and feed ». http://ec.europa.eu/food/plant/protection/resources/qualcontrol_en.pdf

N. Ochiai, K. Sasamoto, H. Kanda, S. Nakamura. 2006. Fast screening of pesticides multiresidues in aqueous samples by dual Stir Bar Sorptive Extraction (dual SBSE) and thermal desorption ». Gerstel, application note : Appnote-12. http://www.gerstel.com/pdf/p-gc-an-2006-12.pdf

K. Zhang, J. Wong, D. Hayward, P. Sheladia, A. Krynitsky. 2009. Multiresidues analysis of wine by Dispersive Solid-Phase Extraction and Ultra High Performance Liquid Chromatography # Tandem Mass Spectrometry. Journal. Agric. Food chem., 57 (10), 4019-4029

CONTRIBUTION PHENOLIQUE DU BOIS ET INFLUENCE SUR LES PERSEPTIONS ORGANOLEPTIQUES DU VIN ROUGE

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Mots clés; Ellagitanins, Vin, Bois de chêne, Rondeur, Astringence, Amertume

RESUME

The level of ellagitannins in wine depends of the species and origin of oak wood as well as its treatment during barrel realization. The impact of ellagitannins concentration on the organoleptic perception of red wine is poorly known and still be under investigation.

In our research, we classified staves according to their ellagitannins level using a NIRS online procedure (Oakscan®) and we were able to correlate the NIRS classification with the level of ellagitannins estimated by HPLC-UV/MS and acidic degradation method. Different types of staves were added to red wine during its ageing and the extraction and evolution of the ellagitannins level was monitored by HPLC-UV/MS. The influence of ellagitannins levels on wine perception was estimated by a trained judge's panel.

The fist results show that the ellagitannins concentration in red wine is correlate with their concentrations in the wood and it appears that the ellagitanins concentration have an influence on the roundness.

Les niveaux d'ellagitanins dans le vin dépendent de l'espèce et de l'origine du bois de chêne aussi bien que des traitements qu'il subit lors de la fabrication de la barrique. L'impact des ellagitanins sur la perception organoleptique du vin rouge est peu connu et les études sont en cours.

Dans notre étude, nous avons classifié les merrains en fonction de leur teneur en ellagitanin en utilisant une procédure NIRS (Oakscan®) et nous avons corrélé cela avec la teneur en ellagitanins estimée par dosages HPLC-UV/MS et par dégradation acide. Les différents types de staves ont ensuite été ajoutés au vin rouge puis l'extraction et l'évolution des ellagitanins pendant l'élevage ont été suivies par HPLC-UV/MS. L'influence des concentrations en ellagitanins des vins sur leurs perceptions organoleptique a été estimée grâce à un panel de juges entrainés.

Les premiers résultats montrent que les concentrations en ellagitanins dans le vin rouge sont en relation avec celles trouvées dans les bois et que celles-ci auraient une influence sur la rondeur des vins.

INTRODUCTION

Les ellagitanins constituent une sous classe des tanins hydrolysable l'autre étant les gallotanins, avec une diversité structurale remarquable. A ce jour, plus de 500 molécules de cette famille dérivée de l'acide gallique ont été isolés dans diverses plantes (Okuda, 2005). La composition en ellagitanins des extraits de bois dépend des espèces de chêne. Par exemple, ils sont en faible quantités dans les espèces de chênes américains. Dans le raisin, les ellagitanins ne sont pas présents mais ils sont retrouvés dans le vin élevé en barrique car ces différentes molécules sont hydrosolubles et passent rapidement dans les milieux hydroalcooliques (Moutounet *et al*., 1989). Les deux ellagitanins majoritaires sont la vescalagine et de son épimère, la castalagine qui ont été isolées il y a trente ans dans les bois de *Castanea* (châtaignier) et *Quercus* (chêne) (Mayer *et al*., 1967). Depuis, d'autres ellagitanins ont été trouvés dans le bois de chêne tels les roburine A, roburine B, roburine C, roburine D, roburine E et la grandinine (Hervé du Penhoat *et al.*, 1991). Ces molécules sont constituées d'un acide hexahydroxydiphénique et d'un acide nonahydroxytriphénique, estérifiés à un glucose non cyclique pour les monomères et de deux monomères pour les dimères. C'est pourquoi, ils libèrent de l'acide ellagique lors de leurs hydrolyses. La concentration de ces tannins hydrolysable dans le vin dépend principalement de l'origine et de l'espèce du bois de chêne aussi bien que de son traitement durant la fabrication de la barrique. Par exemple, lors du vieillissement en fut de chêne, le vin peut extraire quelques centaines de mg d'ellagitanins ainsi que d'autres constituants du bois.

Les impacts des concentrations d'ellagitanins sur les perceptions organoleptiques du vin sont mal connus et contradictoire. Plusieurs auteurs disent que les ellagitanins influencent l'astringence et la perception du vin rouge élève en fût de chêne (Quinn and Singleton, 1985), alors que d'autres suggèrent au contraire que la concentration de ces composés est trop faible pour participer au goût global du vin (Somers, 1990). Plus récemment, le niveau d'astringence des ellagitanins dans l'eau a été estimé (Glabasnia et Hofman, 2006).

Le but de notre étude est d'étudier l'influence de la concentration en ellagitanins sur la perception du vin. Nous avons d'abord classifié les staves de chêne en 3 groupes différents en fonction de leurs concentrations en ellagitanins grâce à une nouvelle procédure NIRS (Oakscan®) (Giordanengo *et al*., 2009). Ensuite, nous avons identifié et quantifié le niveau des principaux ellagitanins de ces staves par HPLC-UV/MS pour corréler la classification par NIRS avec la concentration en ellagitanins. Dans un deuxième temps, les 3 groupes de staves ont été mis en contact avec du vin rouge. Là, l'évolution des principaux ellagitanins a été suivie par HPLC-UV/MS pendant l'élevage. Pour finir, l'influence de la concentration en ellagitanins dans le vin a été estimée par un panel de juges entrainé et les résultats ont été traités sur le logiciel FIZZ grâce à un test de Newman.

MATERIELS ET METHODES

1) Matériels

Echantillons de chêne

Les échantillons de bois sont constitués par le bois de cœur de chêne francais. Ces derniers ont subit les mêmes phases de maturation et de séchage aux mêmes dates. Puis, les bois utilisés pour l'essai sont sélectionnés au sein d'une collection d'environ deux cents

échantillons de merrains. Afin de les choisir, nous avons segmenté les bois en trois classes de teneur en polyphénols à partir des analyses effectués grâce à un appareil proche infrarouge par réflectance (NIRS). Nous avons sélectionné au hasard 7 bois par classe. Ensuite, tous les merrains ont subis une chauffe identique et ont été incorporés au vin.

Pour effectuer les analyses chimiques sur le bois, des écourtures provenant de la découpe des merrains ont été conservées sous forme de copeaux.

Echantillons de vin

Les analyses sont réalisées sur un vin rouge de Cabernet Sauvignon élevé en vin inox pendant 4 mois. Toutes les cuves ont subi les mêmes traitements (3 merrains / hl de vin).

2) Extraction des ellagitanins

Les copeaux sont réduits en poudre de granulométrie inférieure à 0.6 mm grâce à une broyeuse Cyclotec. 4 g de poudre sont extraits par un extracteur Dionex ASE 350 grâce à un mélange de solvants acétone : eau (70 : 30). Les extraits sont ensuite rassemblés et évaporés à sec sous pression réduite. Une solution mère de cet extrait sec est réalisée en reprenant l'extrait sec dans du méthanol.

La solution mère de vin est préparée en reprenant 50 ml de vin évaporé à sec dans du méthanol.

3) Dosages des ellagitanins totaux par dégradation acide

La procédure utilisée est adaptée de celle mise au point par Peng *et al.*, 1991. Elle est basée sur un dosage par HPLC-UV/MS de l'acide ellagique libéré par les ellagitanins lors d'une hydrolyse acide à 100°C au bain marie. Après 2 h d'hydrolyse, on dose l'acide ellagique des tubes hydrolysés ainsi que celui de tubes n'ayant pas subit l'hydrolyse. La différence entre les 2 valeurs correspond aux ellagitanins totaux ayant libéré de l'acide ellagique. Le dosage HPLC est pratiqué sur une chaîne HPLC Hewlett Packard série 1100 (solvant A : H_2O/H_3PO_4 999 : 1, solvant B : MeOH/ H_3PO_4 999 : 1).

4) Dosages des 8 principaux ellagitanins natifs

Echantillons de chêne

Une aliquote de la solution mère de bois est évaporée sous azote. L'extrait sec est repris dans de l'H_2O à 0.4% d'acide acétique. Le dosage HPLC-UV/MS est pratiqué avec le matériel décrit ci-dessus.

Echantillons de vin

Pour doser les ellagitanins natifs du vin, il faut préalablement fractionner celui-ci. La méthode utilisée est adaptée de celle décrite par Cédric Saucier *et al.*, 2006. Pour cela, on utilise une colonne TSK HW 50 F et 3 type de solvants ; de l'H_2O à 0.4% d'acide acétique, du MeOH/H_2O à 0.4% d'acide acétique, de l'acétone/H_2O à 0.4% d'acide acétique.

La fraction obtenue avec le solvant acétone/eau est évaporée sous pression réduite puis reprise dans de l'H_2O à 0.4% d'acide acétique. Le dosage HPLC est pratiqué sur une chaîne HPLC-UV/MS Thermo (solvant A : H_2O/HCOOH 996 : 4, solvant B : MeOH/ HCOOH 996 : 4).

5) Impact organoleptique des ellagitanins dans le vin

Le vin mis en contact avec les bois a été dégusté par un jury entrainé. La dégustation a été organisée par série de trois vins plus un témoin. Elle a été focalisée sur trois marqueurs principaux ; l'amertume, l'astringence et la rondeur. Les résultats ont été traités sur le logiciel FIZZ Traitement grâce à une analyse de variance par un test de Newman-Keuls.

RESULTATS ET DISCUTIONS

1) Dosage des ellagitanins totaux des bois

Le dosage des ellagitanins totaux révèle une grande hétérogénéité dans leurs concentrations puisque celles-ci vont de 5.46 à 32.18 mg d'équivalent acide ellagique/g de poudre de bois (Tab. 1). Ces variations en concentrations d'ellagitanins entre les différentes staves de chêne peuvent être le résultat d'un lavage du à la pluie, de la dégradation par des microorganismes, de l'oxydation chimique, de l'origine du bois de chêne ainsi que du traitement appliqué au bois lors de la fabrication de la barrique (Chatonnet *et al.*, 1995). Les analyses chimiques montrent une bonne corrélation entre le classement des bois par NIRS et celui réalisé grâce aux analyses chimiques. Les classes de bois bas potentiel, moyen potentiel et haut potentiel se chevauchent sur leurs extrémités. Ceci est dû aux incertitudes des deux méthodes utilisées ici. La classe bas potentiel contient des bois qui ont un taux d'ellagitanins totaux allant de 5.46 à 13.20 mg d'équivalent acide ellagique/g de poudre de bois, 9.10 à 25.09 pour la classe moyen potentiel et 21.05 à 32.18 pour la classe haute potentiel (Tab. 1).

Tableau 1 : Dosage des ellagitanins dans le bois

		Dosage des ellagitanins totaux		Dosage de 8 ellagitanins natifs	
	N°	Concentrations en ellagitanins en équivalent acide ellagique (mg/g)	Moyenne des concentrations en ellagitanins en équivalent acide ellagique (mg/g)	Concentrations en ellagitanins en équivalent acide ellagique (mg/g)	Moyenne des concentrations en ellagitanins en équivalent acide ellagique (mg/g)
Classe Potentiel Tannique Bas	48	5,46	9,67 ± 2,5	2,04	4,59 ± 1,95
	23	6,67		1,98	
	32	9,64		4,75	
	21	10,79		5,14	
	33	10,86		5,95	
	52	11,04		4,31	
	19	13,20		7,93	
Classe Potentiel Tannique Moyen	47	9,10	15,74 ± 4,78	4,45	8,15 ± 3,54
	84	12,97		7,72	
	27	13,48		5,68	
	77	13,73		5,47	
	82	16,88		8,03	
	12	18,95		9,95	
	65	25,09		15,72	
Classe Potentiel Tannique Fort	57	21,05	26,32 ± 3,78	11,57	13,91 ± 3,1
	54	21,24		9,97	
	68	26,32		10,44	
	88	26,59		15,66	
	66	27,17		15,00	
	87	29,68		15,65	
	81	32,18		19,10	

2) Estimation de la composition en ellagitanins natifs des bois

Le dosage des ellagitanins natifs montre également une grande disparité des concentrations. Celles-ci vont de 1.98 à 19.10 mg d'équivalent acide ellagique/g de poudre de bois (Tab. 1). Il y a une bonne corrélation entre les classes NIRS et l'estimation de la composition en ellagitanins. Il est observé un chevauchement plus important que précédemment entre les extrémités des classes qui peuvent être dus en plus des incertitudes des deux méthodes, aux variations de quantité des autres ellagitanins que ceux dosés ici. La classe bas potentiel contient des bois qui ont un taux d'ellagitanins natifs allant de 1.98 à 7.93 mg d'équivalent acide ellagique/g de poudre de bois, 4.45 à 15.72 pour la classe moyen potentiel et 9.97 à 19.10 pour la classe haute potentiel (Tab. 1). La classe de bois ayant le potentiel le plus faible à un écart type plus faible que les deux autres classes.

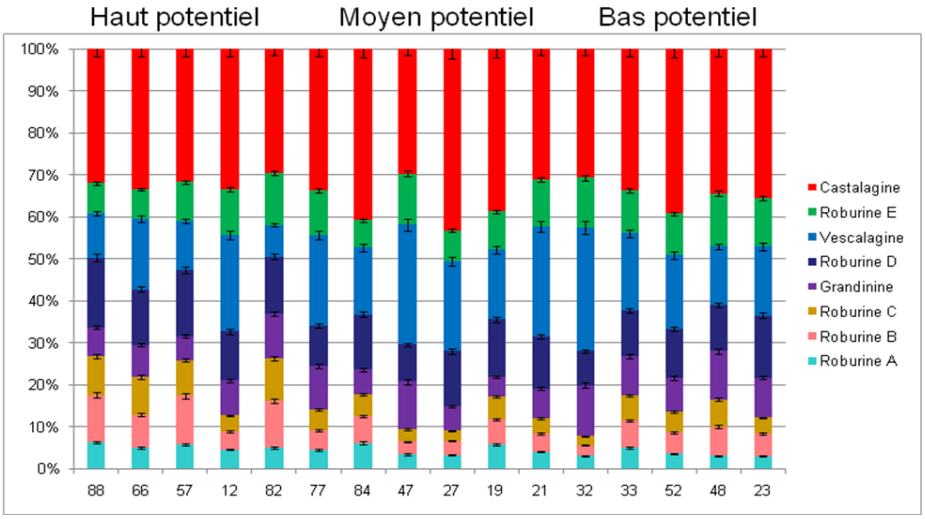

Figure 1 : Composition en ellagitanins natifs

La composition en ellagitanin entre les groupes de bois, estimée par HPLC-UV/MS, montre une similarité entre les groupes de potentiel. La composition en ellagitanins n'influence donc pas le classement des bois par NIRS. Les rapports moyens entre les concentrations des 8 principaux ellagitanins dosés sont ; castalagine (35%), vescalagine (19%), roburine D (12%), roburine E (10%), grandinine (8%) roburine C (6%), roburine B (5%), roburine A (4%) (Fig. 1).

3) Dosage des ellagitanins totaux du vin

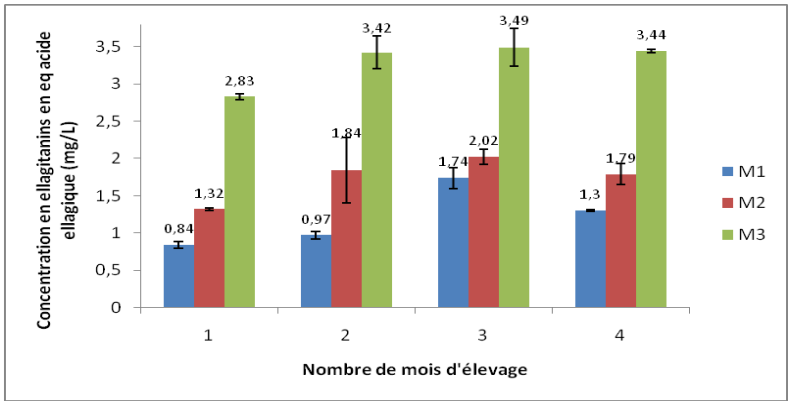

Figure 2 : Concentration en ellagitanins totaux dans les vins

Le vin mis en contact avec les merrains extrait les ellagitanins contenus dans celles-ci. La concentration en ellagitanins dans le vin est fonction du nombre de mois de contact et du type de bois (Fig. 2). Dans cet essai, les concentrations en ellagitanins atteignent leur maximum au troisième mois de contact indépendamment du potentiel du bois. Ce dernier influe sur la concentration en ellagitanins des vins. Les teneurs en ellagitanins de nos vins restent faibles puisque le maximum est de 3.49 mg d'équivalent d'acide ellagique/L de vin. Après 4 mois d'élevage, le vin qui est en contact avec les bois qui ont un potentiel moyen a une concentration d'ellagitanins en moyenne 1.38 fois plus élevée que les vins en contact avec des bois dont le potentiel est bas. La différence entre le potentiel haut et le potentiel moyen est de 1.92 et celle entre le potentiel haut et le potentiel bas est de 2.65. Les cinétiques d'extraction des ellagitanins du bois de chêne sont donc semblables entre les différentes classes de bois, seul la quantité extraite varie en fonction de la concentration en ellagitanins du bois (Fig. 2).

4) Dosage des ellagitanins natifs des vins

Le dosage des ellagitanins natifs dans le vin est en corrélation avec le dosage des ellagitanins totaux du vin. Mais des différences dans les proportions entre les ellagitanins apparaissent. On peut remarquer que la proportion en castalagine retrouvée dans les vins augmente par rapport à ce qu'elle était dans les bois. Deux hypothèses peuvent expliquer cela ; soit la castalagine est mieux extraite par le vin que les autres ellagitanins, soit elle subit de manière moins importante des réactions chimique (oxydation, complexation…). Il a été démontré que la castalagine réagissait moins que la vescalagine, c'est pourquoi nous privilégions ici la seconde hypothèse (Quideau *et al.*, 2005).

5) Influence des concentrations en ellagitanins du vin sur la perception organoleptique

Durant notre étude, nous avons aussi estimé l'impact des concentrations en ellagitanins sur les perceptions organoleptique des vins rouges lors d'une dégustation de type profil sensoriel. Notre panel de juges entrainés a décrit les vins rouges les plus riche en ellagitanins comme étant plus rond ($p < 0.0001\%$). De plus, il apparaît que l'astringence et l'amertume ne sont pas négativement impactées par les concentrations en ellagitanins dans le vin, les différentes notes n'étant pas significativement différentes.

CONCLUSION ET PERSPECTIVE

Cette étude a montré que le classement des bois de tonnellerie en fonction de leurs richesses en ellagitanins était possible. Les analyses, que ce soit pour doser les ellagitanins totaux ou estimer la composition en ellagitanins natifs, ont montré une grande hétérogénéité dans la richesse des bois envers ces molécules. L'analyse moléculaire des 8 principaux ellagitanins n'a montré aucune déviation du classement NIRS en fonction de la composition en ellagitanins natifs. La procédure NIRS est donc fiable pour classer les bois en fonction de leur potentiel ellagitannique total.

Le classement des bois puis leur mise en contact avec le vin a permis d'obtenir des vins avec une richesse en ellagitanins différentes. Cela montre donc l'importance dans le choix des bois de tonnellerie en fonction du vin. D'autant plus que lors de la dégustation, les ellagitanins jouent un rôle dans la perception en bouche au niveau de la rondeur. Pour finir, l'analyse moléculaire des ellagitanins dans le vin a confirmé la grande réactivité des ellagitanins sauf pour le cas de la castalagine.

BIBLIOGRAPHIE :

Chatonnet P., 1995. Influence des procédés de tonnellerie et des techniques d'élevage sur la composition et la qualité des vins élevés en futs de chêne. PhD thesis, Université de Bordeaux, Bordeaux, France.

Giordanengo T., Charpentier J. P., Boizot N., Roussel S., Roger J. M., Chaix G., Robin C. and Mourey N., 2009. OAKSCAN™ : Procédé de mesure rapide et non destructif des polyphénols du bois de chêne de tonnellerie. *Rev. Fr. Oenol.*, 234, 10-15.

Glabasnia A. and Hofman T., 2006. Sensory-directed identification of taste-active ellagitannins in american (*Quercus alba* L.) and european oak wood (*Quercus robur* L.) and quantitative analysis in bourbon whiskey and oak-matured red wines. *J. Agric. Food Chem.*, 54, 3380-3390.

Hervé du Penhoat C. L. M., Michon V. M. F., Peng S., Viriot C., Scalbert A. and Gage D., 1991. Structural elucidation of new dimeric ellagitanins from *Quercus robur*. *J. Chem. Soc. Perkin Trans 1*, 1653–1660.

Mayer W., Gabler W., Riester A. and Korger H., 1967. On tannins compounds from the wood of chestnut and oak wood-IIs. Isolation of castalagin, vescalagin, castalin and vescaline. *Liebigs Ann. Chem.*, 707, 177-181.

Moutounet M., Rabier P., Puech J. -L., Verette E. and Barillere J. P., 1989. Analysis by HPLC of extractable substances oak wood. Application to a chardonnay wine. *Sci. aliments*, 9, 35-51.

Okuda T., 2005. Systematics and health effects of chemically distinct tannins in medicinal plants. *Phytochemistry*, 66, 2012–2031.

Peng S., Scalbert A. and Monties B., 1991. Insoluble ellagitannin in *Castanea sativa* and *Quercus petraea* woods. *Phytochemistry*, 30, 775-778.

Quideau S., Jourdes M., Lefeuvre D., Montaudon D., Saucier C., Glories Y., Pardon P. and Pourquier P., 2005. The chemistry of wine polyphenolic *C*-glycosidic ellagitannins targeting human topoisomerase II. *Chem. Eur. J.*, 11, 6503-6513.

Quinn M. K. and Singleton V. L., 1985. Isolation and identification of ellagitanins from white oak wood and an estimation of their role in wine. *Am. J. Enol. Vitic.*, 36, 148-155.

Saucier C., Jourdes M., Glories Y. and Quideau S., 2006. Extraction, detection, and quantification of flavano-ellagitannins and ethylvescalagin in a Bordeaux red wine aged in oak barrels. *J. Agric. Food Chem.*, 54, 7349-7354.

Somers T. C., 1990. An assessment of the «oak factor» in current winemaking practice. *Tech. Rev. Aust. Wine Res. Inst.*, 67, 3-10.

A NEW RAPID AND ECONOMICAL HPLC METHOD FOR THE SIMULTANEOUS DETERMINATION OF AMINO ACIDS AND BIOGENIC AMINES: APPLICATION IN THE ANALYSIS OF GRAPES AND WINES

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ABSTRACT

This paper reports a new, simple, rapid and economical method for routine determination of 24 amino acids and biogenic amines in grapes and wine. No sample clean-up is required and total run time including column re-equilibration is less than 40 minutes. Following automated in-loop pre-column derivatisation with an *ortho*-phthaldialdehyde-N-acetyl cysteine reagent, compounds were separated on a 3 mm x 25 cm C_{18} column using a binary mobile phase. Grapes were crushed, sieved, centrifuged and diluted 1/20 for analysis; wines were simply diluted ten-fold**.** The method was validated in the range 0.25 - 10 mg/l; repeatability (RSD) was 1 - 2% for most compounds, intermediate precision was 2 - 3% and accuracy 96 - 106%. The LOD varied between 10 µg/l for aspartic and glutamic acids, ethanolamine and GABA, and 100 µg/l for tyrosine, phenylalanine, putrescine and cadaverine. Applied to grapes, the method showed that the amino acid content was strongly correlated with berry volume, moderately correlated with sugar concentration and negatively correlated with total acidity. The method was also applied to the determination of the concentration of the target compounds in two different wine studies.

INTRODUCTION

There is growing interest in the analysis of amino acids in grape juice due their pivotal role as precursors to aromas released during fermentation or ageing. For example isoamyl, isobutyl, phenylethyl alcohols, in addition to tyrosol and tryptophol are derived from respectively leucine, isoleucine, valine, phenylalanine tyrosine and tryptophan. (Hernandez-orte *et al.,* 2002). It has also been shown (Escudero *et al*., 2000) that the amino acids remaining in wine after fermentation have an influence on aromas during the maturing process.

The demand by consumers for better and healthier foods has led to increased interest in biogenic amines, given their importance for human health and food safety. The aliphatic polyamines, putrescine, cadaverine, spermine and spermidine, are pharmacologically active and reportedly toxic (Tabor & Tabor, 1964; Til *et al.*, 1997). Putrescine and cadaverine play an important role in food poisoning as they can enhance the toxicity of histamine (Cinquina *et al*., 2004) and furthermore, they can react with nitrite to form heterocyclic nitrosamines which are carcinogenic (Hotchkiss, 1989).

High performance liquid chromatography is the most widely used technique for the determination of amino acids and biogenic amines in a diversity of matrices. In recent years,

the original HPLC technique of ion-exchange chromatography followed post-column derivatisation with ninhydrin on a dedicated amino acid analyser has been largely supplanted by pre-column derivatisation, due to the flexibility of the technique and relative simplicity of the apparatus without the requirement for a dedicated instrument. Since it was first described as a fluorogenic reagent by Roth in 1971 OPA (o-phthaldialdehyde) has become arguably the most widely used derivatising agent in the chromatographic determination of primary amino acids and biogenic amines. The reaction takes place almost instantaneously at room temperature at alkaline pH in the presence of a thiol-containing reducing agent, the most commonly used of which is as 2-mercaptoethanol (MCE), (Hanczkó *et al.,* 2007). However, the isoindole derivatives produced by OPA-MCE are notoriously unstable, and more bulky thiols such as N-acetylcysteine (NAC) or 3-mercaptopropionic acid (MPA), provide more stable derivatives. The characteristics and stability of OPA-NAC-amine derivatives have been intensively investigated (Molnár-Perl and Vasanits, 1999, Kutlán *et al*., 2002)

There are several publications on the simultaneous analysis of amino acids and biogenic amines, using OPA (Mengerink , 2002; Kutlán *et al.*, 2002; Kutlán & Molnár-Perl, 200;, Hanczkó *et al.*, 2005; Hanczkó *et al.*; 2007, Pereira *et al.*, 2008). These methods provide varying degrees of sensitivity, selectivity and ease of execution, however, in general they involve long analysis times (> 60 minutes) with flow-rates as high as 1.8 ml/min (Koros *et al*., 2007) resulting in high solvent consumption. Therefore, given growing awareness of environmental issues, the rising costs of organic solvents (especially acetonitrile) and of their disposal, the objective of this study was to develop and validate a rapid and economical method for the routine analysis of several amino acids and biogenic amines common to wines, grapes and other food matrices without compromising on selectivity or sensitivity.

MATERIALS AND METHODS

Reagents

The amino acids as their hydrochloride salts and the seven amines in addition to o-phthaldialdehyde (OPA) and n-acetyl cysteine (NAC) were purchased from Sigma (Sigma-Aldrich Chimie, Lyon, France). Doubly distilled water was used to prepare solutions and for washing all consumable materials. The derivatisation reagent, consisting of 2 ml NAC solution and 0.5 ml OPA solution (both 6g/l) was prepared on a daily basis and allowed to stabilise at room temperature for 90 minutes before use.

Analyte Solutions

Stock solutions of the analytes were made with 0.1 M HCl, except for tyrosine which was prepared in 0.1 M NaOH. These solutions stored at –20°C and were stable for several months. A stock mixture containing approximately 40 mg/l of the analytes was prepared in freshly distilled water on a weekly basis; however, it was stable at –20°C for several weeks. Calibration standards were prepared on a daily basis by serial dilution of the stock mixture in freshly distilled water.

Samples

The method was applied to the determination of amino acids and biogenic amines in grapes and wines. Frozen grapes were thawed at room temperature, crushed in a mortar and pestle, sieved to remove solid matter and then centrifuged at 7000 g. The supernatant was diluted 20-fold with freshly distilled water and filtered using a 0.45 µm membrane. Wine was diluted 1

in 10 (v/v) with distilled waterand filtered using a 0.45 µm membrane. Prepared samples were placed in the auto sampler for in-loop derivatisation

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Instrumentation and Operating conditions

A Hewlett-Packard (Agilent Technologies Massy, France) 1100 series HPLC instrument was used, consisting of a model G1322A degasser, a G1312A binary pump, a model G1313A autosampler and a G1321A fluorescence detector set at excitation and emission wavelengths of 330 nm and 440 nm, respectively. The in-loop derivatisation method was as follows: draw 2µl from OPA-NAC reagent, draw 2µl sample (or standard). Mix 15 times in seat. Wait 2 minutes. Draw 3 µl of distilled water. Mix three times in seat. Inject. The injection volume was thus 7 µl. Separations were carried out on a 250 x 3 mm Equisil ® column (CIL, Bordeaux, France). It was protected by a 1 mm C_{18} SecurityGuard ® cartridge supplied by Phenomenx (France). Mobile phase A consisted of 95% 0.05M sodium acetate buffer, pH 6.5 and 5% methanol, filtered under vacuum using a 0.22 µm nylon membrane. Mobile phase B consisted of methanol-acetonitrile 70-30. The flow rate was 0.5 ml/min, the run time was 40 minutes (including re-equilibration of the column), using a binary gradient programme.

Validation

Calibration standards at six concentration points (0.25, 0.5, 1, 2, 5 and 10 mg/l) were prepared in doubly distilled water spiked with the analyte mixture. Standard calibration curves were obtained from unweighted least-squares linear regression analysis of the data. Individual peak areas were interpolated on the calibration graphs to determine the found (back calculated) concentrations as compared to the nominal concentrations. The quality of fit was determined using back-calculated-to-nominal concentrations and the 'lack of fit' test was used to confirm the linearity of the method.

Within-day and between-day precision and accuracy of the method were determined by carrying out replicate analyses of the calibration standards. The repeatability was determined by preparing and analysing each calibration standard five times within a single day (i.e. 30 standards in total) under the same operating conditions. The intermediate precision was determined by carrying out the same operations over five days under different operating conditions. The precision was given by mean relative standard deviation of the back-calculated (found) concentrations and the accuracy of the method was evaluated as 100*[mean found concentration/nominal concentration]. Recovery was carried out grape juice and wine samples with 10 mg/l of the analyte mix. It was determined by comparing the back-calculated (found) concentrations and the nominal concentrations using the standard additions method; it was expressed as 100*[mean found concentration/nominal concentration].

RESULTS AND DISCUSSION

Chromatographic separation

As stated in the introduction, the objective of this study was to develop a rapid and economical method for the simultaneous determination of the principal amino acids and biogenic amines in grape juice and wine. Based on a literature survey the starting point was a method published in 2003 for the simultaneous determination of amino acids and biogenic amines in wine beer and vinegar (Kutlán & Molnár-Perl, 2003). The column used in that study was a Hypersil ODS 5µm dp 200 mm x 4 mm with a 20 x 4 mm guard column. In this study the a more cost-effective 'generic' version was chosen with a column diameter of 3

mm, which enabled a flow rate of 0.5 ml/min to be used (as opposed to 1.3 – 1.7 ml/min in the above reference), thus ensuring further savings on solvents. Initially a ternary gradient program similar to that described in the above reference was employed, however, the program was entirely revised to reduce the amount of acetonitrile in the organic modifier and to enable the method to be executed on a binary pump system (see experimental section). The further advantage of the optimised gradient program is the complete elution of all 24 analytes plus re-equilibration of the column is achieved in less than 40 minutes.

Validation

The repeatability and intermediate precision are summarised in Table 1; precision was given as the relative standard deviation of the back-calculated concentrations from the equation of the linear regression curves. Repeatability precision varied from less than 0.2% RSD at the 10 mg/l calibration point to 5- 7% at 0.25 mg/l, and the intermediate precision varies from less than 0.4% at 10 mg/l to up to 10% at 0.25 mg/l. Amino acids and amines with a low yield under the reaction conditions (serine, tyrosine, phenylalanine, putrescine – **Figure 1**) tended to have higher RSD's at all concentrations and in fact 0.25 mg/l was the limit of quantification (RSD ≈ 20%) for these compounds. The limit of quantification for compounds with higher yields, aspartic and glutamic acids, glutamine and ethanolamine was 0.05 mg/l, and for those of intermediate yield, the limit of quantification was 0.1 mg/l. The precision within the matrix, determined by six replicate analyses of grape juice and wine was greater than 95%. The "lack of fit" test showed no significant deviation from linearity; the bias, calculated as the sum of the residuals (nominal - back-calculated concentration) was non significant. Linear regression of the back-calculated-to-nominal concentrations provided slopes of generally 0.999–1.000 and intercepts equal to 0 (Student's t-test). The accuracy of the method, evaluated as 100 × (mean found concentration/nominal concentration) was approximately 100%.

Applications

Analysis of grape juice

The method was applied to determine the effect of determination of different levels of sunlight in interaction yield on the amino acid and biogenic amine content of Syrah grapes. The grapes received two (high and low) levels of sunlight. Natural sunlight was reduced by 17% using a black canopy, and was increased by 28% using a reflecting surface. For each level of sunlight, three yields were studied: 14 tonnes/ha (the natural yield of the parcel) and 9 and 6 t/ha obtained by removing green bunches before veraison. **Figure 2** shows the effect of shade and yields on the quantity of amino acids and amines (only the high and low yields are presented for ease of interpretation). It may be observed that the majority of amino acids and putrescine (the other biogenic amines were not detected), both shade and yield affected the concentration which decreased in the order Shade/6 tonnes/ha > Reflection/6 tonnes/ha> Reflection 14 t. The total amino acid content was strongly correlated with berry volume (r = 0.88 and 0.99 for the 6 t/ha and 14 t/ha yields, respectively, and was moderately correlated with sugar concentration (r = 0.76 and 0.66, respectively). The total acidity of the berries (data not shown) was reduced by the more intense sunlight modality as was the total amino acid content.

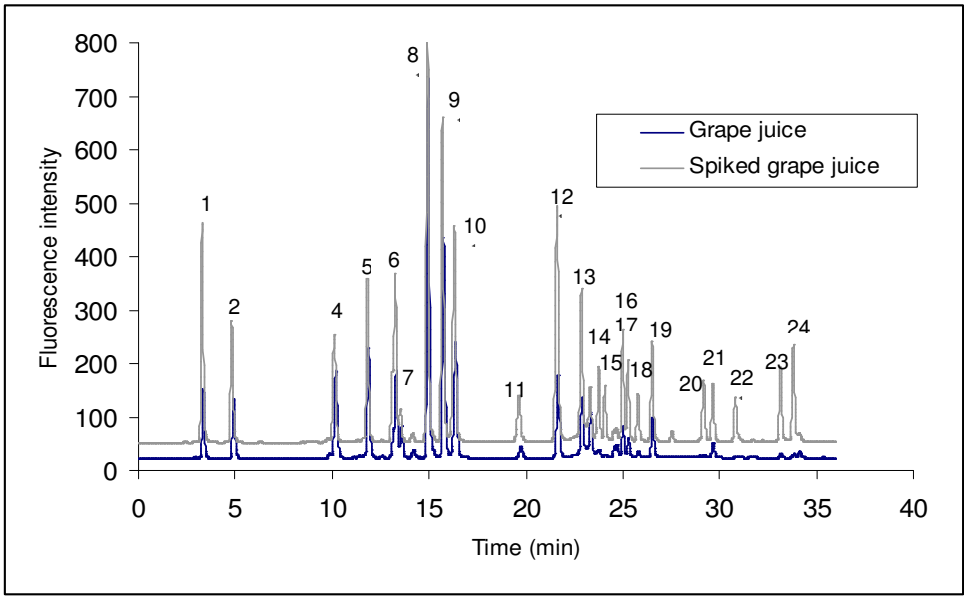

Figure 1: Chromatogram of grape juice unspiked and spiked with 2 ppm of each compound. 1-aspartic acid, 2-glutamic acid 3-serine, 4-asparagine 5- glutamine, 6-threonine, 7-histidine, 8-arginine, 9-alanine, 10-GABA, 11-tryosine, 12-ethanolamine, 13-valine, 14-methionine, 15-phenylalanine, 16-histamine, 17- isoleucine, 18- lysine, 19- leucine, 20-tyramine, 21- putrescine, 22- cadaverine, 23- isoamylamine, 24- phenylethylamine

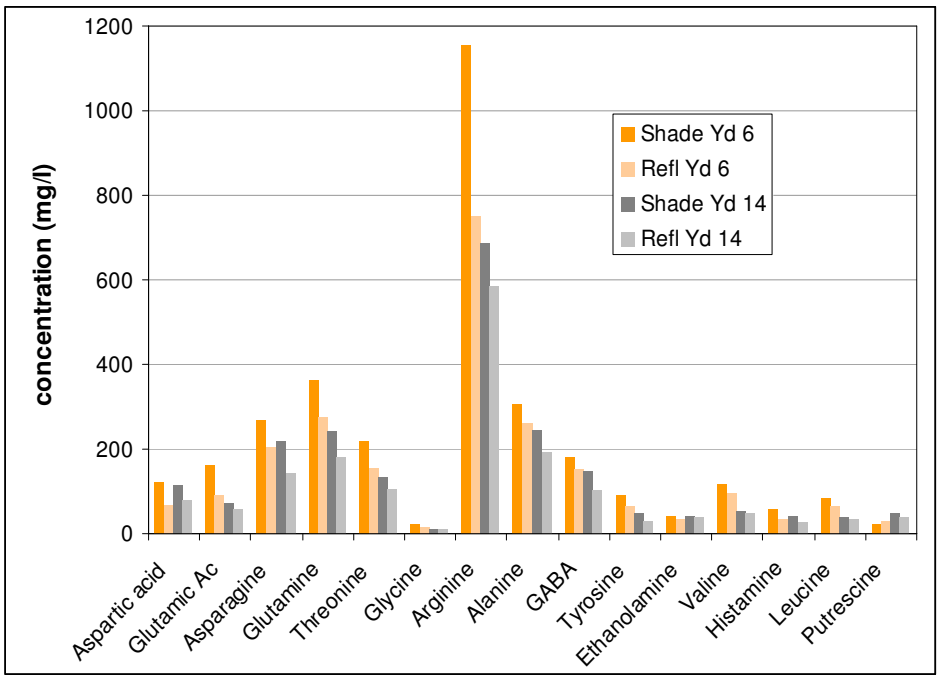

Figure 2: Histogram showing the effect of shade and yield on amino acid and amine concentration of Syrah grapes

Table 1

A: Repeatabity and intermediate precision

	Repeatability (Within-day precision)							Intermediate (between-day) precision						
	0.25 mg/l	**0.5 mg/l**	**1 mg/l**	**2 mg/l**	**5 mg/l**	**10 mg/l**	**Mean CV**	**0.25 mg/l**	**0.5 mg/l**	**1 mg/l**	**2 mg/l**	**5 mg/l**	**10 mg/l**	**Mean CV**
	Mean AF						%	**Mean AF**						**%**
Aspartic Acid	0.245	0.503	1.011	1.995	4.991	10.004	**2.33**	0.232	0.493	1.011	2.013	5.024	9.994	**2.89**
Glutamic Acid	0.248	0.511	0.993	1.998	4.999	10.001	**1.69**	0.251	0.499	1.012	1.982	5.025	9.999	**2.98**
Asparagine	0.252	0.488	0.988	1.989	4.988	9.998	**2.35**	0.251	0.510	0.985	1.977	4.986	9.985	**4.02**
Serine	0.252	0.501	0.987	2.011	5.000	9.999	**1.88**	0.230	0.485	0.975	2.068	5.021	9.989	**7.04**
Glutamine	0.250	0.493	0.997	2.010	5.003	9.997	**1.80**	0.241	0.485	1.013	2.005	5.031	9.992	**4.06**
Threonine	0.247	0.489	1.005	1.999	5.022	9.990	**2.28**	0.233	0.494	0.988	2.017	5.022	9.995	**3.44**
Glycine	0.247	0.505	0.983	2.011	5.007	9.996	**2.40**	0.248	0.505	1.013	1.991	5.001	10.009	**3.05**
Arginine	0.240	0.491	1.001	2.006	5.025	9.987	**2.34**	0.258	0.492	1.028	1.986	4.988	10.015	**2.53**
Alanine	0.234	0.500	1.008	2.013	4.996	9.999	**2.06**	0.241	0.493	1.005	2.011	5.021	9.997	**2.48**
GABA	0.238	0.485	1.016	2.004	5.015	9.991	**2.01**	0.254	0.508	0.990	2.019	4.983	10.014	**2.22**
Tyrosine	0.242	0.502	1.018	2.000	4.978	10.009	**2.19**	0.252	0.508	1.020	1.990	4.977	10.020	**3.04**
Ethanolamine	0.244	0.495	1.011	1.999	5.005	9.997	**1.69**	0.244	0.503	1.018	1.989	5.008	10.005	**1.98**
Valine	0.246	0.485	1.016	2.010	4.993	10.001	**2.26**	0.259	0.487	1.015	2.014	4.976	10.017	**3.14**
Methionine	0.253	0.487	0.999	2.001	5.020	9.991	**1.40**	0.242	0.495	1.019	2.034	4.955	10.024	**2.27**
Phenylalanine	0.246	0.489	1.011	2.005	5.001	9.998	**3.13**	0.231	0.490	1.045	1.972	5.037	9.992	**3.73**
Histamine	0.250	0.491	1.004	1.998	5.013	9.994	**2.04**	0.245	0.506	0.995	1.995	5.034	9.994	**2.76**
Isoleucine	0.246	0.489	1.014	2.001	5.001	9.998	**3.05**	0.257	0.515	1.000	1.991	4.989	10.016	**2.99**
Lysine	0.246	0.487	1.006	1.995	5.032	9.985	**2.44**	0.235	0.487	1.021	2.023	4.995	10.006	**2.91**
Leucine	0.251	0.492	1.009	1.985	5.021	9.992	**1.88**	0.238	0.511	1.000	2.030	4.972	10.017	**2.97**
Tyramine	0.244	0.490	1.010	2.013	4.993	10.001	**2.14**	0.237	0.494	1.002	2.027	5.008	10.000	**2.42**
Putrescine	0.246	0.515	1.011	1.978	4.994	10.005	**2.69**	0.240	0.487	0.987	2.013	5.064	9.977	**4.10**
Cadaverine	0.244	0.499	1.012	2.002	4.988	10.005	**2.03**	0.239	0.491	0.998	2.027	5.016	9.997	**3.03**
isoamylamine	0.240	0.491	1.006	2.001	5.024	9.988	**1.76**	0.237	0.496	0.998	2.008	5.041	9.987	**3.01**
Phenylethylamine	0.244	0.494	1.003	2.001	5.018	9.991	**1.46**	0.243	0.509	1.010	1.995	5.005	10.006	**3.00**

Mean AF: Mean amount found (n = 5)

Analysis of wine

The method was applied to the analysis of red wines in two different studies. In the first study, a comparison was made between wines produced at one site in France and one site Romania. The winemaking was supervised by the same consultant at both sites. In total 11 wines were analysed: four Cabernet sauvignons and three Merlots from Romania, and two Merlots and two Syrahs from France. The predominant amino acids were aspartic and glutamic acids, glycine, threonine, alanine, GABA and ethanolamine. Putrescine was the principal biogenic amine (and the only biogenic amine in the Merlots) with small quantities of cadaverine in the other wines. Within a given variety, the chromatographic profiles were very similar with differences observed only in concentrations (**Figure 3**), though as indicated in the Figure, the French Merlot contained residual arginine and a higher concentration of putrescine. The total concentration of amino acids and biogenic amines varied from approximately 300 mg/l to over 1 g/l in the case of one of the Cabernet sauvignon wines. The Romanian Merlot wines generally contained lower concentrations amino acids and amines than the French Merlots or the Syrah wines, though this was in no way related to overall wine concentration as all wines contained between 13.5 and 14% alcohol.

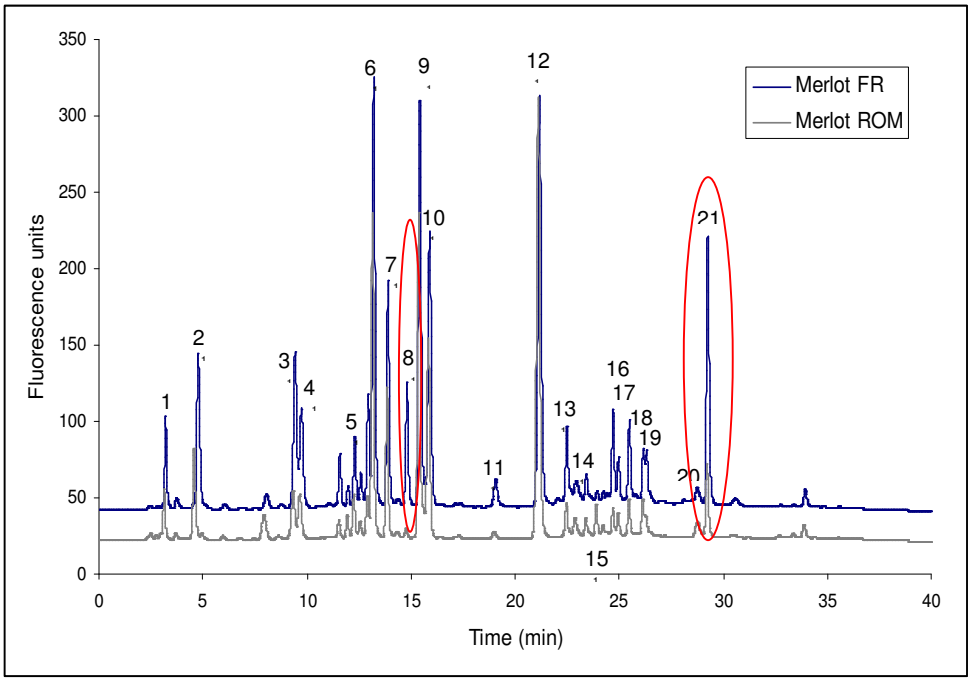

Figure 3: Chromatogram of Merlot wines from France (FR) and Romania (ROM). Peak numbers as per Figure 1.

In the second study, the aim was to observe the impact of sulphur dioxide in interaction with malolactic fermentation and ageing in oak on the amino acid and amine concentrations in wines made from the same Syrah grapes. **Figure 4** presents chromatograms of two wines from the same harvest in 2009 - one to which sulphur dioxide had been added before and during fermentation and the other which no sulphur dioxide was added. Both wines were entirely fermented and aged in stainless steel tanks. It may be seen that the wine without the sulphur dioxide treatment is slightly richer in all compounds, both amino acids and amines. It is interesting to note that this effect is less marked in the pair of wines (with and without sulphur dioxide) aged in oak barrels (**Figure 5**) and this trend was actually reversed in the pair of wines whose malolactic fermentation took place in barrels and which also were oak aged (data not shown).

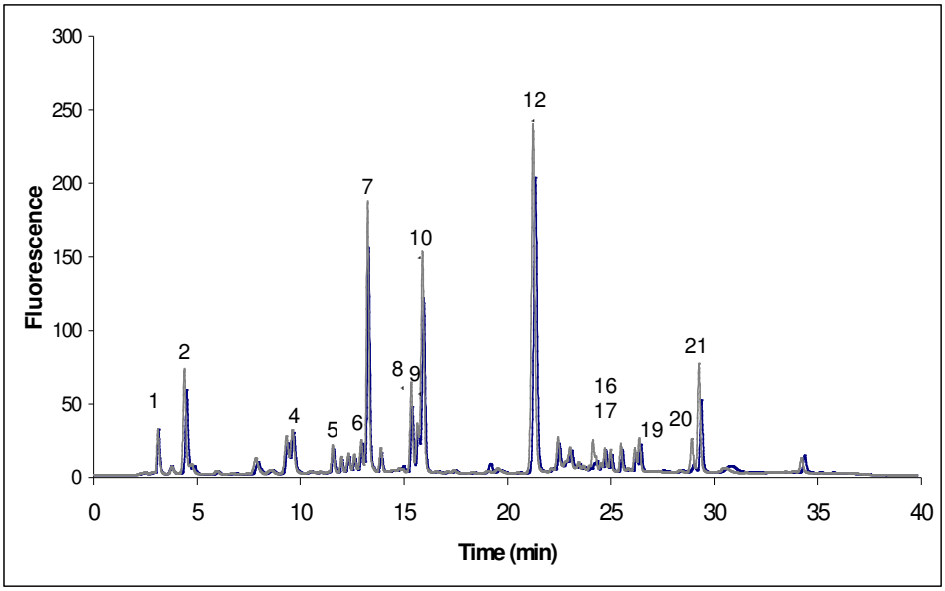

Figure 4: Chromatogram of Syrah wine with (blue trace) and without (grey trace) the addition of SO_2. Peak numbers as per Figure 1

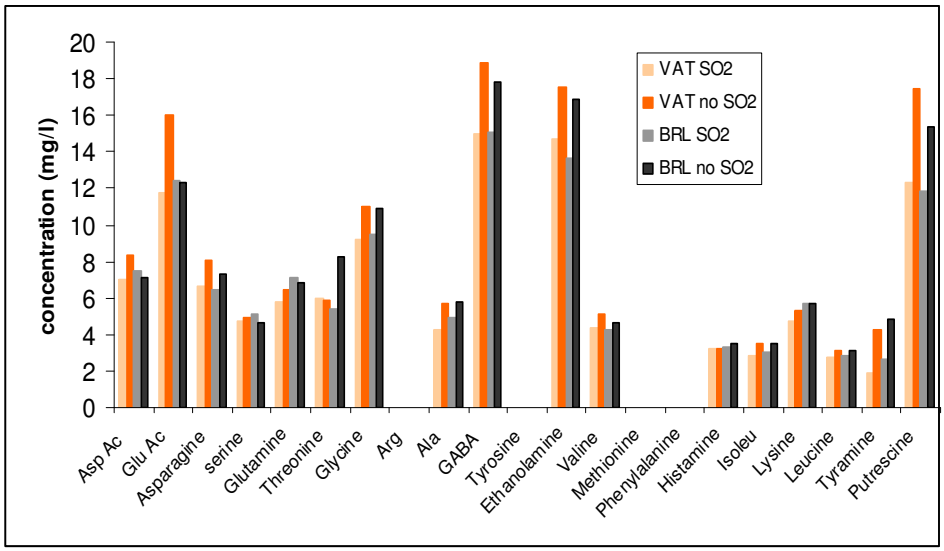

Figure 5: Histogram of amino acid and amine concentrations as a function SO_2 addition and exposure to oak. BRL = wine aged (but not fermented) in oak

CONCLUSION

A new rapid and economical method has been described for the simultaneous determination of amino acids and biogenic amines. The method was statistically validated and applied to the determination of these compounds in grapes and wines. Due to the high sensitivity of the method, no sample preparation, other than a simple dilution is required before derivatisation, which is carried out using a fully automated in-loop procedure. The method has proved suitable for the analysis of grapes and wines and will used in several other applications in the future.

REFERENCES

Cinquina, A. L., Calı, A., Longo, F., De Santis, L., Severoni, A., & Abballe, F., 2004. Determination of biogenic amines in fish tissues by ion-exchange chromatography with conductivity detection. *Journal of Chromatography A*, 1032: 73–77.

Escudero, A., Hernandez-Orte, P., Cacho, J., & Ferreira, V., 2000. Clues about the role of methional as character impact odorant of some oxidized wines. *Journal of Agricultural and Food Chemistry*, 48: 4268–4272.

Hanczko R, Korös A, Tóth F, and Molnár-Perl I., 2005. Behavior and characteristics of biogenic amines, ornithine and lysine derivatized with the o-phthalaldehyde-ethanethiol-fluorenylmethyl chloroformate reagent. *Journal of chromatography. A* 1087(1-2):210-22.

Hanczkó, R. Jámbor, A. Perl, A. Molnár-Perl I, 2007. Advances in the o-phthalaldehyde derivatizations: Comeback to the o-phthalaldehyde–ethanethiol reagent. *Journal of Chromatography A,* 1163, 25-42.

Hernandez Orte, P., Cacho, J., & Ferreira, V. 2002. Relationship between varietal amino acid profile of grapes and wine aromatic composition. Experiments with model solutions and chemometric study. *Journal of Agricultural and Food chemistry*, 50, 2891–2899.

Hotchkiss, J. H. 1989. Preformed N-nitroso compounds in foods and beverages. *Cancer Surveys*, 8: 295–321.

Kőrös, Á, Varga Zs., Molnár-Perl,I., 2008. Simultaneous analysis of amino acids and amines as their o-phthalaldehyde-ethanethiol-9-fluorenylmethyl chloroformate derivatives in cheese by high-performance liquid chromatography. *Journal of Chromatography A*., 1203; 146-152.

Kutlan, D., Presits, P., Molnar-Perl, I., 2002. Behavior and characteristics of amines derivatives obtained with o-phthaldialdehyde/3-mercaptopropionic acid and with o-phthaldialdehyde/N-acetyl-l-cysteine reagents. *Journal of Chromatography.* A 949: 235–248.

Kutlán, D., Molnár-Perl, I., 2003. New aspects of the simultaneous analysis of amino acids and amines as their o-phthaldialdehyde derivatives by high-performance liquid chromatography: Analysis of wine, beer and vinegar *Journal of Chromatography A*, 987: 311-322.

Mengerink, Y. Kutlán, D., Tóth, F., Csámpai, A. , Molnár-Perl, I., 2002. Advances in the evaluation of the stability and characteristics of the amino acid and amine derivatives obtained with the o-phthaldialdehyde/3-mercaptopropionic acid and o-phthaldialdehyde/N-acetyl--cysteine reagents: High-performance liquid chromatography–mass spectrometry study. *Journal of Chromatography A*, 949, 99-124.

Molnár-Perl, I., Vasanits, A., 1999. Stability and characteristics of the o-phthaldialdehyde/3-mercaptopropionic acid and o-phthaldialdehyde/N-acetyl--cysteine reagents and their amino acid derivatives measured by high-performance liquid chromatography. *Journal of Chromatography A*, 835, 73-91.

Pereira, M., Pontes, J.S., Camara, J.C., Marques, J.C., 2008. Simultaneous analysis of free amino acids and biogenic amines in honey and wine samples using in loop orthophthalaldeyde derivatization procedure. *Journal of Chromatography. A*, 1189: 435-43.

Roth, M., 1971, Florescence reactions of amino acids. Analytical Chemistry, 43: 880-882.

Tabor, H., Tabor, C. W., 1964. Spermidine, spermine and related amines. *Pharmacology. Reviews*., 16: 245-300.

Til, H. P., Falke, H. E., Prinsen, M. K., & Willems, M. I., 1997. Acute and subacute toxicity of tyramine, spermidine, spermine, putrescine and cadave´rine in rats. *Food and Chemical Toxicology*, 35, 337-348.

EXTRACTION OF WINE COMPOUNDS FROM CORK STOPPERS

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ABSTRACT

The importance of the interactions between packaging and foodstuffs is well reported in literature as it may affect the overall quality of the product. In this perspective, wine development after bottling has been a subject of intense research. Studies concerning wine post-bottling development invariably consider closures determinant to the process of wine ageing. In most cases, the effect of closures on oxygen exposure of wine is evaluated and while scalping or sorption of compounds by packaging material is one of the most important mechanisms in the interaction between packaging and foodstuffs, it has often been referred but never thoroughly analyzed when considering closures/wine systems. Moreover, different levels of certain volatile sulphur compounds have been reported in wines presenting the same degree of oxygen exposure. An experimental protocol was then established in order to determine whether the origin of such differences is the adsorption/absorption of these molecules by certain types of closures, namely cork closures.

INTRODUCTION

Post-bottling development of wine is closely related to the type of closure used. For centuries, natural cork closures constituted the privileged mean for bottle sealing as a result of their remarkably adequate physical properties like flexibility, high impermeability, lightness and chemical inertness. Technological advances led to the appearance of other alternatives, as an attempt both to avoid cork problems like off-flavour odours caused mainly by 2,4,6-TCA (cork taint) and to lower bottling costs. Not only technical cork stoppers, essentially made of composite cork, where developed by the cork industry but also a whole range of synthetic closures, which may now be used as wine stoppers; although not without some inconvenient, in the case of synthetic closures: high oxygen permeability, scalping of wine volatiles, and migration of some of their components into wine over time (Capone *et al.*, 2003, Godden *et al.*, 2005, Lopes *et al.*, 2007). Scalping phenomenon, described as the packaging ability to directly remove compounds through sorptive processes (Nielsen *et al.*, 1994) has also been recently mentioned as a probable cause for saranex liners screw-caps differentiated behaviour towards wine components volatile components (Lopes *et al.*, 2009). Flavour scalping was also noted in wines sealed under Tetrapack and "bag-in-box" containers, which have a strong sorption capacity of nonpolar compounds (Blake *et al.*, 2009). Thus, wine evolution is related to the type of closure used not only because of its direct interaction with wine regarding compound migration but also as a consequence of closures influence on oxygen management after bottling (Godden et al, 2001, Lopes et al., 2003, Lopes et al., 2005, Skouroumounis et al., 2005).

Therefore, the present study aims to evaluate scalping phenomena ocurring between wine closures. An experimental protocol was established in order to determine whether the origin of such differences is the adsorption/absorption of these molecules by certain types of

closures, namely cork stoppers. Macerations followed by solid-liquid high pressure extraction and HPLC-UV or GC-FPD analysis were conducted using granulated cork and cork stoppers in both model wine solution and red wine itself.

MATERIAL AND METHODS

Experiments with granulated cork in synthetic wine solution

Dimethyl sulphide (DMS) was chosen as a representative volatile sulphur compound. Maceration solution was an hydroalcoholic solution (12% ethanol) containing 100 µg/L of DMS. 5 g of granulated natural cork where used. Flasks containing the macerations where kept at 21ºC, away from light, for a period of 4 months. Experiments were carried out in 5 repetitions. DMS level in the solution was evaluated through headspace sampling followed by quantification using gas chromatography coupled with a flame photometric detection (GC-FPD) according to the method proposed by Lavigne *et al.* (1993).

Chromatographic experiments were performed using a Hewlett-Packard 5980-I coupled with a HP 19256-A flame photometric detector . The column was Chromosorb WHP (4 m x 3 mm). The oven temperature was kept at 65ºC for 5 min and programmed at a rate of 6ºC min/L to 110ºC. The carrier gas was hydrogen (15.5 mL/min). Its flow rate in the flame was 93 mL/min and a mixture of nitrogen/oxygen (80/20) at 100 mL/min was used. The make-up gas was nitrogen at 55 mL/min.

After solvent and operatory conditions (extraction time, temperature) optimization, DMS contents in granulated cork were assessed by performing a solid-liquid extraction (HP-SLE) with an ethanol solution 12% (v/v) using an high-pressure Dionex extractor followed by a liquid-liquid extraction (LLE) with CH_2Cl_2. Extracts were concentrated to 200 µL under N_2 stream and kept at -20ºC in complete obscurity for further characterization by GC with mass spectrometry (MS) detection.

Experiments with cork stoppers and wine

Cork stoppers having previously been in contact with red wine of the Merlot cv. were submitted to an HP-SLE in the same conditions as the granulated cork.

Extracts were evaporated under reduced pressure at 30 °C, the residue was dissolved in water, and freeze-dried to obtain a powder extract. This extract was solubilized in 1 mL of a methanol/water solution (50:50, v/v) before analysis by high performance liquid chromatography with ultraviolet detection (HPLC-UV).

Wine samples were filtered through GHP Acrodisc 25 mm, 0.45 µm filters, immediately prior to injection into the HPLC system. The column was a Lichrospher 100 RP-18 (5-rm packing, 250 X 4 mm i.d.). Elution conditions were as followed: flow rate 1 mL/min at room temperature, 20 µL sample loop; Solvent A, water/formic acid (99:1 v/v); solvent B, acetonitrile/formic acid (99:1 v/v). The gradient elution profile was: 0 to 3 min 3% B, 3 to 13 min 3 to 5% B, 13 to 50 min 5 to 40% B, followed by column recnditioning. Eluting peaks were monitored at 320 nm.

A control assay was conducted by following the same procedure with similar cork stoppers which had not been in contact with wine.

RESULTS AND DISCUSSION

Experiments with granulated cork in synthetic wine solution

After 4 months in contact with granulated cork, DMS concentration in the headspace tends to diminuish (Fig. 1).

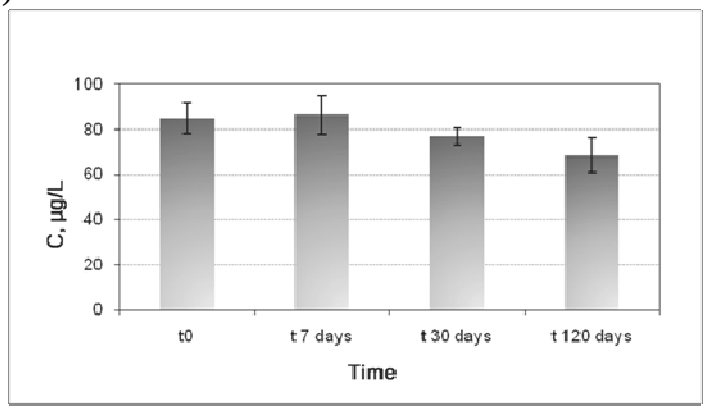

Figure 1: Evolution of DMS concentration in the headspace.

Presented values are corrected so that they don't include losses due to its high volatility. This may suggest that DMS has been sorbed by cork.

Further analysis by GC-MS will allow the direct quantification of DMS extracted from cork and confirm this hypothesis.

Experiments with cork stoppers and wine

Considering phenolic compounds assessment data, cork presents phenolic compounds which may migrate to wine and/or react with other wine substances. Furthermore, there is evidence of the presence of wine phenolic compounds in cork stoppers since closures present higher levels of these compounds when in contact with wine (Tab. 1).

Table 1: Levels of phenolic compounds in cork stoppers extracts.

Phenolic compounds	Concentration (Control), µg/g cork closure	Concentration, µg/g cork closure
Gallic acid	14.61 (1.50)	22.09 (2.01)
Vanillic acid	2.88 (0.24)	16.21 (0.92)
Caffeic acid	4.51 (0.12)	10.63 (1.07)
Ferulic acid	6.43 (0.87)	5.12 (0.65)
Vanillin	(8.72 (1.02)	6.64 (0.98)
Unknown (total)	189.31 (5.22)	163.87 (7.82)

Standard deviations of 5 replicates are given in parentheses

CONCLUSION

Analysis of the data seems to indicate the existence of scalping of the volatile sulphur compound studied – DMS – and of phenolic compounds, with suggested migration from wine into the stopper. Further analysis are necessary in order to clarify this tendency. Optimization of the GC-MS analysis of the cork extracts (in course) could confirm these results and allow to enlarge the experience to several other wine compounds. Another interesting point would be to identify by means of HPLC-MS several unknown phenolic compounds detected by HPLC-UV.

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REFERENCES

Blake, A., Kotseridis, Y., Brindle, I., Inglis, D., Sears, M., Pickering, G.J., 2009. Effect of closure and packaging type on 3-alkyl-2—methoxypyrazines and other impact odorants of Riesling and Cabernet Franc wines. *Journal of Agricultural and Food Chemistry*, 57: 4680-4690

Capone, D., Sefton, M., Pretorius, I., Hoj, P., 2003. Flavour "scalping" by wine closure-the "winemaking" continues post vineyard and winery. *Australian Journal of Grape and Wine Research*, 18: 16-20

Godden, P., Lattey, K., Francis, L., Gishen, M., Cowey, G., Holdstock, M., Robinson, E., Waters, E., Skouroumounis, G., Sefton, M., Capone, D., Kwiatkowski, M., Field, J., Coulter, A., D'Costa, N., Bramley, B., 2005. Towards offering wine to the consumer in optimal condition - the wine, the closures and other packaging variables. A review of AWRI research examining the changes that occur in wine after bottling. *Wine Industry Journal*, 20: 20-30

Kwiatkowski, M., Skouroumounis, G.K., Lattey, K.A., Waters, E.J., 2007. The impact of closures, including screw cap with three different headspace volumes, on the composition, colour and sensory properties of a Cabernet Sauvignon wine during two years'storage. *Australian Journal of Grape and Wine Research*, 13: 81-94.

Lavigne, V. , Boidron, J. N. , Dubourdieu, D. 1993. Dosage des composés soufrés volatils légers dans les vins par chromatographie en phase gazeuse et photométrie de flamme. *Journal International des Sciences de la Vigne et du Vin*, 27: 1-12.

Lopes, P., Saucier, C., Teissedre, P.-L., Glories, Y., 2007. Main routes of oxygen ingress through different closures into wine bottles. *Journal of Agricultural and Food Chemistry*, 55: 5167-5170

Lopes, P., Silva, M.A., Pons, A., Tominaga, T., Lavigne, V., Saucier, C., Darriet, P., Teissedre, P.-L., Dubourdieu, D., 2009. Impact of oxygen dissolved at bottling and transmitted through closures on the composition and sensory properties of a Sauvignon blanc wine during bottle storage. *Journal of Agricultural and Food Chemistry*, 57: 10261-10270

Nielsen, T. Jägerstad, M., 1994. Flavour scalping by food packaging. *Trends in Food Science and Technology*, 5: 353-356

Skouroumounis, G.K., Kwiatkowski, M.J., Francis, I.L., Oakey, H., Capone, D., Duncan, B., Sefton, M.A., Waters, E.J., 2005. The impact of closure type and storage conditions on the composition, colour and flavour properties of a Riesling and a wooded Chardonnay wine during five years'storage. *Australian Journal of Grape and Wine Research*, 11: 369-384

INFLUENCE OF DIFFERENT COMPOUNDS AND TECHNOLOGICAL OPERATIONS ON PHYSICAL – CHEMICAL, ORGANOLEPTIC PARAMETERS AND THE CONTENT OF HEALTHY COMPONENT-ANTIOXIDANTS OF SAPERAVI WINE

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ABSTRACT

One of the main goals of the modern wine industry is to supply customers with the stable and high quality wine. In the recent years, besides the organoleptic characteristics a special attention is paid to the nutritional values and healthy properties of industrially produced wines. The healthy properties of wine are associated with phenolic compounds found in most red wines (which is well known for its antioxidant properties).

In the present work the influence of various compounds and technological processes on the physical-chemical and organoleptic properties as well as on the antioxidant activities of Saperavi brand wine is discussed. Based on the studies presented in the paper the optimal conditions of wine treatment were found. These optimal conditions allow preserving high quality and stability of wine in combination with its nutritional values and healthy properties.

Ein von wichtigsten Zielen moderner Weinindustrie ist die Bestellung stabilen und qualitativen Weine an Kunden. In der letzter Zeit ausser organoleptischen Eigenschaften Hilfseffekt von Wein und sein Wert als Nahrungsmittel sehr stark beachtet ist, was seinerseits dadurch verursacht wird, dass es im Wein antioxidativ aktive phenolische Verbindungen gibt.

Durchgefuehrte arbeit befasst sich die Untersuchung von Einfluss verschiedener Behandlungsmittel und technologischen Prozesse zusammen und einzeln auf organoleptische und physikalisch-chemishe Eigenschaften und antioxidativen Aktivitaet von Saperavi. Bestimmt sind solche Bedingungen, dass ausser den Stabilitaet und Qualitaet der Wine auch maximale Erhaltung der pharmakologisch aktiven Stoffe und seines Werts als Nahrungsmittel moeglich ist.

INTRODUCTION

It is well known that free organic radicals are very harmful for the living organisms. These free radicals are commonly produced as a result of the metabolic activities of the living organisms. The other sources of organic free radicals may be associated to the polluted environment, the sun irradiation, the cigarette smoke, fast food, smoked food products, etc. [1, 2]. The living organisms have its unique fermentation systems to scavenge these free organic radicals [3, 4].

It is believed that nearly 60 human diseases (Alzheimer's disease, cancer, atherosclerosis, etc.) are associated with the high levels of organic free radicals present in the body [3, 5].

The identification of the novel natural antioxidants and the development of the medical treatment models based on these natural antioxidants represent an important goal of medicine. In the meanwhile these novel natural antioxidants must be relatively non-toxic and its reaction products with harmful free organic radicals must be easily eliminated from the human body [2]. The above-mentioned criteria are met by some natural antioxidants isolated from the plants. As we mentioned above, besides its organoleptic properties the most wines has some healthy properties. The healthy properties of wine are mostly associated with flavonoids (myricetin, catechin, etc.) and natural stilbene compounds: cis- and trans-resveratrol (glucoside as well as aglucone forms) found in it. Most flavonoids and resveratrol are concentrated in the flesh and seeds of the grape. During the fermentation most of these compounds are extracted into the wine. The concentration of above mentioned phenolic compounds in white wines produced using the traditional European technology are minimal. This is because during the fermentation of white wines (using the European method) the grape juice is isolated from the flesh and seeds [6, 2]. On the other hand the concentration of these antioxidants in the red wines and wines produced using "kakhetian" technology are in significant amounts because the flesh and seeds being present during the fermentation process. These flavonoids and natural stilbenes are 10-20 times more powerful antioxidants than the vitamins C and E [7, 8, 2].

For example, flavonole quercetine increases the positive pharmacological action of resveratol even after a period of 30 min (half-life for resveratol decomposition/degradation) [9]. It is also proved that the effectiveness of resveratol as an antioxidant is enhanced with the moderate consumption of the alcohol with it (wine) [10]. The antioxidant properties of resveratrol are diminished when used alone (as ingredient of some antioxidant medication formulation).

The enhancement of the antioxidant properties of resveratol in the presence of alcohols perhaps can be explained by the permeation property changes of the cell at membrane and mitochondrial levels. The usual concentration of the resveratol in red wines is ~ 0.2 – 0.8 g/L. [11]

Resveratol decreases the risk of Cardiovascular, Alzheimer's, and Parkinson's, diseases. It also lowers the cholesterol levels [12]. It also cures the skin cells and has properties similar to the phytoesterogen compounds. Resveratol also has the pronounced anticancer properties and it prevents the formation and growth of the tumor in humans [10].

The high dosage of resveratol helps to decrease the sugar levels in the blood [13]. Resveratol is also used against some fungal diseases, for the treatment of various skin inflammations and for some liver diseases [14].

Quercetin acts as antihistamine (anti allergic compound) and posses anti-inflammatory properties. In diabetic patients it has been found that quercetin lowers the concentration of those ferments that cause the accumulation of sorbitol in the blood thus lowering the risk of

kidney and eye damage. Similar to resveratol, quercetin also acts as phytoesterogen. It also lowers the risk of cancer [10]. Quercetin enhances the immune system of the body and slows down the "aging" process in humans. Quercetin also has useful preventive properties against arthritis, asthma, stomach ulcer, and major allergy symptoms. It also protects the brain cells and helps to fight the Alzheimer's disease [15].

After 8 years of extensive studies and research it has been discovered that these flavonoids lowers the risk of pancreatic cancer by almost 23% [14]. Also, myricetin decrease the risk of breast cancer in females [16].

MATERIALS AND METHODS

Thought the studies the wine "Saperavi" of 2007 vintage was used (Based on the red grapes of cv *Saperavi* from the "Kindzmarauli's Marani" LTD).

Determination of stilbenes - cis- and trans-resveratrol, flavonols - myricetin and quercetin, as well as anthocyans was performed using High Performance Liquid Chromatographic - HPLC methods with UV-Vis detection at 280, 370 and 518 nm respectively on the RP 18 (5 μm) LiChrocart 250-4 chromatographic column.

Measuring of antioxidant activity of wines was performed using Electron Spin Resonance ESR Spectrometer РЭ-1301. This method has been chosen since it is the only direct method for registration and identification of free radicals. The method provides possibilities to record directly absorption spectrum of the free radicals, to identify free radicals as well as to measure the rate of change of their concentration under the influence of different antioxidants. If hydroxyl radical, spin trap and antioxidant are placed all together in the water solution, antioxidant will partially neutralize hydroxyl radical. This will reduce adduct's ESR spectrum intensity, which will decrease proportionally with antioxidant activity of the antioxidant. As adduct Fremy's salt was used.

RESULTS AND DISCUSSION

The goal of the presented work is to study the influence of various substances and technological processes on physical-chemical and organoleptic parameters and antioxidant activities of cv *Saperavi* brand wine.

In this work we selected Saperavi brand wine (2007 vintage). For the wine treatment during the experiments we mainly used chemicals supplied by the "Institut oenologique de champangne**"** (France**)**: Sodium bentonite - Bentostab (BS); animal origin entirely hydrolysed gelatine Colle Perle**;** animal origin entirely hydrolysed gelatine – Colfine; animal origin partially hydrolysed gelatine - Inocolle**;** Insoluble polyvinylpyrrolidone (PVPP); soluble Casein; Creme de Tartare (Potassium bitartrate); Kísilgúr filter (diatomite); In addition, we used sodium bentonite (Askangel) produced in Georgia; paper filter- cartons (EK) of Zeitz-production and membrane filters. Throughout the work the cold treatment method was used.

On the selected wine sample more than 210 trial treatments were performed using above-mentioned compounds (separately and in combination) at various concentration and doses. Treatment has been conducted at several stages (9 stages). Initially all the samples were

tested for their organoleptic parameters. Degustation has been conducted by 5 mark (German) system. The best-selected samples (there were at least 8 samples per stage), were than tested for their stability.

Since the objective of our studies was to determine the influence of the various treatment chemicals and conditions on the antioxidant properties of Saperavi brand wine we also determined the amounts of the natural antioxidants such anioxidants (cis- and trans-resveratrol, myricetine, quercetin, anthociane) present in the wine after treatment.

Finally, the following 14 samples were selected:

1. Saperavi, 2007 harvest (control)
2. treated 5 g / dal by PVPP
3. treated 1.7 g / dal by Colle Perle
4. treated 4 g / dal by Cazein
5. treated 20 g / dal by Bentostab (BS)
6. treated 5 g / dal by PVPP, Cazein - 2 g/dal; BS-25 g/dal . Removed from deposit, filtered through diatomite, undergone cold treatment (5^0C) for 5 days and then filtered through membrane filter;
7. treated 7 g / dal by PVPP, Cazein - 2 g/dal; BS-25 g/dal . Removed from deposit without filtration;
8. sample 7, filtered through firstly diatomite and then paper filter.
9. sample 8, filtered through membrane filter.
10. sample 7, filtered through diatomite, undergone cold treatment (-5^0C) for 5 days and then filtered through membrane filter.
11. Treated 7 g/dal by PVPP, Colle Perle - 1.7 g/dal, BS-25 g/dal and removed from deposit without filtration.
12. sample 11, filtered through paper filter
13. Treated 7 g/dal by PVPP, Colle Perle _ 1.7 g/dal, BS-15 g/dal and removed from deposit without filtration;
14. sample 13, filtered through paper, undergone cold treatment (-5^0C) for 10 days and then filtered through membrane filter.

It should be noted that during cold treatment, potassium bitartrate was added in order to reach the optimal acid stability of the studied wines.

In the above-mentioned 14 samples cis- and trans resveratrol, myricetien and quercetin concentrations were determined using high performance liquid chromatography (HPLC).

Typical chromatogram is shown on Fig. 1. Results are shown in Tab.1.

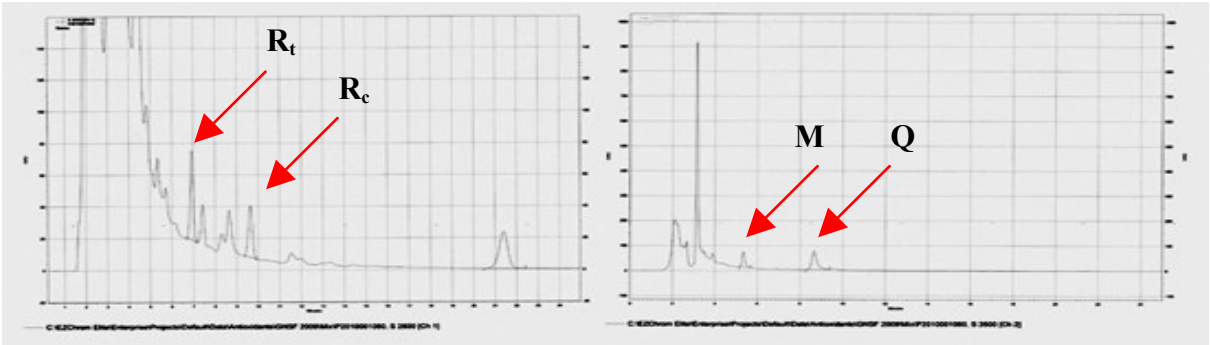

Fig. 1. Chromatograms of wine sample for determination of cis (R_c)- and trans-resveratrol (R_t) and myricetin (M) and quercetin (Q) respectively. First chromatogram is zoomed.

According to the Tab.1, PVPP, Colle Perle, cazein and bentonite when used separately, have different impact on flavanoids and stilbens (for wine treatment average doses of the substances were used).

When using only PVPP (1), the concentration of trans-resveratrol decreased by 74 %, cis-resveratrol – 46 %, myricetin – 56%, quercetin – 57%. Trans- and cis-resveratrol concentrations did not change when treated by Kol-pearl (3) and casein(4) and myricetin and quercetin decreased by only 10 %. Treatment with bentostab (5) did not change concentration of cis-resveratorl and trans-resveratrol, but myricetin and quercetin concentrations decreased by 13,3 and 18% respectively.

Tab.1 Concentration of antioxidants in wine samples

Compound / Sample N	trans-Resveratrol (mg/l)	cis-Resveratrol (mg/l)	Myricetin (mg/l)	Quercetin (mg/l)
1	1.96	1.11	8.25	11.9
2	0.51	0.6	3.6	5.1
3	1.97	1.2	7.6	10.5
4	1.9	1.07	7.4	10.65
5	1.7	1.13	8.0	9.8
6	0.45	0.46	2.35	3.25
7	0.28	0.37	1.75	2.4
8	0.27	0.36	1.2	1.82
9	0.3	0.38	1.45	2.2
10	0.3	0.37	1.5	1.8
11	0.3	0.35	1.65	2.0
12	0.31	0.38	1.6	2.35
13	0.3	0.38	1.95	2.8
14	0.25	0.4	1.7	2.3

According to the results, PVPP has the strongest influence on the concentrations of flavanoids and stilbens in wine. Its negative impact was also noticeable when wine was treated using combination of different treatment chemicals. During complex treatment (6) we used 5 g/dal of PVPP and analysis showed, that the concentrations of trans-resveratrol is reduced by 77%, cis-resveratrol – 58%, and myricetin and quercetin – 71 and 73% respectively. Similar treatment when PVPP dosage was increased to 7g/dal, the concentrations of trans-resveratrol is reduced by 86%, cis-resveratrol – 66%, myricetin and quercetin – 78 and 80% respectively.

The amounts of Trans- and cis-resveratrol do not change when filtered through diatomite and paper filters (8) while concentrations of myricetin and quercetin decreased by 31 and 24% respectively. No changes in concentrations of above mentioned compounds have been observed when wine filtration was conducted using the membrane filter (9).

We also investigated the influence of cold treatment method (at various time periods) on the changes in concentrations of the wine antioxidants. Concentration of Antioxidants in wines (10) and (14) did not practically change when treated at -5^0C for 5 and 10 days.

During combined treatment it was also interesting to determine what is the influence of bentonite dosage on the concentration of antioxidants in (11) and (13). According to the results, when increasing bentonite dosage from 15 to 25 g/dal, the concentrations of trans- and cis- resveratrols are similarly reduced in comparison to the control sample. In myricetin and quercetin there is difference: (13) -76 and 76 % and in (11) – 80 and 83%.

With paper filtration (12) the concentrations of antioxidants do not change.

We also investigated how the wine treatment with various compounds may affect content of antocyans. Among 14 samples we selected those with extensive colour and high percentage of resveratrol and flavanoides and conducted HPLC analysis. The results are shown in Tab.2. We considered antocyans of sample (1) as 100% and calculations in Tab.2 are done in relation with the sample (1).

Tab.2 De – Delphynidine; Cy – Cyanidine; Pt – Petunidine; Po – Peonidine; Mv – Malvidine; Po-ac - Acetylated peonidine; Mv-ac – Acetylated Malvidine; Po-cu - cumarylated Peonidine; Mv-cu - cumarylated Malvidine.

	De	Cy	Pt	Po	Mv	Po-ac	Mv-ac	Po-cu	Mv-cu
2	86.0	76.0	91.3	98.9	94.8	78.7	95.9	56.2	31.1
3	97.3	100	97.6	100	96.1	83.0	96.8	96.2	95.7
4	87.7	71.1	93.7	93.0	93.9	78.7	93.8	90.3	92.3
5	90.3	79.3	90.1	93.3	88.2	78.7	89.4	83.8	83.4
6	68.6	73.6	75.4	83.4	75.8	80.2	66.0	68.6	70.6
7	72.8	71.9	75.4	81.7	76.3	70.2	65.1	63.8	67.7
8	71.6	70.9	74.8	80.9	73.7	72.3	63.0	62.7	63.2
9	64.8	62.0	68.9	78.3	70.1	68.1	58.7	59.5	61.5
10	72.3	66.1	74.1	80.5	76.4	74.4	73.3	63.8	65.8
11	70.3	79.3	72.2	79.1	73.4	70.2	61.3	63.2	64.8
12	71.2	76.0	75.1	84.1	75.5	76.6	64.5	63.8	65.8
13	79.6	92.6	84.4	91.5	84.7	78.7	72.6	69.2	74.9
14	79.5	81.8	84.1	85.7	84.4	70.2	72.7	64.9	65.8

As shown from Tab. 2, treating the wine with Colle Perle does not affect antocyans. Tretment with bentonite and cazein lowers antocyans amount with the same level. PVPP considerably affects antocyans, especally cumulated Peonidine and Malvidine amount. They decrease by 44 and 69 % respectiveley. As for complex treatment, antocyan amount decrease by approximately the same amount – 20-35%.

Antioxidant activity of wines, which means their medical treatment effect were measured by Electron Spin Resonance (ESR) method. In Tab. 3 are shown results for antioxidant activity for wine samples.

Tab. 3. Antioxidant activity of wine samples expressed in % in relation with the absorption signal of pure Fremy's salt.

Sample N	Antioxydant activity (%)
1	86.6
2	74.5
3	83.3
4	84.0
5	81.0
6	73.2
7	71.4
8	70.2
9	71.2
10	66.7
11	71.0
12	70.8
13	71.9
14	66.4

Correlation between the antioxidant activity of wine polyphenolic compounds and their concentration has been evaluated. There is not direct linear correlation between antioxidant activity and content of measured major antioxidants, as well between antioxidant activity and anthocyan content, but some proportional interdependency with both groups simultaneously can be nevertheless observed. Achieved data confirm that wine sample 6 is the best compromise between stability, organoleptic characteristics and quality, content of antioxidants and antioxidant activity of wine.

CONCLUSIONS

Among various wine treatment chemicals the PVPP has the most negative impact on preservation of antioxidants in wines and usually it reduces them by 46-74%. Although treatment with PVPP improves wine stability against polyphenolic and phenolic cloudiness and softens organoleptic parameters of young wines, PVPP's negative influlence on antioxidants must be taken into account during wine treatment.

It has been stated that filtrations do not affect antioxidants significantly. Cold treatment of wine insignificantly lowers the amount of healthy components.

Casein and Colle perle practically do not affect the concentrations of trans- and cis-resveratrols. Only myricetin and quercetine concentrations are reduced by 10%.

When wine is treated with bentostab, amount of trans-resveratrol concentration is reduced by 13%, cis-resveratrol do not change and myricetin and quercetin concentrations are lowered by 3-18%.

On the basis of research presented in this work the sample (6) was identified as the best quality wine in terms of maintaining health beneficial components, stability and best organoleptic parameters. However, conducted studies also showed that it is possible to maintain more antioxodants in wines by selecting proper treatment method.

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BIBLIOGRAPHY

[1] Sava I.G., Bataglia V., Rossi C.A., Salvi M. and Toninelo A., 2006. "Free radical scavenging action of the natural polyamine spermine in rat liver mitochondria". *Free Radical Biology and Medicine*. 41 : 1187.

[2] Shakulashvili N., Chikvaidze E., 2008."Free Radicals, Antioxidants, Rezveratrol, Vine, and Georgian Vine". *Modern Medicne*. 7 : 27-30.

[3] Freeman B.A., Crapo I.D. 1982. "Biology of Dseases. Free radicals and tissue injury". *Lab.Invest*. 47 : 412-426.

[4] Katzitadze A., Kiladze N., Kiladze S., Chikvaidze E., Lebanidze A., Kirikashvili I. 2004. "Photosensitization and skin photoaging." Experimental Clinic Medicine Int. Conference "Dermatocosmetology-21 Century. Tbilisi. April 20-23.

[5] Maxwell S.R., Lip G.Y. "Free radicals and antioxidants in cardiovascular disease". 1997. *Br.J.Clin. Pharmacol*. 44 : 307-317.

[6] Goldberg D.M. 1995. "Does wine work" *Clin Chem*. 41 :14-16.

[7] Hertog M.G. et al 1993. "Dietary antioxidant flavonoids and risk of coronary heart disaese: the Zetphen Elderly study" *Lancet.* 342 : 1007-1011.

[8] Sato M., Ramarathnam N., Suzuki Y., Ohkubo T., Takeuchi M., Ochi H. 1996. "Varietal differences in the phenolic content and superoxide radical scavenging potential of wines from different sources" *J.Agric. Food Chem*. 48 : 220.

[9] Pace-Asciak C.R., *et al.* 1995. "The red Wine phenolics trans-rezveratrol and quercetin block human platelet aggregation and eicosanoid synthesis: Implications for protection against coronary heart disease" *Clin Chim Acta.* 235 : 207-19.

[10] Baur JA. Sinclair DA. 2006. "Therapeutic potential of resveratrol: the in vivo evidence". *Nat Rev Drug Discov.* 5(6) : 493-506.

[11] Gu X., Creasy L., Kester A., Zeece M., 1999. "Capillary electrophoretic determination of resveratrol in wines". *J Agric Food Chem*. 47 (8): 3223-3227.

[12] Renaud S., 1992. "Wine, Alcohol, Platelets and French Paradox for coronary Heart Disiase." *The Lancet*. 339 : 1523-1526

[13] Miodini P., Fioravanti L., di Fronzo G., Capelletti V. 1999. The two phyto-oestrogenes genistein and quercetin exert different effects on oestrogen receptor function. *Br J Cancer.* 80 : 1150-5.

[14] Nothlings U., Murphy SP., Wilkens LR., Henderson BE., Kolonel LN., 2007. "Flavonols and pancreatic cancer risk: the multiethnic cohort study". *Am.J.Epidemiol*. 166 (8) : 924-31.

[15] Arichi H. et al. 1982. "Effects of stylben components of the roots Polygonum cuspidatum… on lipid metabolism" *Chem Pharm Bul.l* 30 : 1766-70.

[16] Beato M., Truss M & Chavez 1996 Control of transcription by steroid hormones. *Annals of the New York Academy of Sciences.* 784 : 93-123.

Evolution de la teneur en amines biognene durant conservation des échantillons-.optimisation de méthodes pour l'analyse des amines biogènes.

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RESUME

Le document suivant présente le développement d'une nouvelle méthode d'analyse simultanée de 8 amines biogènes (histamine, la méthylamine, l'éthylamine, la tyramine, la putrescine, la cadavérine, phenethylamine, et isoamylamine). Elle est basée sur une méthode développée par Gomez-Alonso et al. (2007). La méthode analytique proposée présente les avantages suivants: dérivation facile de vins, quantification des amines biogènes, et dégradation complète du réactif de dérivation en excès pendant la préparation des échantillons afin de préserver la colonne. Elle se compose d'une séparation par CLHP en phase inverse et une détection en UV-vis des aminoenones formé par la réaction de composés aminés avec le réactif de dérivation diethyl ethoxymethylenemalonate (DEEMM). La technique a été validée, avec une méthode alternative d'analyse œnologique pour la validation, le contrôle qualité et l'évaluation des incertitudes (OIV Oeno 10/2005). Comme application de la méthode proposée, la teneur en amines biogènes des vins de la vallée du Rhône a été étudiée. En outre, selon les résultats qui ont découvert une variation dans la concentration des amines biogènes pendant le temps de stockage, un même échantillon a été analysé au moment de la préparation, après 1, 2 et 4 jours et après 1, 2 et 4 semaines. Différentes températures ont été étudiées. Pour déterminer la stabilité des dérivés, la même expérience a été réalisée avec et sans dérivation. Comme on peut le constater, les composés produits par la réaction de dérivation ont été parfaitement stables, alors que le vin conservé a montré au fil du temps une variation dans la concentration des amines, notamment de l'histamine. Les résultats indiquent que la durée de stockage est un facteur important qui détermine le contenu en amines biogènes des vins dosés.

The present paper reports the development of an optimized method for simultaneous analysis of 8 biogenic amines (histamine, methylamine, ethylamine, tyramine, putrescine, cadaverine, phenethylamine, and isoamylamine). It is based on a method developed by (Gomez-Alonso, et al., 2007). The proposed analytical method has the following advantages: easy derivatization of wines, quantification of biogenic amines, and complete degradation of excess derivatization reagent during sample preparation to preserve column. It consists of reversed phase separation by HPLC and UV-vis detection of the aminoenones formed by the reaction of amino compounds with the derivatization reagent diethyl ethoxymethylenemalonate (DEEMM). The technique was confirmed with an alternative oenological analysis method for the validation, quality control and uncertainty assessment (OIV Oeno 10/2005). As a specific application of the proposed method, the biogenic amine content of Rhône valley wines was investigated. Moreover, according to results which found

a variation in the concentration of biogenic amine during storage time, a same sample was analyzed at the time of preparation, after 1, 2, and 4 days and after 1, 2, and 4 weeks. Different storage temperatures were studied. To determine the stability of the derivatives, the same experiment was done with and without derivatization. As can be seen, the compounds produced by the derivatization reaction were perfectly stable, instead of wine conserved which showed a variation in the concentration of amines, particularly histamine. The results indicated that storage time was an important factor affecting biogenic amine content.

INTRODUCTION

Le mot biogène signifie « engendré par la vie ». L'appellation « amines biogènes » est donc donnée pour toutes les amines provenant du métabolisme de cellules vivantes, animales, végétales ou microbiennes. Les amines biogènes du vin sont majoritairement d'origine microbienne (Lonvaud-Funel, 2001; Moreno-Arribas, et al., 2003). Les principales sont l'histamine, la putrescine, la cadavérine, la tyramine. Ces composés du vin susceptibles d'avoir une incidence sur la santé humaine sont de plus en plus étudiés (Ancin-Azpilicueta, et al., 2008; Marques, et al., 2008; Martin-Alvarez, et al., 2006).Parmi celles-ci, un groupe de composés biologiquement actifs sur le système nerveux central et sur le système vasculaire de l'homme est pointé du doigt. Ces molécules étant naturellement présentes dans le vin (principalement formées lors de la fermentation malolactique par décarboxylation des acides aminés précurseurs), une réglementation sur la teneur maximale est actuellement à l'étude. De plus, les amines biogènes, outre leur effet sur la santé humaine, jouent également un rôle de masque d'arômes, dépréciant la qualité organoleptique des vins.
Dans ce contexte, il devient donc indispensable de développer une méthode d'analyse fiable et rapide permettant de quantifier la présence de ces composés dans les vins.

RESULTATS ET DISCUSSION

Les amines biogènes étudiées ici sont des amines primaires, secondaires ou tertiaires, aliphatiques ou aromatiques. Or, seules les amines aromatiques absorbent en UV. En effet, la détection des molécules par UV nécessite la présence d'un chromophore dans la molécule, généralement une séquence de doubles liaisons conjuguées. De nombreuses méthodes d'analyse sont actuellement disponibles (Önal, 2007).
Dans notre cas, nous nous sommes penchés sur une méthode utilisant la technologie HPLC/DAD couplé au un chromophore diéthyl ethoxymethylenemalonate (DEEMM) (fig 1). Ce dernier par une étape d'alkylation, aussi appelée dérivation, nous permet d'obtenir des amines biogènes visibles par le détecteur à barrette de diodes suivant un protocole modifié issue d'une méthode développée par Gomez-Alonso et al. (2007) (fig 2).

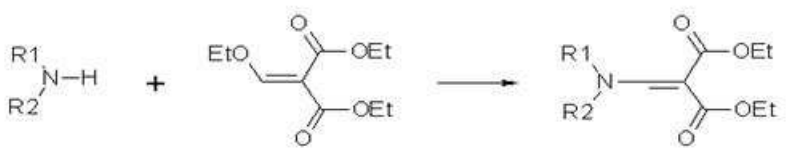
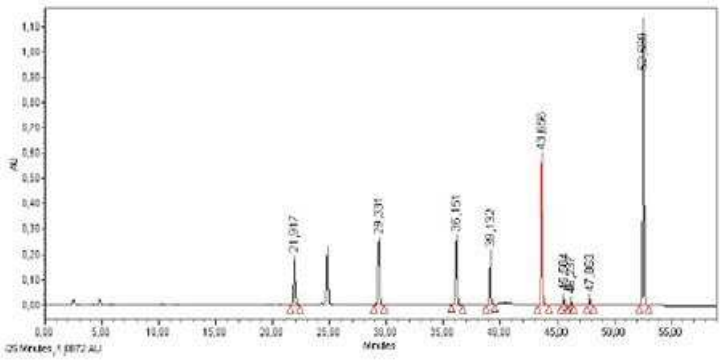


Fig 1 : réaction de dérivatisation

Le rendement da la dérivation est calculé grâce à l'ajout d'un étalon interne (2, 4, 6 – TrimethylPhenethylAmine hydrochloride ou 2, 4, 6 TPA). La quantification de chacune des amines biogènes est réalisée contre une gamme étalon (0-0,5-1-5-10-20 mg/L).



Amines Biogènes	Temps de rétention (min)
Histamine	21,92
Méthylamine	29,33
Ethylamine	36,15
Tyramine	39,13
Putrescine	44,66
Cadavérine	45,58
Phénéthylamine	46,23
Isoamylamine	47,86

Fig 2 : chromatogramme obtenu par le protocole modifié d'une méthode développée par Gomez-Alonso et al. (2007)

La technique a été validée, avec une méthode alternative d'analyse œnologique pour la validation, le contrôle qualité et l'évaluation des incertitudes (OIV Oeno 10/2005). Comme application de la méthode proposée, la teneur en amines biogènes des vins de la vallée du Rhône a été étudiée sur 84 vins issus de 3 millésimes (2005-2006-2007) (Tab 1).

mg/L	Moyenne	Ecart type	Max	Min
Histamine	5,04	3,07	14,05	<0.5
Methylamine	9,18	7,33	36,64	0,37
Ethylamine	3,84	1,63	10,46	1,66
Tyramine	4,70	2,98	12,35	<0.5
Putrescine	13,27	8,93	48,72	3,71
Cadaverine	0,40	0,25	1,82	0,14
Phenethylamine	0,56	0,51	2,67	<0.5
Isoamylamine	0,28	0,24	1,23	<0.5

Tab 1 : état des lieux du contenu en amines biogènes des vins de la vallée du Rhône (N=84)

Comme déjà décrit, l'amine biogène retrouvée majoritairement se trouve être la putrescine (Jimenez Moreno, et al., 2003). En ce qui concerne l'histamine, seule amine amenant actuellement à une réglementation, on se retrouve avec des quantités moyennes de 5 mg/L. tout les amines biogènes se retrouvent donc en quantités assez faibles mais il est à remarquer l'existence de vins comprenant des quantités inhabituelles. Aucun effet millésime n'a pu être mis en évidence.

D'après la littérature sur les amines biogènes, la conservation des échantillons ne semble pas poser de problème. Au cours des différentes étapes de validation de la méthode, nous avons néanmoins remarqué des variations, notamment une diminution des concentrations sur des échantillons issus d'un même vin analysés à plusieurs jours d'intervalles. Partant de ce constat, il a donc été décidé d'étudier la conservation des échantillons avant le processus de dérivation mais aussi après ce processus (Fig 1). Afin de vérifier que le résultat ne dépend pas d'une matrice particulière, six vins, contenant des teneurs en amines biogènes variables ont été sélectionnés.

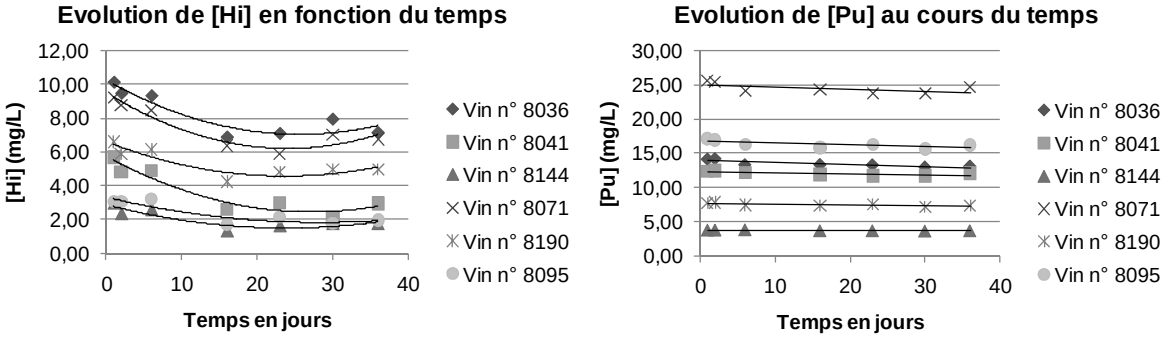


Fig 3 : Evolution au cours du temps des teneurs en Histamine et en Putrescine des échantillons avant dérivation.

Lorsqu'on observe les résultats, on s'aperçoit rapidement que la concentration en histamine dans les différents échantillons diminue de façon substantielle au cours du temps. En revanche, en ce qui concerne les sept autres amines biogènes, leur concentration reste sensiblement la même au cours du temps. Différentes températures de stockage de l'échantillon (+20°C, +4°C, -20°C) ont été étudiées : aucun impact sur cette dégradation n'a pu être établi. D'après ces observations, il apparait qu'il n'existe donc pas de moyen efficace pour conserver les échantillons en attente d'analyse.
Il est à remarquer que l'instabilité dans le temps de l'histamine est un phénomène déjà observé lors des étapes d'élevage et de vieillissement en bouteilles (Jimenez Moreno, et al., 2003). Les mécanismes impliqués dans cette diminution sont actuellement inconnus.

Il a donc semblé intéressant de connaître l'évolution des teneurs en amines biogènes dans les échantillons dérivés afin de savoir si cette méthode pourrait constituer une alternative avantageuse (fig 3). Les résultats ont permis de montrer que la dérivation permettait de stabiliser les échantillons.

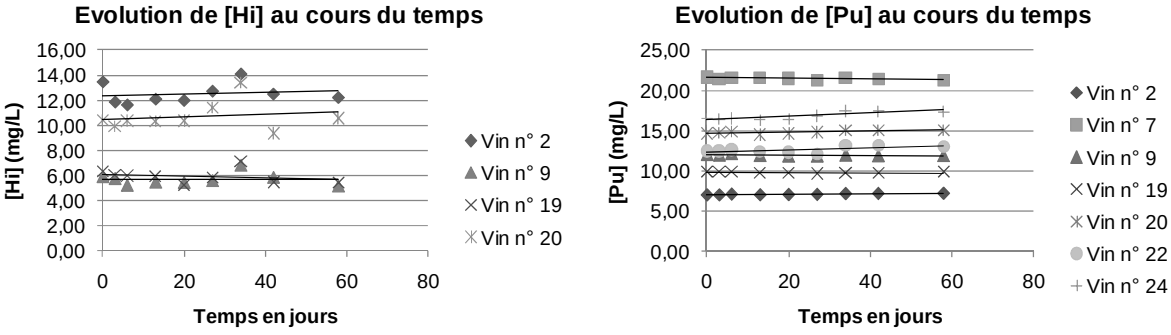


Fig 4 : Evolution au cours du temps des teneurs en Histamine et en Putrescine des échantillons après dérivation

Au final, la méthode d'analyse des amines biogènes par HPLC-DAD après dérivation au DEEMM a été choisie par noter laboratoire par rapport aux méthodes existantes (dérivation pré ou post colonne à l'OPA) pour les raisons suivantes :

- Limitation des coûts de consommables : la colonne peut permettre l'analyse de plusieurs centaines d'échantillons, les réactifs de dérivation sont très stables et ne colmatent pas la colonne. Il n'est donc pas nécessaire de grouper l'analyse des échantillons, et ils peuvent par conséquent être analysés au fil de l'eau afin de diminuer les délais d'analyse
- Nous avons montré que les teneurs en histamine dans un vin peuvent diminuer de 30 % dans le premier mois après réception des échantillons (stockage de l'échantillon à +20°C, +4°C, -20°C). Afin de parer à ce problème, nous avons étudié la conservation des échantillons après dérivation. Les résultats montrent qu'après 2 mois de stockage, la variation en histamine est inférieure à 10% et stable pour les autres amines biogènes d'intérêt ;
- Cette méthode permet l'analyse des précurseurs des amines biogènes (acides aminés) (Gomez-Alonso, et al., 2007) ;
- Les amines biogènes dérivées au DEEMM sont détectées par un détecteur à barrette de diodes à 280 nm. Ce matériel est généralement plus répandu dans les laboratoires qu'un fluorimètre, rendant la diffusion de la méthode plus aisée.

REMERCIEMENTS

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REFERENCES

Ancin-Azpilicueta, C., Gonzalez-Marco, A. and Jimenez-Moreno, N. (2008). Current knowledge about the presence of amines in wine. *Crit Rev Food Sci Nutr* **48,** 257-75.

Gomez-Alonso, S., Hermosin-Gutierrez, I. and Garcia-Romero, E. (2007). Simultaneous HPLC analysis of biogenic amines, amino acids, and ammonium ion as aminoenone derivatives in wine and beer samples. *J Agric Food Chem* **55,** 608-13.

Jimenez Moreno, N., Torrea Goni, D. and Ancin Azpilicueta, C. (2003). Changes in amine concentrations during aging of red wine in oak barrels. *J Agric Food Chem* **51,** 5732-7.

Lonvaud-Funel, A. (2001). Biogenic amines in wines: role of lactic acid bacteria. *FEMS Microbiol Lett* **199,** 9-13.

Marques, A. P., Leitão, M. C. and San Romão, M. V. (2008). Biogenic amines in wines: Influence of oenological factors. *Food Chemistry* **107,** 853-860.

Martin-Alvarez, P., Marcobal, A., Polo, C. and Moreno-Arribas, M. (2006). Influence of technological practices on biogenic amine contents in red wines. *European Food Research and Technology* **222,** 420-424.

Moreno-Arribas, M. V., Polo, M. C., Jorganes, F. and Munoz, R. (2003). Screening of biogenic amine production by lactic acid bacteria isolated from grape must and wine. *Int J Food Microbiol* **84,** 117-23.

Önal, A. (2007). A review: Current analytical methods for the determination of biogenic amines in foods. *Food Chemistry* **103,** 1475-1486.

Effect of nitrogen addition during alcoholic fermentation on the final content of biogenic amines in wine

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ABSTRACT

An aspiration of the wine industry is to control critical technological factors to produce wines with low levels of biogenic amines. Among these factors, amino acids and ammonium ions are essential growth factors for growth of yeasts and lactic acid bacteria during alcoholic and malolactic fermentations, but also they are potential biogenic amines precursors. Nitrogen is often a limiting nutrient for Saccharomyces cerevisiae during batch alcoholic fermentation and must be sometimes corrected. But this action can be contradictory with the aim of controlling biogenic amine content. To rationalized nitrogen addition, fermentation experiments at the pilot scale (100 L) were conducted with grapes (Syrah and Grenache) coming from the Rhône Valley by varying the concentration and type of nitrogen added (mineral nitrogen, yeast hulls, inactivated yeast). The purpose of this work was to assess the effect of nitrogen addition on amino acid and final biogenic amine concentration during wine-making conditions.

Une des volontés de la filière vinicole est de produire des vins de faibles teneurs en amines biogènes en contrôlant les facteurs techniques critiques. Parmi ces facteurs, les acides aminés et les ions ammoniums sont des facteurs de croissance essentiels pour la croissance des levures et des bactéries lactiques au cours de la fermentation alcoolique et malolactique, mais également des précurseurs potentiels d'amines biogènes. L'azote est souvent un élément nutritif limitant pour Saccharomyces cerevisiae au cours de la fermentation alcoolique et doit donc parfois être corrigé. Mais cette intervention peut être en contradiction avec l'objectif de contrôler la teneur en amines biogènes. Pour rationaliser cet ajout d'azote, des fermentations à l'échelle pilote (100 L) ont été menées en utilisant en Vallée du Rhône (Syrah et Grenache) en faisant varier la concentration et le type d'azote ajouté (azote minéral, écorce de levure, levures inactivées). Le but de ce travail a été d'évaluer l'effet de l'azote sur les acides aminés et la teneur finale en amines biogènes.

INTRODUCTION

The occurrence of biogenic amines in fermented foods such as cheese, sausages, beer and wine, is mainly due to the decarboxylation of certain amino acids by the action of micro-organisms. Histamine, tyramine, putrescine, cadaverine and phenylethylamine are biogenic amines most frequently found in wines from respective decarboxylases activities of lactic acid bacteria during malolactic fermentation (Önal, 2007). The content of biogenic amines in wine is a serious subject of a toxicological point of view, because they can cause adverse physiological reactions in susceptible individuals, such as headache, nausea, hypotension reactions or hypertension, heart palpitations, and anaphylactic shock (Bodmer, et al., 1999; Jansen, et al., 2003). Thus, high levels of biogenic amines in the future could be an obstacle to the marketing of certain wines.

In this way, one of the wishes of the wine industry is to produce wines with low levels of biogenic amines in controlling the critical technical factors. The production of biogenic amines in wine depends not only on the presence of bacteria potentially producing biogenic amines, but also other parameters such as the presence of amino acid precursors, pH, or duration of malolactic fermentation (Martin-Alvarez, et al., 2006). Thus, among these factors, amino acids and ammonium ions are essential growth factors for multiplication of yeast and lactic acid bacteria during alcoholic fermentation and malolactic fermentation. Nitrogen is often a limiting nutrient in *Saccharomyces cerevisiae* during alcoholic fermentation. Nitrogen, and notably Yeast Available Nitrogen, is essential for a successful fermentation, insufficient nitrogen levels in musts lead to sluggish alcoholic fermentation even to stop fermentation. Various studies have shown that a minimum of 120-140 mg YAN /L was required to yield optimum fermentation kinetics (Sablayrolles, et al., 1996). It is recommended to supplement these deficient musts in nitrogen to ensure a good fermentation (Bely, et al., 1990). The most common intervention applied is the addition of ammonium to increase nitrogen content in the legal limit of 1000 mg/l in Europe and 950 mg/l in USA (Taillandier, et al., 2007) of diammonium phosphate (DAP) or sulphate (21% of nitrogen). Nitrogen amount is important, but the quality of the nitrogen source is equally important. Every day research is carried out into new additives and it is therefore of interest to know how the addition of nitrogen sources affects amino acid uptake, especially of those related to the production of higher alcohols and their corresponding esters, and how the addition affects the synthesis of these aroma compounds (Hernandez-Orte, et al., 2006).
But these interventions can be in contradiction with the objective of controlling the content of biogenic amines, the addition of nitrogen corresponding to an addition of potential precursors of biogenic amines. To streamline the addition of nitrogen, fermentation at pilot scale (100 L) were conducted using grapes of the Rhone Valley (Syrah and Grenache). The levels of amino acids and biogenic amines have been monitored as well as bacterial populations. The purpose of this study was to evaluate the effect of nitrogen and amino acids on biogenic amines content in the final wine.

RESULTS AND DISCUSSION

All fermentations were performed without incident, whatever the variety. Despite severe nitrogen starvation, no stopped fermentations were observed. As expected, fermentation times were reduced in relation to nitrogen intake (Figure 1).

No feature related to the grape has been observed. It is noteworthy that the shortest fermentations (10 days) were obtained with a dose of 300 mg YAN/L. Fermentation kinetics remained stable for higher additions. It should be noted that the impact of adding complex form of nitrogen (inactivated dry yeast) was obtained for a dose of 400 mg/L which corresponded to quantity usually used in enological practices. That can be explained by the fact that they may include a soluble fraction mostly formed by yeast cytoplasm metabolites (proteins, peptides, amino acids, polysaccharides: glucanes, mannoproteins, sterols and fatty acids) and an insoluble fraction of inactive inert support, mainly cellulose in their composition. In addition, other compounds, such as vitamins, and minerals are often included in most inactivated yeast preparations (Pozo-Bayón, et al., 2009).

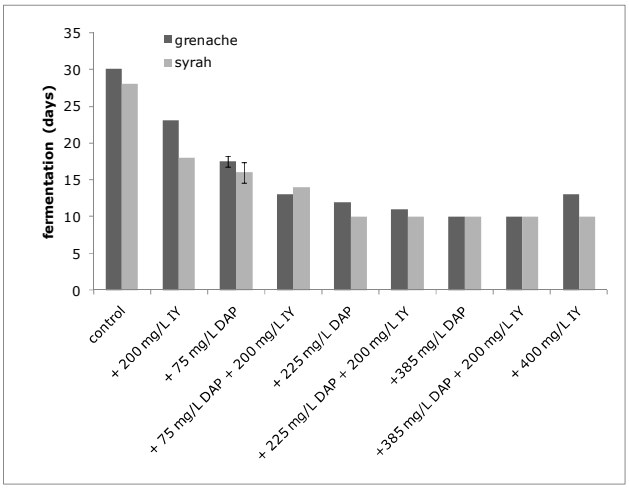

Figure 1: duration of alcoholic fermentation observed depending on grape (Syrah □/ Grenache ■) and added nitrogen (Diammonium Phosphate and / or inactivated yeast –IY).
Control (natural levels of nitrogen in must) = 75 mg/L. N=2 independent experiments.

It has been well observed that the higher the fermentation was slow to finish (in a nitrogen deficiency), most bacterial population at the end of alcoholic fermentation is important (Figure 2).

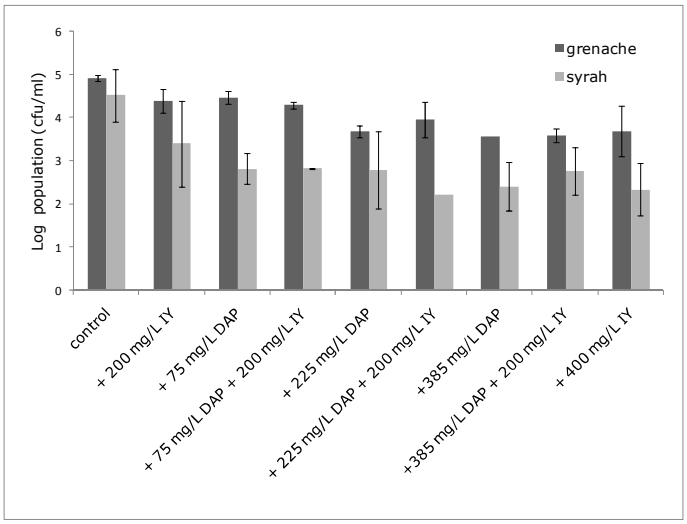

Figure 2: Bacteria population at alcoholic fermentation end depending on grape
(Syrah □/ Grenache ■) (N=2 independent experiments).

For both cultivars, when nitrogen additions increased, the level of lactic bacteria at the alcoholic fermentation populations decreased. Whatever, different behaviors could be observed according to the variety: lactic acid bacteria population in Grenache was more important (about 1 log) as compared in Syrah. Thus, bacterial populations tended to grow more easily on the Grenache musts. The lactic acid bacteria populations were monitored during fermentation (Figure 3).

During the alcoholic fermentation, bacteria population increased, and especially during the last stage, when the yeast activity slowed down, as expected.

At the end of alcoholic fermentation, biogenic amines (histamine, tyramine, putrescine, cadaverine and phenylethylamine) were not detected (< 0,5 mg/L) indicating that biogenic amines producing lactic acid bacteria did not impact the level of biogenic amine during alcoholic fermentation in spite of their development.

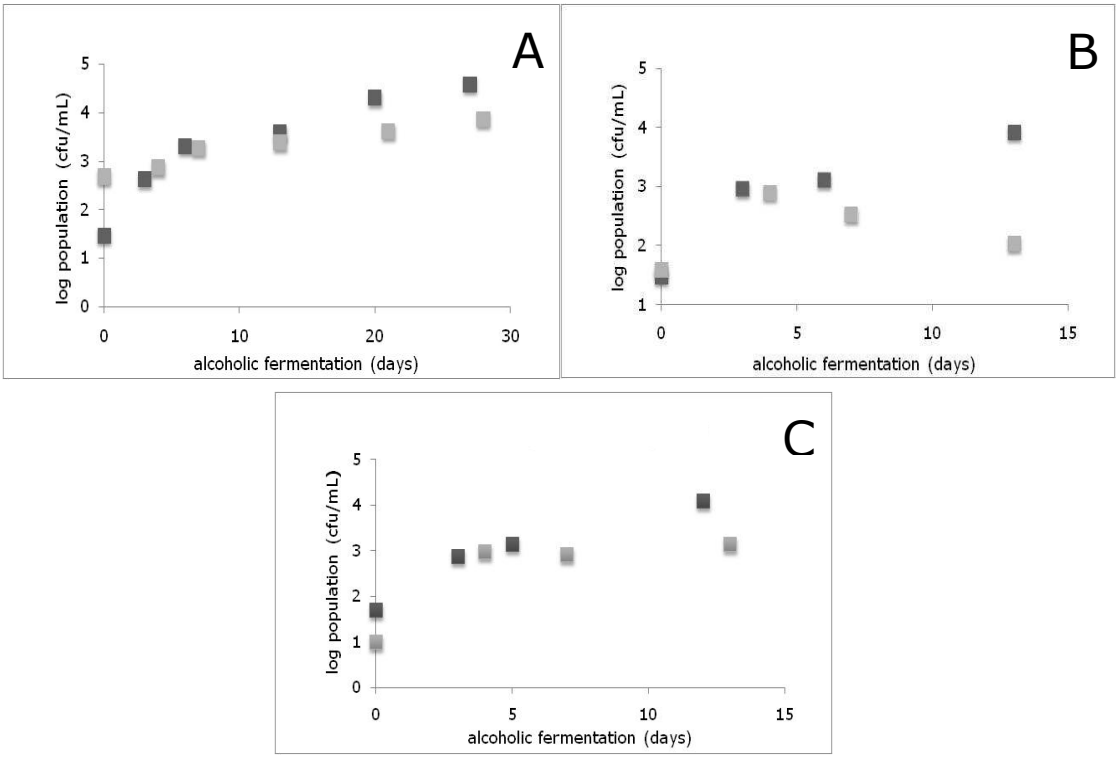

Figure 3: Population kinetics of lactic acid bacteria during the alcoholic fermentation (days) depending on grape (Syrah □/ Grenache ■) and adding nitrogen (A-control 75 mg/L, B- +225 mg/L of DAP and C- +400 mg/L with inactivated yeast IY).

After a stint at 10 °C in order to synchronize the fermentation, wines were transferred into 5 L flask and placed at 18 °C in order to achieve the malolactic fermentation. The remaining wine was stored at -4 °C for further experiments. The malolactic fermentation took place over a period of 15-20 days irrespective of modality (data not shown). Malolactic fermentations completed rather quickly, suggesting that growth inhibitors of lactic acid bacteria were not produced. Production of such inhibitors were however reported previously during sluggish fermentations (Alexandre, et al., 2004). It seems that there has not been present in the case of formation of growth inhibitors of lactic acid bacteria, inhibitors formed by yeasts during sluggish fermentation. The character of HDC+ lactic acid bacteria performed by quantitative PCR after malolactic fermentation was completed. At the end of malolactic fermentation, 90 to 95% of bacteria were potentially producing histamine. However, strains have been not identified: it may therefore be either inoculated strain or strains present in the native must.

A determination of biogenic amines content was achieved once degradation of malic acid was completed (Figure 4). No amount of tyramine, putrescine, cadaverine and phenylethylamine could be detected (< 0,5 mg/L). However, the histamine content over malolactic fermentation dramatically increased to reach close from 10 mg/L in some modalities. Moreover, significant differences were observed between modalities. It has been

clearly shown that the addition of nitrogen led to an increase of histamine in wines. Similarly, the type of addition seems to be important on the behavior of fermentations. For instance, the addition of 400 mg/L of inactivated yeast increased the level of histamine and shortened the fermentation period. It seems that the addition of complex forms of nitrogen that is to say, amino acids, leads to add more precursors.

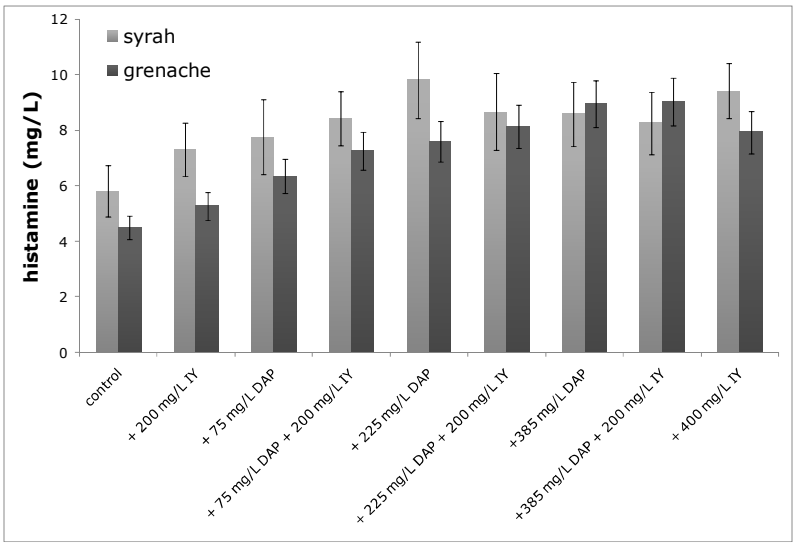

Figure 4: histamine (mg / L) depending on grape (Syrah □/ Grenache ■)
and adding nitrogen (DAP and / or inactivated yeast IY). N=2 independent experiments.

We have been able to correlate the amount of nitrogen added to the amount of histamine produced. It is interesting to relate these data to quantities of precursors present at the end of fermentation. In our case, we determined the amounts of histidine, precursor of histamine. However, no significant correlation could be demonstrated. The levels of histidine did not appear increased with the addition of nitrogen. It is nevertheless noteworthy that the assay of amino acids that we used only allows the determination of free amino acids. But lactic acid bacteria are known for their ability to assimilate peptides (Remize, et al., 2006; Ritt, et al., 2008). This could be a way of explanation to correlate levels between different compounds (Herbert, et al., 2005; Herbert, et al., 2006).

The addition of nitrogen allows rapid fermentation limiting bacterial growth (Figure 2). However the corollary of this supplement is a precursor to enrichment. Our results demonstrate that these two opposing mechanisms are translated into reality by increasing the final concentration of biogenic amines. If it was just a matter of biogenic amines, should be avoided while nitrogen. However, the nitrogen reduces the duration of fermentation and to avoid arrest. Moreover, lack of nitrogen causes yeast synthesis of higher alcohols, responsible for heavy flavors (Carrau, et al., 2008; Hernández, et al., 2006). Difficulties fermentation can also lead to the formation by the yeast fatty acid in short and medium chains. These compounds are known to inhibit bacterial growth, which may jeopardize the completion of malolactic fermentation (Alexandre, et al., 2004). But excessive preventive supplementation can lead to repression phenomena and in some cases decrease the efficiency of fermentation (Taillandier, et al., 2007). It can also lead to a lower synthesis of higher alcohols and formation of ethyl carbamate too, or microbial instability if the residual concentration of

ammonium is too high with possibility to have precursors for lactic bacteria potentially biogenic amines producer. In fact, addition of nitrogen must be reasonable in order to find a balance between fermentation and smooth the risk of producing biogenic amines.

We have shown a correlation between the amount of nitrogen added and the production of biogenic amines without being provided related to the concentration of amino acid precursors. Thus, by level of nitrogen added, the histamine levels have increased to the value of 10 mg/L limit for the export of wine in Switzerland at the time of writing this article. We can thus understand how an operation that appears benign may jeopardize the marketing of wine produced. It has been already shown that musts supplemented with a yeast autolysate had greater concentrations of some biogenic amines (tyramine and cadaverine)(González and Ancín, 2006).
It is also important to remember that this study was conducted with a limited number of strains. The evidence is that in these cases, only histamine was produced. Because of the existence of tools for characterization of genetic traits associated with the production of other biogenic amines (tyramine, putrescine and cadaverine) (Nannelli, et al., 2008), we now will test new starters on the same base wine from this study that we could keep. Also, one of our aims is to better characterize the pool of free amino acids and related oligopeptide form for each modality. Similarly, this study posing the question of the possible presence of precursors in wine-based products of yeast cell, more commercial products should be considered.
In fact, other yeast derivatives are commercialized to be used in wines to improve fermentation and wine organoleptic characteristics. Their composition is highly variable, but most of them are constituted by inactivated yeast, metabolites from yeast autolysis (such as amino acids, peptides, proteins, polysaccharides, nucleotides and fatty acids), yeast walls, vitamins and minerals. In addition, yeast autolysates composition may vary depending on yeast culture conditions (Guilloux-Benatier and Chassagne, 2003). All of these yeast derivatives products are offered by different companies and under many different names promising very specific improvements in wine. Nevertheless, in spite of the growing interest in these products by the oenological sector, the information related to their composition is often unclear and can impact as we showed on the final biogenic amine content.

In addition, other factors such as pH of wine and the characteristics of the vintage may also play a key role in the biogenesis of the amines. This is what we observed between the two varieties with a particular variation in the kinetics of bacterial. These elements should also be taken into account in particular the management of malolactic starters.

In conclusion, this study shows that some widely used oenological practices can lead to an increased risk of obtaining wines contain significant levels of biogenic amines. Thus, this study shows that the new risk management "biogenic amines" is often at odds with the desire to promote the quality of wine. Indeed, we could show our work, even if it can reduce bacterial populations, the addition of nitrogen can lead to increased production of these compounds. Other recent studies have also shown that many actions to increase the complexity of wine, such as skin maceration and aging on lees, strongly influenced the final concentration of biogenic amines in wines (Alcaide-Hidalgo, et al., 2007). Cannot reasonably overcome completely these qualitative techniques, make a diagnosis of bacterial populations indigenous proved decisive. In case of presence of bacteria producing the only solution currently available to limit the level of biogenic amines in wine is the use of malolactic

starters. However, the result depends on the level of indigenous bacterial population and characteristics of wine that can cause a bad location.

ACKNOWLEDGMENTS

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REFERENCES

Alcaide-Hidalgo, J. M., Moreno-Arribas, M. V., Martín-Álvarez, P. J. and Polo, M. C. (2007). Influence of malolactic fermentation, postfermentative treatments and ageing with lees on nitrogen compounds of red wines. Food Chemistry 103, 572-581.

Alexandre, H., Costello, P. J., Remize, F., Guzzo, J. and Guilloux-Benatier, M. (2004). Saccharomyces cerevisiae-Oenococcus oeni interactions in wine: current knowledge and perspectives. Int J Food Microbiol 93, 141-54.

Bely, M., Sablayrolles, J.-M. and Barre, P. (1990). Automatic detection of assimilable nitrogen deficiencies during alcoholic fermentation in oenological conditions. Journal of Fermentation and Bioengineering 70, 246-252.

Bodmer, S., Imark, C. and Kneubuhl, M. (1999). Biogenic amines in foods: histamine and food processing. Inflamm Res 48, 296-300.

Carrau, F. M., Medina, K., Farina, L., Boido, E., Henschke, P. A. and Dellacassa, E. (2008). Production of fermentation aroma compounds by Saccharomyces cerevisiae wine yeasts: effects of yeast assimilable nitrogen on two model strains. FEMS Yeast Res.

Gomez-Alonso, S., Hermosin-Gutierrez, I. and Garcia-Romero, E. (2007). Simultaneous HPLC analysis of biogenic amines, amino acids, and ammonium ion as aminoenone derivatives in wine and beer samples. J Agric Food Chem 55, 608-13.

González, M. and Ancín, A. (2006). Amine concentrations in wine stored in bottles at different temperatures. Food Chemistry 99, 680-685.

Guilloux-Benatier, M. and Chassagne, D. (2003). Comparison of components released by fermented or active dried yeasts after aging on lees in a model wine. J Agric Food Chem 51, 746-51.

Herbert, P., Cabrita, M. J., Ratola, N., Laureano, O. and Alves, A. (2005). Free amino acids and biogenic amines in wines and musts from the Alentejo region. Evolution of amines during alcoholic fermentation and relationship with variety, sub-region and vintage. Journal of Food Engineering 66, 315-322.

Herbert, P., Cabrita, M. J., Ratola, N., Laureano, O. and Alves, A. (2006). Relationship between biogenic amines and free amino Acid contents of wines and musts from Alentejo (Portugal). J Environ Sci Health B 41, 1171-86.

Hernandez-Orte, P., Bely, M., Cacho, J. and Ferreira, V. (2006). Impact of ammonium additions on volatile acidity, ethanol, and aromatic compound production by different Saccharomyces cerevisiae strains during fermentation in controlled synthetic media. Australian Journal of Grape and Wine Research 12, 150-160.

Hernández, T., Estrella, I., Carlavilla, D., Martín-Álvarez, P. J. and Moreno-Arribas, M. V. (2006). Phenolic compounds in red wine subjected to industrial malolactic fermentation and ageing on lees. Analytica Chimica Acta 563, 116-125.

Jansen, S. C., van Dusseldorp, M., Bottema, K. C. and Dubois, A. E. (2003). Intolerance to dietary biogenic amines: a review. Ann Allergy Asthma Immunol 91, 233-40; quiz 241-2, 296.
Lucas, P. M., Claisse, O. and Lonvaud-Funel, A. (2008). High frequency of histamine-producing bacteria in the enological environment and instability of the histidine decarboxylase production phenotype. Appl Environ Microbiol 74, 811-7.
Martin-Alvarez, P., Marcobal, A., Polo, C. and Moreno-Arribas, M. (2006). Influence of technological practices on biogenic amine contents in red wines. European Food Research and Technology 222, 420-424.
Nannelli, F., Claisse, O., Gindreau, E., de Revel, G., Lonvaud-Funel, A. and Lucas, P. M. (2008). Determination of lactic acid bacteria producing biogenic amines in wine by quantitative PCR methods. Lett Appl Microbiol 47, 594-9.
Önal, A. (2007). A review: Current analytical methods for the determination of biogenic amines in foods. Food Chemistry 103, 1475-1486.
Pozo-Bayón, Á., M., Andújar-Ortiz, I. and Moreno-Arribas, M. V. (2009). Scientific evidences beyond the application of inactive dry yeast preparations in winemaking. Food Research International 42, 754-761.
Remize, F., Gaudin, A., Kong, Y., Guzzo, J., Alexandre, H., Krieger, S. and Guilloux-Benatier, M. (2006). Oenococcus oeni preference for peptides: qualitative and quantitative analysis of nitrogen assimilation. Arch Microbiol 185, 459-69.
Ritt, J. F., Guilloux-Benatier, M., Guzzo, J., Alexandre, H. and Remize, F. (2008). Oligopeptide assimilation and transport by Oenococcus oeni. J Appl Microbiol 104, 573-80.
Sablayrolles, J.-M., Dubois, C., Manginot, C., Roustan, J.-L. and Barre, P. (1996). Effectiveness of combined ammoniacal nitrogen and oxygen additions for completion of sluggish and stuck wine fermentations. Journal of Fermentation and Bioengineering 82, 377-381.
Taillandier, P., Ramon, F., Fuster, A. and Strehaiano, P. (2007). Effect of ammonium concentration on alcoholic fermentation kinetics by wine yeasts for high sugar content. Food Microbiology 24, 95-100.

COMPOSES CLEFS DE L'AROME DES VINS ROSES DE PROVENCE

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SUMMARY
Important exploratory work has been conducted on the aromas of Provence rosé wines at the Centre for Research and Experimentation on Rosé Wine (Centre de Recherche et d'Expérimentation sur le Vin Rosé). It required implementation of precise analyses and sensorial analyses specific to rosé wine. This study demonstrated the olfactory contribution of various volatile compounds originating both from fermentation and grape variety.
An original experiment in dearomatisation and reconstitution of odour demonstrated the predominance of ethyl esters and high acetate alcohols, producing the fruit and amylic notes to be found in rosé wines. In addition, the search for certain varietal compounds well known in other types of wines led to the identification of two volatile thiols (3-mercapto-hexanol and 3-mercapto-hexyl acetate), two furanic compounds (furaneol and homofuraneol) and a C13 nor-isoprenoid (ß-damascenone) as constituting key compounds in rosé wine aromas. They improve our understanding of the fruit component without however completely justifying the aromatic complexity of the rosé wines under examination.

These compounds are now measured in most of the experiments conducted at the Centre du Rosé and are used as quality indicators in protocols to evaluate vine management methods and rosé wine-making techniques. They make it possible to quantify the effect that racking or the fermentation temperature has on amylic compounds, the impact of the harvesting date on furanic compounds or the influence of "reductionist" wine-making techniques (inertage, maceration of must deposits, etc.) on production of volatile thiols.

RÉSUMÉ
Un important travail exploratoire sur les arômes des vins Rosés de Provence a été conduit au Centre de Recherche et d'Expérimentation sur le Vin Rosé. Il a nécessité la mise en œuvre d'analyses fines et d'analyses sensorielles spécifiques au vin Rosé. Cette étude a permis de démontrer la contribution olfactive de différents composés volatils à la fois d'origine fermentaire et variétale.
Une expérience originale de désaromatisation et reconstitution d'odeur a montré le caractère prépondérant des esters éthyliques et acétates d'alcools supérieurs, à l'origine des notes fruitées et amyliques des vins Rosés. Par ailleurs, la recherche de certains composés variétaux, bien connus dans d'autres types de vins, a permis d'identifier comme composés clés de l'arôme des vins Rosés deux thiols volatils (3-mercapto-hexanol et acétate de 3-mercapto-hexyle), deux composés furaniques (furanéol et de l'homofuranéol) et un C13 norisoprénoïde (béta-damascénone). Ils donnent des éléments de compréhension de la composante fruitée sans toutefois pouvoir justifier la complexité aromatique des vins Rosés étudiés.

Ces composés sont pris en compte dans les protocoles expérimentaux et permettent, par exemple, de quantifier l'effet du débourbage ou de la température de fermentation sur les composés amyliques, l'impact de la date de récolte sur les composés furaniques ou l'incidence de techniques de vinification « réductrices » (inertage, macération de bourbes…) sur la production de thiols volatils.

INTRODUCTION

La particularité des vins Rosés est d'être centré sur le fruit. Bien sûr, les vins blancs et les vins rouges présentent très souvent des notes fruitées et par ailleurs, un grand nombre de vins Rosés sont mis en valeur par des odeurs florales ou épicées. Cependant, le dénominateur commun des vins Rosés du monde semble être cette signature fruitée. Des travaux récents ont démontré la contribution d'un certain nombre de composés volatils. A côté des composés fermentaires bien connus comme les esters éthyliques, les alcools supérieurs et leurs acétates (CACHO, 2004), d'autres composés contribuent à l'arôme des vins Rosés et notamment le 3-mercaptohexanol et son acétate, dans les vins Rosés de Bordeaux (MURAT *et al.,* 2001, DUBOURDIEU et MURAT, 2004, MURAT, 2005) et ceux de Grenache (FERREIRA *et al.,* 2002, CACHO, 2004). Dans ce type de vins, d'autres composés, apportant des nuances olfactives intéressantes ont été également identifiés : la béta-damascénone et le furanéol.
Ainsi, parmi les composés contribuant à l'arôme fruité des vins, un certain nombre sont des composés variétaux, dont la présence et la teneur peut dépendre de la variété utilisée pour la vinification. Il semblait donc important, dans le contexte des vins de Rosés de Provence, vins issus d'un assemblage de différentes variétés, d'identifier les composés clefs de l'arôme fruité afin de pouvoir, ultérieurement, guider le choix des variétés, des pratiques viticoles et des procédés œnologiques pour favoriser leur présence.

MATÉRIEL ET MÉTHODES

Vins

Dix vins Rosés AOC Côtes de Provence distingués par une médaille à l'occasion du concours des Vins de Saint Tropez ont été prélevés en 2004, 2005 et 2006.

Essai de reconstitution d'odeur

Un des dix vins sélectionnés en 2004, vin jugé particulièrement aromatique, est désaromatisé selon la méthode décrite par MASSON et SANCHEZ (2005). L'efficacité de la désaromatisation est estimée par l'analyse des principaux composés volatils d'origine fermentaire.
Le vin désaromatisé est alors additionné de 15 composés volatils à des concentrations proches de celles que présentait le vin témoin. Les trois vins (témoin, désaromatisé, reconstitué) sont alors soumis au jury expert du Centre du Rosé, qui en fait l'analyse sensorielle, selon la méthode du profil quantifié, à l'aide d'une fiche de dégustation sur laquelle il a été entraîné.

Techniques d'Analyse instrumentale :

Analyse des principaux composés volatils d'origine fermentaire :

Les vins font l'objet d'une extraction liquide/liquide par un mélange éther/hexane (50/50) puis d'une analyse semi quantitative CPG/FID inspirée de MURAT (2001).

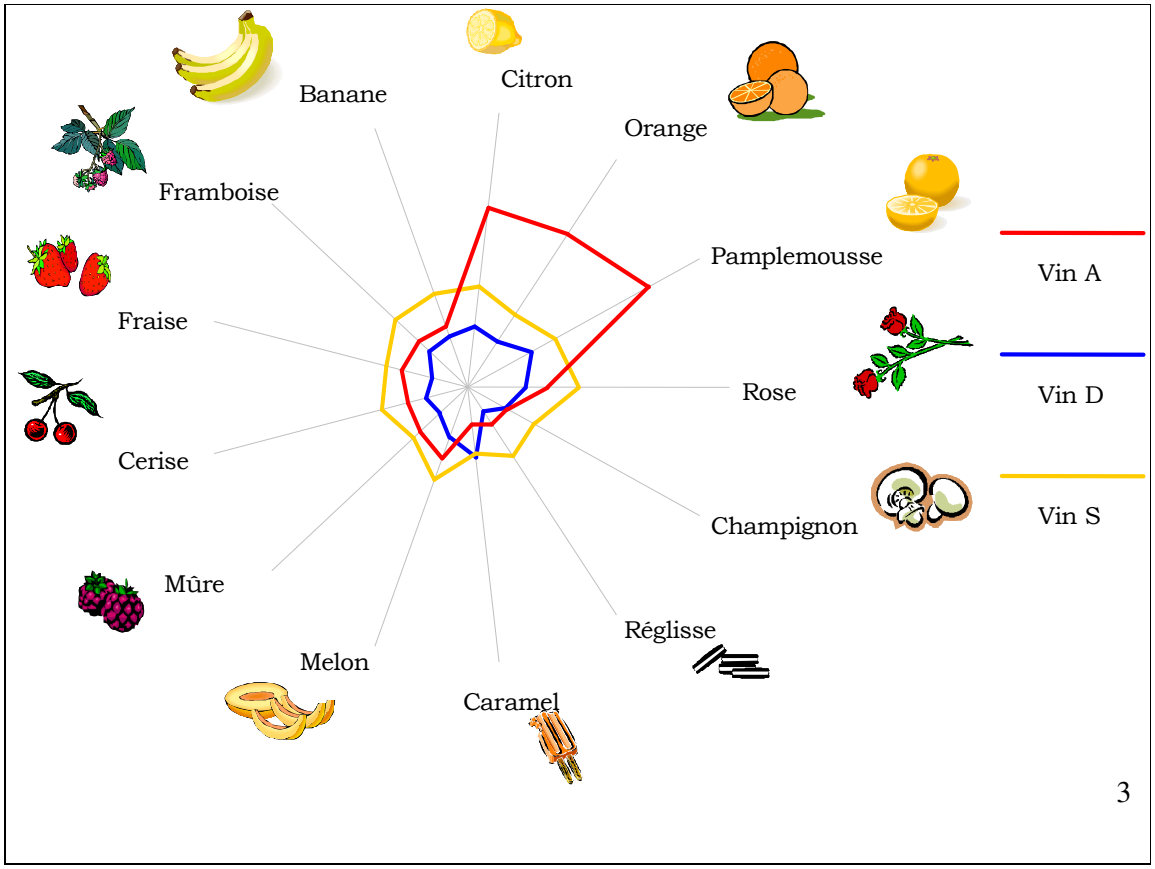
Analyse des thiols variétaux, de la béta-damascenone et de la béta-ionone
Ces analyses sont réalisées par le laboratoire SARCO (Floirac, France). Les thiols variétaux sont dosés selon la méthode décrite par TOMINAGA et al. (1998). La béta-damascenone et la béta-ionone sont analysées selon le protocole décrit par MURAT (2001).
Analyse du furanéol et de l'homofuranéol
L'analyse de ces deux furanones est réalisée par le laboratoire Nyseos (Montpellier, France) selon la méthode proposée par SCHNEIDER *et al.* (2002).

RÉSULTATS ET DISCUSSION

L'impact sensoriel des molécules rajoutées séparément les unes des autres a été évalué et les résultats ont été récemment publiés (MASSON et SCHNEIDER, 2009).

Reconstitution d'odeur
La complémentation du vin désaromatisé est réalisée de façon à retrouver dans le vin, les 15 composés volatils testés à des concentrations proches de celles déterminées dans le vin initial. La vérification est faite par leur dosage dans le vin de départ et le vin supplémenté.

Les résultats, présentés sur la figure 1 montrent que le vin S (vin reconstitué) présente une odeur significativement plus intense que le vin D (désaromatisé). La supplémentation a permis d'augmenter significativement la plupart des notes olfactives au delà même de celles trouvées sur le vin initial.
Il semble donc bien que les composés rajoutés, participent à la qualité olfactive du vin, et peuvent donc être classés parmi les composés clefs de l'arôme de ce type de vin. Trois notes cependant restent nettement en retrait malgré la supplémentation : citron, orange, pamplemousse. Ces notes olfactives rappellent celles des composés de type thiols (3-mercaptohexanol et son acétate notamment) dont il a été démontré qu'ils sont également des



composés clefs de certains vin Rosés comme ceux issus de Merlot, Cabernet Sauvignon et Cabernet Franc (MURAT *et al.*, 2001) ou ceux issus de Grenache (FERREIRA *et al*., 2002). L'option choisie pour valider cette hypothèse n'a pas été celle de l'ajout vu la difficulté de manipulation de ces molécules facilement oxydables, et l'impossibilité de vérifier « en ligne » la supplémentation. En revanche, nous en avons réalisé le dosage dans un certain nombre de vins afin de vérifier si leur concentration permettait de les valider en tant que composés clefs.

Figure 1. Comparaison du profil olfactif réalisé par le jury expert du Centre du Rosé sur les 3 vins de l'essai : A (aromatique), D (désaromatisé), S (supplémenté).

Teneurs en thiols variétaux des vins Rosés de Provence

Le dosage des 3 thiols variétaux (4MMP, 3MH, et A3MH) a été réalisé sur les échantillons de 2003 et 2004. Les résultats sont présentés dans le tableau 1.
La 4-méthyl-4-mercaptopentan-2-one (4MMP), thiol volatil responsable des arômes de buis du cépage Sauvignon (DARRIET, 1995) n'a été détecté dans aucun des échantillons analysés.
Le 3MH et l'A3MH (3-mercapto-hexanol et acétate de 3-mercapto-hexyle) sont connus pour leurs odeurs intenses de pamplemousse et de fruit de la passion et ont des seuils de perception faibles, respectivement de 60 ng/L et 4 ng/L (TOMINAGA, 1998).
Le 3MH est présent dans tous échantillons analysés et sa concentration est supérieure au seuil de perception dans 90% des cas, ce qui laisse supposer une contribution significative de ce composé à l'arôme des vins Rosés provençaux. L'A3MH est mise en évidence dans 14 vins et sa teneur y est systématiquement supérieure au seuil de perception.
Si l'on considère le millésime, il est évident que les vins de 2003 présentent des teneurs en thiols nettement inférieures à ceux de 2004. Ces différences peuvent être liées au fait que l'origine des vins n'est pas la même. Cependant on retrouve cet écart entre les vins 4 et 10 de 2003 et 20 et 18 de 2004, qui proviennent de la même cave. Il semble donc plus vraisemblable que cette faible teneur en thiols des vins de 2003 soit la conséquence de la forte canicule. Cette observation est confirmée par d'autres résultats (SCHNEIDER, communication personnelle; DAGAN 2006). Son explication pourrait résider dans une moindre biosynthèse de précurseurs de thiols dans les raisins mais nous ne disposons pas des données sur les précurseurs de thiols des raisins à l'origine de ces vins pour pouvoir le confirmer.

Tableau 1 : Teneurs en thiols volatils (ng/L) des vins des millésimes 2003 et 2004 (- = non détecté)

	3-mercaptohexanol ng/L	3-mercaptohexyl acetate ng/L
2003		
Ech° 1	88	-
Ech° 2	20	-
Ech° 3	20	-
Ech° 4	519	81
Ech° 5	207	27
Ech° 6	581	13
Ech° 7	89	-
Ech° 8	70	-
Ech° 9	317	-
Ech° 10	197	37
2004		
Ech° 11	194	23
Ech° 12	1026	152
Ech° 13	868	126
Ech° 14	1300	248
Ech° 15	1059	38
Ech° 16	246	30
Ech° 17	671	-
Ech° 18	388	45
Ech° 19	95	7
Ech° 20	899	136

Les valeurs présentées dans le tableau 1 sont en cohérence avec les résultats de MURAT (2005) obtenus sur 30 vins bordelais et 10 vins provençaux, même si les vins ne sont pas issus du même millésime. On note toutefois une teneur plus importante en acétate de 3-mercaptohexyle dans les vins Rosés de Provence que dans les vins Rosés bordelais. Cette différence, déjà apparente dans MURAT (2005) peut être due à l'effet du millésime ou, plus probablement, à des cépages spécifiques à chaque région. Parmi les cépages provençaux, des différences notables ont en effet été mises en évidence (MASSON, 2006). Les vins Rosés de Syrah et de Grenache sont plus riches en thiols volatils que les vins Rosés de Cinsaut et la proportion d'A3MH paraît plus importante dans le cas du cépage Syrah.

Malgré les effets marqués du millésime, il apparaît donc que le 3-mercaptohexanol, et dans une moindre mesure son acétate, sont des composés clefs de l'arôme des vins Rosés. Ce résultat confirme celui obtenu par CACHO (2004) pour les vins Rosés de Grenache. Il est également en mesure d'expliquer le mauvais score obtenu pour les notes d'agrumes, par la supplémentation du vin de l'essai de reconstitution d'odeur. Le vin reconstitué sans l'ajout de 3-mercaptohexanol et de son acétate présente en effet des notes d'agrumes significativement plus faibles que le témoin, alors que leur dosage (échantillon 4 du millésime 2003) montre qu'ils sont présents à des teneurs bien supérieures à leur seuil de perception.

Teneur en ß-damascénone et en ß-ionone des vins Rosés de Provence

Afin de compléter l'étude des composés clefs de l'arôme des vins Rosés de Provence, nous avons effectué le dosage d'autres composés dont l'impact sur l'arôme des vins Rosés a été signalé par FERREIRA *et al.* (2005) et MURAT (2001) : la béta-damascénone et la béta-ionone ainsi que les furanéol et homofuranéol.

La béta-damascénone, composé odorant puissant isolé pour la première fois dans une huile essentielle de rose bulgare (DEMOLE *et al.,* 1970), a été identifié dans les vins par SCHREIER et DRAWERT (1974). Elle est présente dans de nombreux cépages et dans les vins correspondants, et est formée par hydrolyse acide de nombreux précurseurs, comme récemment rappelé par BAYONOVE (1998).

Connue pour son odeur de compote de pomme, ce composé dépasse son seuil de perception dans tous les vins Rosés analysés (tableau 2). Ce composé pourrait jouer un rôle significatif dans le bouquet des vins Rosés de Provence. Force est de constater cependant que les seuils de détections publiés dans la littérature sont très variés, selon la matrice sur laquelle ils ont été déterminés (de 45 ng/L selon KOTSERIDIS, 1999 en solution hydo-alcoolique modèle à 4,5 µg/L dans des vins doux selon ETIEVANT, 1991). Il est donc difficile de statuer à partir de ces valeurs. Une étude plus récente (PINEAU *et al.,* 2007) a établi les seuils dans un vin blanc désaromatisé au charbon (140 ng/L) et dans différents vins rouges désaromatisés par évaporation sous vide ou non (de 850 à 7000 ng/L). Dans le cas des vins Rosés, aucune donnée n'existe, mais vu la nature de ce type de vin, on est tenté d'utiliser le seuil olfactif des vins blancs. Dans ce cas, il est évident que la béta-damascénone constitue un composé clef de l'arôme des vins, au vu des teneurs retrouvées (entre 1,4 et 4,6 µg/L).
Par ailleurs, PINEAU *et al.* (2007) signalent le rôle d'exhausteur des notes fruitées rempli par la béta-damascénone dans les vins rouges, qu'il pourrait également jouer dans le cas des vins Rosés.

A l'inverse, la béta-ionone, composé norisoprénoïde dont l'origine supposée est la dégradation directe du β-carotène au cours de la vinification présente des teneurs trop faibles pour apporter une contribution olfactive. Il semble donc que sa contribution à l'arôme des vins Rosés soit relativement spécifique des Rosés issus de cépages bordelais comme le Merlot, le Cabernet sauvignon et le cabernet Franc.

Tableau 2. Résultats du dosage de la béta-damascénone et la béta-ionone dans les 10 vins Rosés AOC Côtes de Provence du millésime 2004

	b-damascénone (ng/L)	b-ionone (ng/L)
2004		
Ech° 11	4617	16
Ech° 12	2835	19
Ech° 13	2258	19
Ech° 14	2930	20
Ech° 15	1445	6
Ech° 16	6607	13
Ech° 17	1399	14
Ech° 18	2268	11
Ech° 19	2461	10
Ech° 20	1816	12
Moyenne	***2864***	***14***

Teneurs en furanéol et homofuranéol des vins Rosés de Provence

Ces composés sont connus pour leur odeur agréable de caramel et fraise (furanéol), de caramel et pain grillé (homofuranéol). Le furanéol a d'abord été identifié dans les vins de

Vitis labrusca (RAPP, 1980) et semble caractériser les vins d'hybrides (RAPP, 1980; GUESDES DE PINHO, 1995). Sa présence dans les vins de *Vitis vinifera* n'a été démontrée que récemment (GUTH, 1997; CUTZACH, 1998a; CUTZACH, 1998b), certainement à cause de son analyse mal aisée (GUESDES DE PINHO, 1995). L'homofuranéol est également un composé volatil important pour les vins de Merlot et Cabernet Sauvignon [KOTSERIDIS, 1998]. Un précurseur glycosidique du furanéol a été identifié dans la fraise. Dans le raisin aucune preuve structurale n'a encore été fournie bien que des arguments étayant son existence soient avancés (GUESDES DE PINHO, 1995; KOTSERIDIS, 1998).

Les vins Rosés de Provence de 2005 présentent tous des teneurs en furanéol supérieures au seuil de perception de 37 µg/l (tableau 3). Pour l'homofuranéol, les valeurs enregistrées sont légèrement inférieures à son seuil de perception (10 µg/l). A la lumière de ces résultats, on peut supposer que ces composés jouent un rôle significatif dans l'arôme des vins Rosés analysés. Ces observations confirment les conclusions de FERREIRA *et al.* (2002) ; ce travail a en effet montré que l'élimination de ces deux molécules dans un vin Rosé de Grenache diminuait nettement les notes fruitées et caramel.

Tableau 3 : Résultats du dosage du furanéol et de l'homofuranéol dans les 10 vins Rosés AOC Côtes de Provence du millésime 2005.

	furanéol (µg/L)	homofuranéol (µg/L)
2005		
Ech° 21	201	7
Ech° 22	60	2
Ech° 23	145	5
Ech° 24	42	5
Ech° 25	59	3
Ech° 26	95	3
Ech° 27	80	2
Ech° 28	103	2
Ech° 29	69	2
Ech° 30	81	6
Moyenne	***94***	***4***

Application aux expérimentations sur vins Rosés

Ces composés sont désormais dosés dans la plupart des expérimentations mises en place au Centre du Rosé et servent d'indicateurs qualitatifs dans les protocoles d'évaluation des modes de conduite de la vigne ou des techniques de vinification des vins Rosés. Ils ont par exemple permis de démontrer qu'un débourbage clair favorisait la production d'esters éthyliques (MASSON, 2004). L'influence de la température de fermentation a également été évaluée. De basses températures (13 à 14 °C) favorisent la production de composés amyliques alors que des températures plus élevées (17 à 18°C) sont profitables aux thiols volatils (CAYLA et MASSON, 2010). Concernant l'impact de la date de récolte, les dosages des composés furaniques dans les vins obtenus à partir de différentes dates de récolte montrent que la maturation du raisin s'accompagne d'une augmentation de ces composés aromatiques (CAYLA, communication personnelle). L'étude de techniques de vinification « réductrices » a aussi permis de démontrer l'influence favorable d'un inertage au pressoir sur la production de thiols volatils (CAYLA, 2008) et d'une macération de bourbes à basses températures (MASSON, 2008).

CONCLUSION

L'ambitieux travail de recherche sur l'arôme des vins Rosés de Provence présenté ci-dessus a abouti à la mise en évidence de quelques composés clés. Ces molécules sont d'origine fermentaire (esters éthyliques et acétates d'alcools supérieurs) et variétale (3-mercapto-hexanol et acétate de 3-mercapto-hexyle, furanéol et homofuranéol, béta-damascénone). Elles servent aujourd'hui de marqueurs qualitatifs dans la plupart des protocoles expérimentaux mis en place par le Centre du Rosé et portant sur des facteurs aussi variés que les terroirs, les cépages, l'extraction des jus, la fermentation et la conservation des vins Rosés.
D'autres composés analysés (béta-ionone, terpènes, certains alcools supérieurs) sont présents dans les vins Rosés à des teneurs faibles et nettement inférieures à leur seuil de perception.
Les recherches se poursuivent pour tenter d'identifier les molécules responsables de certaines notes aromatiques souvent décrites dans ces vins, notamment dans le registre floral, épicé et fruits à chairs jaunes.

REFERENCES BIBLIOGRAPHIQUES

BAYONOVE C. 1998. L'arôme variétal : le potentiel aromatique du raisin. In : œnologie : fondements scientifiques et techniques, Flanzy C. (Ed), Lavoisier Tec&Doc : Paris, 165-181.

CACHO J., 2004. Relation entre l'analyse physico-chimique et sensorielle des vins Rosés, *Rencontres Internationales du Rosé,* juin 2004, Toulon, 51-63.

CAYLA L., 2008. Presser sous gaz inerte est favorable à la qualité des Rosés de Grenache. *Rosé.com,* **13**, 5-7.

CAYLA L. et MASSON G., 2010. Conséquences des fermentations à basses températures sur les vins Rosés, *Rosé.com*, 16, 5-7.

CUTZACH I, CHATONNET P, and D. DUBOURDIEU. 1998a. Etude sur l'arôme des vins doux naturels non muscatés. 1ière parie : analyse qualitative de l'arôme des Banyuls et Rivesaltes au cours de leur vieillissement. J. Int. Sci. Vigne Vin, 32: 99-110.

CUTZACH I, CHATONNET P, HENRY R, PONS M, and D. DUBOURDIEU. 1998b. Etude sur l'arôme des vins doux naturels non muscatés. 2ième partie : dosages de certains composés volatils intervenant dans l'arôme des vins doux naturels au cours de leur vieillissement. J. Int. Sci. Vigne Vin, 32: 211-221.

DAGAN L. 2006. Potentiel aromatique des raisins de Vitis vinifera L. cv. Petit Manseng et Gros Manseng. Contribution à l'arôme des vins de pays Côtes de Gascogne. PhD Thesis. Ecole Nationale Supérieure Agronomique de Montpellier (France). 225 p.

DARRIET P. 1995. Recherche sur l'arôme et les précurseurs d'arôme du Sauvignon. PhD Thesis. University of Bordeaux II (France). 170 p.

DEMOLE E., ENGGIST P., SAUBERLI U., STOLL M. et E. KOVATS. 1970. Structure et synthèse de la damascénone, constituent d'odeur de l'essence de rose bulgare (*Rosa damascena* Mill.). Helv. Chim. Acta, 53 : 541-551.

ETIEVANT P. 1991. Wine. In : Volatile compounds in Foods and Beverages, Maarse H. (Ed), Dekker Inc. : New-York, Basel, Hog-Kong, 483-546

FERREIRA V., ORTIN N., ESCUDERO A., LOPEZ R. et CACHO J., 2002. Chemical Characterization of the Aroma of Grenache Rosé Wines: Aroma Extract Dilution Analysis, Quantitative Determination, and Sensory Reconstitution Studies. *J. Agric. Food Chem.*, 50, 4048-4054.

GUEDES DE PINHO P, and BERTRAND A. 1995. Analytical determination of furaneol (2,5-dimethyl-4-hydroxy-3(*2H*)-furanone). Application to differenciation of white wines from hybrids and various Vitis vinifera cultivars. Am. J. Enol. Vitic., 46: 181-186.

GUTH H. 1997. Identification of character impact odorants of different white wine varieties. J. Agric. Food Chem., 45: 3022-3032.

KOTSERIDIS Y. 1999. Etude de l'arôme des vins de Merlot et Cabernet Sauvignon de la région bordelaise. PhD thesis. University of Bordeaux II (France), 189 p.

MASSON G., 2005. Le débourbage, une clé de la réussite. *Rosé.com,* **8 :** 14-16.

MASSON G., 2006. Quoi de neuf sur l'arôme des vins rosés ? *Rosé.com,* **9**, 3-7.

MASSON G., 2008. Débourbage et valorisation des bourbes. *Rosé.com,* **13**, 10-14.

MASSON G. et SANCHEZ J., 2005. Impact des composés volatils d'origine fermentaire sur l'arôme des vins Rosés. *In Vino Analytica Sciencia*, juillet 2005, Montpellier.

MASSON G, SCHNEIDER R., 2009. Key aroma compounds of Provence Rosé wines. *Am. J Enol. Vitic*., 60:1.

MURAT M.L. 2001. Recherches sur la vinification des vins Rosés et clairets de Bordeaux. Diplôme d'études et de Recherches de l'université de Bordeaux II.

MURAT M-L., 2005. Acquisitions récentes sur l'arôme des vins Rosés. Partie 1 : caractérisation de l'arôme, étude du potentiel aromatique des raisins et des moûts, *revue des œnologues*, 117, 27-30.

MURAT M-L., TOMINAGA T. et DUBOURDIEU D., 2001. Mise en évidence de composés clefs dans l'arôme des vins Rosés et clairets de Bordeaux. *J. Int. Sci. Vigne Vin*, *35*, 99-105.

PINEAU B., BARBE JC., VAN LEEUWEN C., and D. DUBOURDIEU. 2007. Which Impact for β-Damascenone on Red Wines Aroma? J. Agric. Food Chem.**,** 55: 4103-4108

RAPP A, KNIPSER W, ENGEL L, ULLEMEYER H, and W. HEIMANN. 1980. Off-flavor compounds in the berry and wine aroma of grape vine hybrids. I. The strawberry-like aroma. Vitis, 19: 13-23.

SCHNEIDER R., KOTSERIDIS Y., BELANCIC A., AUGIER C., et A. RAZUNGLES. 2002. Quantification of Furaneol and Homofuraneol in *Vitis vinifera* Wines by Stable Isotope Dilution Assay using GC-MS/MS. In : 10th Weurman Flavour Research Symposium, Le Quéré et Etiévant (Ed.), Lavoisier Tec&Doc, Paris, 710-714.

TOMINAGA T., FURRER F., HENRY R., DUBOURDIEU D., 1998. Identification of new volatile thiols in the aroma of *Vitis vinifera* L. Var. Sauvignon. *Flavour fragrance J. ,* 13, 159-162.

Thème : Nouveaux produits, nouvelles technologies, nouveaux défis
Sous-thème : Oenologie - Méthodes d'analyse : Innovations et perspectives

Connaissance et maîtrise de la couleur des vins Rosés

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RÉSUMÉ

Chaque année, plus de six cent vins Rosés commerciaux français et étrangers sont mis à disposition par l'Union des Oenologues et analysés par le Centre de Recherche et d'Expérimentation sur le Vin Rosé. Les résultats montrent une très grande diversité de couleur, du blanc taché au rouge pâle. Un gradient de couleur, fonction de l'origine géographique, peut être établi du Nord au Sud aussi bien à l'échelle mondiale que française. Les vins Rosés les plus pâles sont généralement situés dans les zones les plus septentrionales.
Plusieurs systèmes d'analyses permettent de quantifier la couleur. Afin de décrire avec précision cette diversité, notamment en analyse sensorielle, différentes palettes de couleur ont été développées au fil des années. Tout dernièrement une charte de couleur spécifique à la description des Rosés du monde conjugue représentation visuelle et description sémantique.
Cette diversité de couleur se justifie en grande partie par les cépages et les terroirs, facteurs sur lesquels les patriciens n'ont qu'un pouvoir limité. Mais l'étape clé de la maîtrise de la couleur reste les modalités de contact entre les peaux et le jus (durée, température, action mécanique, pour les trois facteurs les plus influents). Une macération à l'échelle de la paillasse informe sur le potentiel couleur (extractibilité) de la vendange. Cet indicateur permet au vinificateur de prévoir la couleur du moût. Les autres étapes de la vinification (souche de levure, collage, sulfitage …) peuvent modifier également la couleur finale du vin, mais dans une moindre mesure.

SUMMERY

Every year, more than six hundred commercial French and foreign rosé wines are made available by the national French oenologists association and analysed by the Centre for Research and Experimentation on Rosé Wine. The results show tremendous variety of colour, from stained white to pale red. A colour gradient depends on the geographic origin and can be established North-South both for the world and for France. The palest rosé wines are generally produced in the northernmost areas.
Several analysis systems can be used to quantify colour. Over the years, a number of different colour ranges have been developed to describe this diversity precisely, particularly for sensorial analysis. A recently released colour chart created specifically to describe the world's rosé wines combines visual representation with semantic description.
This diversity of colour is brought about largely by the grape varieties and wine-growing areas, factors that wine-growers can only influence to a limited degree. The key stage for controlling colour resides in the interaction between the grape skins and juice (duration, temperature, mechanical action are the three most influential factors). Maceration at the bench level indicates the potential colour (extractability) of the grape harvest. This indicates

to the wine-grower the future colour of the must. The other wine-making stages (yeast strain, fining, sulphiting, etc.) can also modify the wine's final colour, but to a lesser extent.

INTRODUCTION

Il est unanimement reconnu que la couleur est une dimension fondamentale pour le vin Rosé. En premier lieu, la vue est bien le premier sens que met en éveil une bouteille de vin. En effet, la très grande majorité des bouteilles de vin Rosé sont conçues en verre blanc ce qui permet un accès direct à la couleur. Par ailleurs, de par ses modes d'élaboration et ses origines diverses, le vin Rosé présente une gamme de couleur plus variée que les autres vins : il couvre le très large espace qui sépare les vins blancs des vins rouges.

MATÉRIELS ET MÉTHODES

Vins Rosés du monde

La couleur de 1190 vins Rosés du monde fournis l'Union des Œnologues de France est analysée en 2004 et 2005. Ces vins proviennent de 27 pays différents, la France représentant la plus grande partie de l'échantillonnage. L'Italie et l'Espagne sont également bien représentées.

Vins Expérimentaux

Les vinifications sont pour la plupart menées à la cave expérimentale du Centre du Rosé dans des cuves de 120L, à partir de raisins ou moûts issus de la zone Provençale,

Techniques d'Analyse instrumentale

- Les mesures de couleur sont réalisées par spectrophotométrie (Lambda 20, Perkin Elmer). Un module de calcul permet d'extraire les coordonnées tristimulaires à partir des spectres.
- Plusieurs nuanciers (grilles de notation de la couleur) sont utilisés. La position sur le nuancier représente le barycentre des positions données par au moins 5 opérateurs. Le vin est observé dans un verre, sous lumière contrôlée.

RÉSULTATS ET DISCUSSION

1- Une large palette de couleur influencée par l'origine géographique

Les 1190 Rosés du monde sont positionnés sur le nuancier de couleur. La figure 1 permet de visualiser la très grande variété de couleur représentée sous la forme d'un nuage de point en diagonale. Dans le but d'établir une relation entre la couleur des vins et leur origine géographique, une analyse statistique est effectuée sur la base des mesures colorimétriques et notamment la valeur a* représentant l'intensité de la teinte rouge. Les résultats (non présentés), dont la portée doit être relativisée au regard du faible nombre d'échantillons disponibles pour certains pays, permettent de formuler l'hypothèse d'un gradient géographique de couleur des vins Rosés (MASSON, 2006a). La couleur devient plus intense et plus rouge en se déplaçant du nord vers le sud. Les vins français occupent une position intermédiaire (figure 2). Le gradient de couleur nord-sud évoqué au plan mondial semble se confirmer à l'échelle de la France. Cependant, dans cette classification la Provence fait exception de par sa tradition et sa politique volontariste de production de vins Rosés pâles.

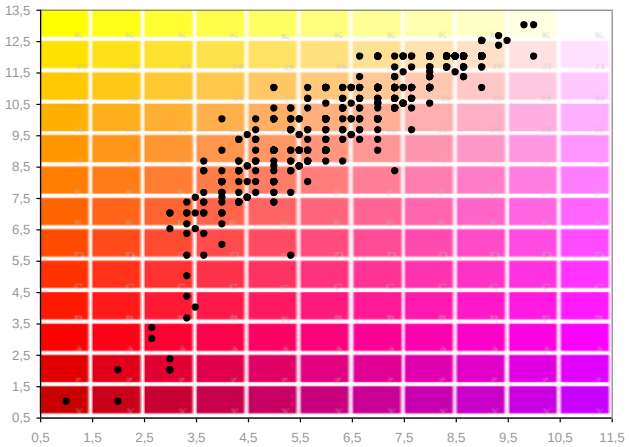

Figure 1. Représentation des 556 échantillons de 2004 sur le nuancier de couleur des vins Rosés.

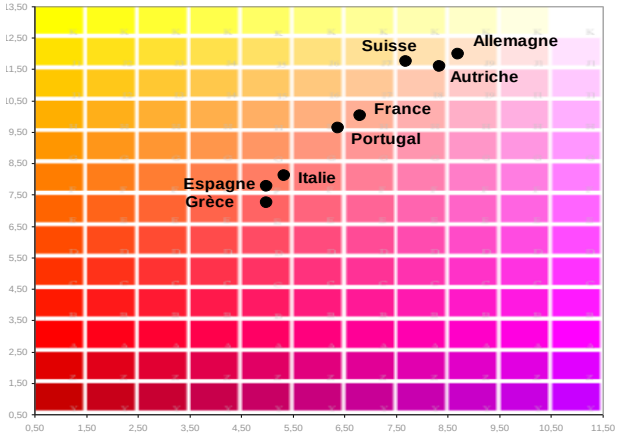

Figure 2. Représentation sous forme de moyennes des pays les plus représentés. Attention, ces points moyens cachent une disparité parfois importante dans un même pays producteur.

2- Des outils pour décrire la couleur

Les analyses de la couleur par spectrophotométrie ou colorimétrie restent les données de référence. Elles permettent de quantifier la couleur en intensité et nuance. Il n'est toutefois pas aisé de relier ces données à l'aspect visuel. C'est pourquoi les praticiens apprécient d'utiliser le nuancier papier sur lequel ils peuvent matérialiser leur impression visuelle. La palette de couleur est déclinée pour les vins Rosés au sens large (figure 3 partie gauche) ou recentrée sur des couleurs plus pâles pour la Provence (figure 3 partie droite) (POUZALGUES, 2006). Cet outil offre un choix important de couleur (intensité et teinte) mais ne permet pas de qualifier (nommer) les couleurs et oblige à comparer un liquide à une surface opaque. Une version liquide a donc été développée. Le nuancier des vins Rosés de Provence (figure 4) rassemble trois sources d'information de la couleur (observation visuelle, description sémantique et donnée colorimétrique chiffrée). Il permet la comparaison et la description de la couleur par les professionnels (MASSON, 2006b). C'est également un formidable outil pédagogique pour parler de ce vin encore méconnu, de son origine, de son élaboration et de sa consommation.

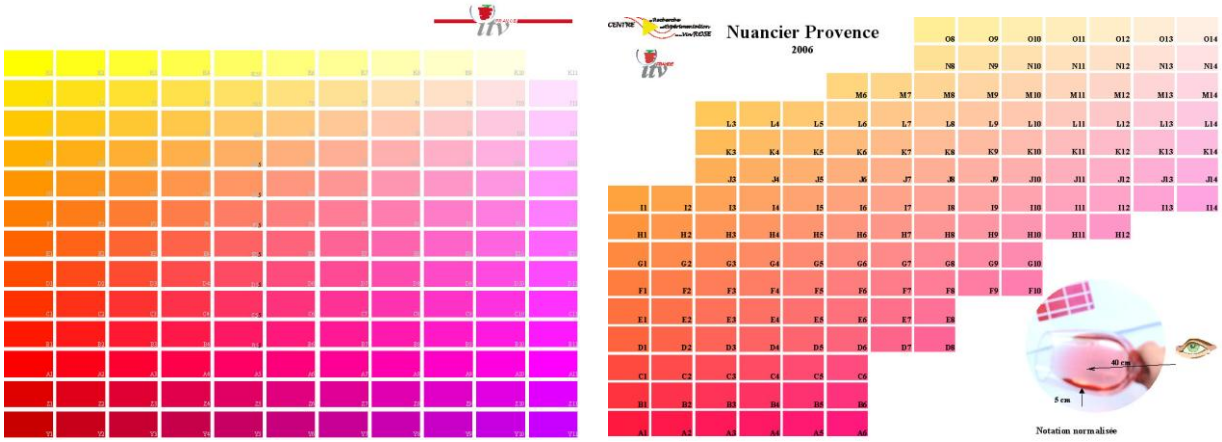

Figure 3. Nuancier papier présentant les principales couleurs des vins Rosés à gauche (version 2000) adapté aux vins Rosés de Provence à droite (version 2006)

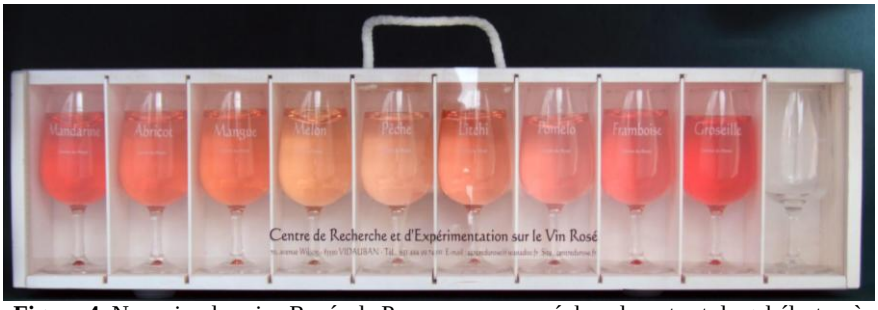

Figure 4. Nuancier des vins Rosés de Provence composé de colorants et de gel élastomère.

Tout dernièrement une charte de couleur spécifique à la description des Rosés du monde (figure 5) conjugue représentation visuelle et description sémantique, en offrant une plus grande variabilité que le nuancier des vins Rosés de Provence. Sept couleurs (litchi, pèche, saumon, abricot, corail, framboise et cerise) sont déclinées en 3 tonalités (clair, moyen et intense). Elles permettent de désigner (par un terme et une vignette de couleur) la diversité des vins Rosés au niveau mondial. Cette palette est particulièrement destinées aux concours et autres guides sur les vins.

Figure 5. Palette de couleur des vins Rosés – description visuelle et sémantique.

Ces outils sont tous développés sur la base d'analyses de vins ; les couleurs sont choisies par tris statistiques (analyse de variance et classification hiérarchique) et le vocabulaire est défini par consensus de techniciens ou à partir du travail fourni par le jury expert du Centre du Rosé.

3- Les sources de variabilité de la couleur des vins Rosés

Les vinificateurs disent souvent du vin Rosé qu'il est très difficile à élaborer. Il nécessite en effet un grand savoir faire et impose de maîtriser un ensemble de techniques. Les variantes de production du Rosé sont nombreuses et représentent une source de variabilité importante. Elles sont classées ci-dessous par ordre décroissant d'influence sur la couleur.

3.1- La macération pelliculaire : durée, température et actions mécaniques

La durée de la macération pelliculaire paraît être la principale source de variabilité de couleur des vins Rosés. Un contact très réduit entre les pellicules et le jus peut donner un vin Rosé proche du blanc alors qu'avec ce même raisin, une macération de plus de 24 heures donne naissance à un vin quasiment rouge. D'autres paramètres peuvent sensiblement

augmenter l'intensité de la macération et notamment la température à laquelle elle se déroule. Des températures élevées favorisent la diffusion des composés colorés de la pellicule. Des travaux du Centre de Recherche et d'Expérimentation sur le Vin Rosé (CAYLA, 2005a) ont montré que la couleur du vin pouvait varier du simple au double en passant d'une température de macération de 12 à 18 °C (photographie 1). C'est la raison pour laquelle, les vignobles producteurs de Rosés pâles comme la Provence, privilégient de plus en plus les vendanges de nuit de façon à récolter des raisins frais. Les actions mécaniques engendrées par les étapes de récolte, transport, transferts vers le pressoir, éraflage et foulage, cycle de pressurage … altèrent l'intégrité des baies et favorisent le contact entre les pellicules et le jus. Ils jouent un rôle essentiel dans l'extraction de la matière colorante (CAYLA, 2005b).

Photographie 1. Couleur de 5 vins Rosés issus du même lot de raisin de Cinsaut dont les durées et les températures de macération pelliculaire augmentent de gauche à droite : pressurage direct, 8 h à 12 °C, 8 h à 18 °C, 20 h à 12 °C, 20 h à 18 °C.

Il peut être intéressant d'estimer le potentiel de couleur de la vendange avant récolte pour gérer au mieux les conditions d'obtention du moût : limiter ou au contraire favoriser la diffusion de la couleur en modifiant les calendriers d'apports, durée et température de macération …). La méthode ITV-Rosé, réalisable par un laboratoire en routine, a pour principale caractéristique de simuler à l'échelle de la paillasse des conditions proches de la vinification réelle. Elle consiste à écraser 200 baies de manière reproductible à l'aide d'un fouloir de paillasse et à mesurer la couleur des jus après un contact avec les pellicules de 2 heures à température ambiante (CAYLA, 2008).

3.2- Les cépages

La couleur du vin est souvent étroitement corrélée à la couleur de la peau du raisin et à son épaisseur. Ainsi, les cépages roses (Clairette rose) ou gris (Grenache gris) donnent généralement des vins Rosés très pâles alors que pour d'autres (Syrah, Carignan ou Merlot, par exemple) la prise de couleur est très rapide.

Le cépage influence non seulement l'intensité de la couleur du vin Rosé mais aussi sa teinte. Plus une variété est acide et plus sa couleur est vive, c'est-à-dire à dominante rose franc et éventuellement des reflets bleutés. Les raisins moins acides donnent des vins Rosés à tonalité jaune orangée. D'autres composés sont susceptibles d'influencer la teinte des vins Rosés. Des résultats récents (MASSON, 2009a) montrent que certains cépages provençaux (Tibouren, Grenache, Cinsaut) connus pour donner des vins Rosés à teinte orangée présentent des teneurs élevées en acides hydroxycinnamiques et des teneurs faibles en glutathion. Cette composition particulière engendre des mécanismes d'oxydation intenses et des phénomènes de brunissement dans les moûts et dans les vins.

3.3- Terroir d'origine

Les résultats présentés au paragraphe 1 ont montré l'influence de l'origine géographique sur la couleur des vins Rosés. Les pays et les régions françaises étudiées ont en effet des sols et des climats différents qui donnent naissance à des raisins puis à des vins de composition variée. Au sein d'une même région viticole, d'un même bassin de production ou d'une même commune, des « micro-terroirs » présentant des conditions pédologiques et climatiques différentes peuvent engendrer différents styles de vin Rosé.
Une expérimentation originale conduite en Provence (MASSON, 2009b) apporte des preuves objectives à ces observations de terrain. Quatorze parcelles de Cinsaut et 14 parcelles de Grenache sélectionnées sur des terroirs variés des « Côtes de Provence » et des « Coteaux Varois en Provence » ont fait l'objet pendant 4 ans de modes de conduite de la vigne et de vinifications expérimentales standardisées. Le bilan analytique des vins ainsi obtenus laisse apparaître une forte variabilité de composition parmi les vins Rosés analysés tant au plan physico-chimique que sensoriel. La couleur en est l'élément le plus visible (photographie 2).

Photographie 2. Diversité des couleurs des vins Rosés de Grenache (terroirs différents, vinification standardisée), millésime 2000

3.4- Maturité des raisins

La teneur en composés phénoliques, notamment en anthocyanes, molécules responsables de la couleur rouge des raisins et des vins, augmente au cours de la maturation. Le vin Rosé, dans des conditions identiques de vinification, présente une couleur d'autant plus soutenue que la récolte est décalée dans le temps (CAYLA, 2009).

3.5- Autres sources de variabilité

Les sources de variations de la couleur des vins Rosés sont très nombreuses. Le millésime et ses conditions météorologiques toujours spécifiques engendrent des modifications importantes. La règle générale est que plus l'année est sèche et chaude, plus les raisins et les vins sont colorés. Dans certaines conditions extrêmes, on peut toutefois observer des blocages de maturité. A l'inverse, plus un millésime est froid et pluvieux, moins les vins sont colorés.
Le rendement agronomique est également un facteur de variation significatif. La concentration en composés colorants est d'autant plus élevée que la charge des vignes est faible.

Les collages mis en œuvre sur les vins Rosés pour assurer une bonne clarification et une bonne stabilisation ont pour conséquences une diminution de l'intensité colorante des moûts et des vins (TOURREL et CAYLA, 2009). La nature de la colle utilisée, la dose et le moment

d'emploi sont autant d'éléments déterminants pour la couleur du vin Rosé.
Au cours de la fermentation alcoolique, la chute de couleur est généralement importante. Cela rend le travail du vinificateur particulièrement difficile car il doit anticiper cette diminution en élaborant un moût plus coloré que l'objectif de couleur final du vin. Si de manière systématique, la moitié des anthocyanes sont perdues dans les trois premiers jours de la fermentation alcoolique, cette perte de matière colorante n'entraine pas une chute constante de couleur (COTTEREAU, 2004). La baisse d'intensité colorante au cours de la fermentation alcoolique est évaluée 50 % en moyenne (TOUZET, 2008) avec une très forte variation d'une cuve à l'autre. La souche de levure, la teneur en alcool, l'acidité et la concentration en tanins pourraient expliquer certaines variations.
Enfin, la décoloration consécutive aux sulfitages post-fermentaires, bien que partiellement réversible, est également à considérer. La présence de SO_2 entraîne une décoloration des anthocyanes et une sous-évaluation de la couleur rouge. Il est possible de s'affranchir de cette décoloration partielle des anthocyanes, par ajout de quelques gouttes d'éthanal (FLANZY et CAYLA, 2006) ; la couleur rouge potentielle est alors révélée (apparente).

CONCLUSION

L'analyse des vins Rosés au plan mondial montre que le monde du vin Rosé est vaste. Les différences de couleur constatées dans cette étude suggèrent des différences de goût au moins aussi importantes. Cette diversité doit être considérée comme une grande richesse qui mérite d'être préservée coûte que coûte.
Les travaux qui sont présentés dans cet article illustrent bien le double objectif du Centre du Rosé : des études scientifiques et techniques afin de mieux connaitre, élaborer et parler des vins Rosés, débouchant sur des outils à la fois professionnel et grand public.

BIBLIOGRAPHIE

- CAYLA L., 2005a. Maturité et macération, un couple indissociable. Rosé.com 8, 11-13.
- CAYLA L., 2005b. Une phase sensible du pressurage : le remplissage. Rosé.com 8, 20-22.
- CAYLA L., 2008. Itinéraires d'élaboration des vins Rosés. Actes de Mondiaviti, Bordeaux,
- CAYLA L., 2009. Maturité des raisins et qualité des vins Rosés français. Le vin Rosé : entité, élaboration, perception. Edition Féret. 139-141
- COTTEREAU P., 2004. Synthèse des études « vins Rosés », influence sur la couleur des principaux paramètres de vinification. Entretiens Rhône Méditerranée.
- FLANZY C. et CAYLA L., 2006. Elaboration des Vins Rosés, résultats d'expérimentation. Cahiers Itinéraires d'ITV-France, 11-40 pages.
- MASSON G., 2006a. Panorama des Rosés du monde. Actes des Rencontres Internationales du Rosé, Aix en Provence, 30 juin 2006, 33-37.
- MASSON G., 2006b. Le nuancier des vins Rosés. Genèse, mise au point et développement. Académie Amorim, Prix coup de cœur 2006.
- MASSON G., 2009a. Oxydabilité des cépages provençaux ; conséquences sur les arômes des vins Rosés. Rosé.com 15, 11-13.
- MASSON G., 2009b. Expression de différents terroirs en Provence. Le vin Rosé : entité, élaboration, perception. Edition Féret, 105-107.

- POUZALGUES N., 2006. Comment décrire les vins rosés de Provence, les descripteurs visuels, Rosé.com 9.
- TOURREL B. et CAYLA L., 2009. Collages des vins Rosés. Le vin Rosé : entité, élaboration, perception. Edition Féret, 177-181.
- TOUZET F., 2008. Couleur des rosés : des repères à connaître, des pièges à éviter. Rosé.com 14, 12-14.

Pesticide multiresidue SPME-GC-MS-MS method

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RÉSUMÉ

Ce travail décrit une méthode de microextraction en phase solide (SPME) chromatographie en phase gazeuse et spectrométrie de masse en tandem (MS / MS) pour quantifier 25 pesticides dans des vins de liqueur blanc et des vins de liqueur rouge. La méthode d'analyse montre une bonne linéarité présentant coefficients de corrélation (R^2) généralement supérieurs à 0.99. Les limites de detection (LD) et quantification (LQ) sont de 0.1 à de 72.4 ug/l et de 0.2 à de 219.2 ug/l, respectivement. Les LQ sont inférieures aux valeurs limites maximales de résidus (LMR) fixées par le règlement européen (CE Règlement Nº 396/2005) pour les raisins et la plupart de ces valeurs sont inférieures à LMR/10 (niveaux suggérés pour le vin). La méthode proposée a été appliquée à 17 vins de liqueur. Les pesticides analysés n'ont pas été détectés dans ces vins.

ABSTRACT

The present work describes a solid phase microextraction (SPME) gas chromatography and tandem mass spectrometry (MS/MS) method to quantify 25 pesticides in fortified white wine and fortified red wine. Analytical method showed good linearity presenting correlation coefficients (R^2) mostly higher than 0.99. Limits of detection (LOD) and quantification (LOQ) values in the range 0.1 – 72.4 ug/l and 0.2 – 219.2 ug/l, respectively, were obtained. LOQ values are below the maximum residue levels (MRL) established by European Regulation (EC Regulation Nº 396/2005) for grapes and most of these values are below MRL/10 (levels suggested for wine). The proposed method was applied to 17 fortified wines. Analyzed pesticides were not detected in the tested wines.

INTRODUCTION

Monitoring of pesticides has received much attention in the last few years. In case of vineyard protection, the use of these compounds may result in the presence of their residues in the wine, thus potentially compromising the safety of this product (Correia *et al*., 2000; Patil *et al*., 2009).
Besides the health hazards that may be caused by pesticides residue, the sensorial quality of wine may also be affected (Patil *et al*., 2009).
According to some authors, vinification include a number of steps which reduce significantly the pesticide residue levels that may be present in grapes. Thus their contents in wines are significantly lower than in grapes (Cabras *et al*., 1997; Otteneder H.,Majerus P., 2005; Flamini R., Panighel A., 2006).
Until now, the European Commission (EC) has established pesticide maximum residue levels (MRL's) in grapes, but not for wine (EC Regulation Nº 396/2005). However, pesticides MRL's for wine have been suggested in order to guarantee as much as possible the safety of the beverage. Otteneder and Majerus (2005) suggested that a limiting value for residue levels in wine

could be a reduction of 90% of the pesticide maximum residue levels in grapes, thus reaffirming the necessity of effective and sensitive methods to detect pesticide residues in wine.
Solid phase microextraction (SPME) is an alternative extraction method to traditional techniques, allowing complete elimination of solvents, blanks reduction, extraction time reduction. This method does not require complete removal of the analyte from the liquid matrix (Kataoka *et al*., 2000) and can thus be alpplied to a wide range of applications than other techniques such as solid phase extraction (SPE), wich requires an exhaustive extraction (Pawliszyn J., Arthur C., 1990).
The need for higher selectivity and sensitivity as well as the necessity for confirmation have been successfully achieved by coupling liquid chromatography (LC) or gas chromatography (GC) with mass spectrometry (MS) and tandem mass spectrometry (MS-MS) (Flamini R., Panighel A., 2006; Economou *et al*., 2009). The use of these chromatographic techniques coupled with MS or MS-MS, for the determination and/or confirmation of pesticides in still wine has been reported for some authors (Vitali *et al*., 1998; Cunha *et al.*, 2009; Patil *et al.*, 2009).
In this work, a SPME-GC-MS-MS method was validated for the determination of 25 pesticides (azoxystrobin, beta-cyfluthrin, chlorothalonil, chlorpyriphos, chlorpyriphos-methyl, cypermethrin, cyprodinil, deltamethrin, diazinon, dicofol, fenhexamid, fenitrothion, fenthion, fenoxycarb, flusilazole, folpet, iprodione, kresoxim-methyl, lambda-cyhalothrin, malathion, metalaxyl, procymidone, tebuconazole, trifloxystrobin and vinclozolin) in fortified wine.

MATERIAL AND METHODS

Solutions and reagents

All pesticide standards were high purity (> 95%) supplied by Sigma – Aldrich (Seelze, Germany). Individual pesticide stock solutions (1g/l) where prepared in methanol (99.9%), supplied by Sigma – Aldrich (Seelze, Germany), and stored under refrigeration (2-6 ºC). A stock standard mixture solution with all pesticides was also prepared in methanol, weekly. The stock standard mixture solution was stored also under refrigeration (2-6 ºC).

SPME procedure

SPME extraction procedure was performed in a Combipal MH 01-00B autosampler (CTC Analytics AG, Zwingen, Switzerland). SPME fibers (Supelco, Bellefonte, USA) were conditioned according to the supplier's instructions. The extraction procedure was performed using 20 ml clear glass vials (La-Pha-Pack, Langerwehe, Germany). The samples of 19 ml were extracted by immersion of a 100um Polydimethylsiloxane (PDMS) coated fiber, under the following conditions: extraction temperature 35 ºC, agitator speed 250 rpm and extraction time 60 min. After extraction and desorption, fiber conditioning was performed for 5 min, in the presence of nitrogen (99.995%).

Gas chromatographic analysis

Gas chromatographic analyses were performed in a FocusGC, equipped with a split/splitless injector (Thermo Fisher Scientific, Waltham, MA, EUA). The analytical column used was a TR-5MS (30m x 0.25mm ID x 0.25um film thickness) coated with 5% Phenyl Methylpolysiloxane stationary phase (Thermo Fisher Scientific, Waltham, MA, EUA). The split/splitless injection port was maintained in splitless mode for 3 min and set at a fixed temperature of 250 ºC. The SPME desorption was carried out in the injector port for 6 min. The oven temperature

programme used for the analyses was the following: initial temperature 80 ºC for 5 min, raised to 300 ºC at a rate of 5 ºC/min and kept for 10 min.

Mass spectrometry detection

Retention times of each pesticide were determined in full scan mode. A PolarisQ ion trap mass spectrometer (Thermo Fisher Scientific, Waltham, MA, EUA), operated in the electron impact (EI) mode was used. The ion source and transfer line temperatures were set at 250 ºC and 280 ºC, respectively. The analyses were carried out with a filament-multiplier delay of 5 min.

RESULTS AND DISCUSSION

Calibration and linearity

Matrix-matched standards were prepared in fortified wine samples, previously analyzed for the absence of compounds. The calibration curves were obtained for all pesticides by spiking the fortified wine samples at least at five concentration levels. This procedure was repeated in five different days, for fortified white wine (FWW) and fortified red wine (FRW). At the end, five calibration curves were obtained for each pesticide and for each matrix. Good linearity was achieved for the majority of the pesticides in FWW and FRW, with correlation coefficients of regression (R^2) >0.99. The lowest value of R^2 was obtained for fenoxycarb in FRW (0.9808) and the highest value for lambda-cyhalothrin in FWW (0.9999).

Repeatability, intermediate precision and recovery

The repeatability (*r*) was determined in fortified wine samples, spiked with pesticides at different concentration levels. The test was performed at least in three independent preparations, in FWW and FRW. The *r* results, expressed as relative standard deviation (RSD%), are summarized on Tab. 1. Good results for the *r* were obtained for almost all the tests (RSD ≤ 20%), according to EC SANCO/2009/10684 values reported. Values of RSD ≤ 5% were obtained for some pesticides, even at low concentration levels. The lowest value of *r* was obtained for iprodione in FRW, spiked at 246.84 ug/l and for trifloxystrobin in FRW, at 19.00 ug/l (0.8%). The highest value for *r* was obtained for folpet, in FRW, spiked at 119.19 ug/l (27.3%).

The intermediate precision (IP) was determined in fortified wine samples, spiked with pesticides at different concentration levels. The test was performed at least in four independent preparations, in FWW and FRW. Each preparation was performed in different days. IP results, expressed as relative standard deviation (RSD%), are presented on Tab. 1. Good results for the IP were obtained for almost all the tests (RDS ≤ 20%). The lowest value of IP was obtained for deltamethrin in FWW, spiked at 11.27 ug/l (1.1%) and the highest value for IP was obtained for diazinon, in FRW, spiked at 1.58 ug/l (27.8%).

Recovery (R) results were obtained from the same values used for the IP study. Good recoveries were achieved for the majority of the studied pesticides, according to EC SANCO values reported (R values between 70 and 120%). R results, expressed in %, are presented also on Tab. 1. The lowest R value was obtained for folpet in FWW, at 747.38 ug/l (69.9%) and the highest R value for tebuconazole in FRW, at 138.71 ug/l (127.5%).

Limits of detection (LOD) and quantification (LOQ)

LOQ for each pesticide were validated considering values used for the IP and R studies, and the French Standard NF T 90-210. LOD values were estimated considering the LOQ values.

LOQ values for each pesticide, expressed as ug/l, are presented in Tab. 2, as well as the respective MRL according to EC Regulation N^{er} 396/2005 and MRL/10 (pesticide levels suggested for wine). Good LOQ were obtained for all pesticides. The lowest LOD and LOQ values were obtained for fenthion, 0.1 and 0.2 ug/l, respectively, in both matrices. The highest LOD and LOQ values were obtained for azoxystrobine, 72.4 and 219.2 ug/l, respectively, in FRW.

Lower LOQ values may be achieved if lower concentration levels were tested. Furthermore, analyzing the chromatograms (Fig. 1) obtained for low concentration levels, for each pesticide, it can be shown that the equipment sensibility allows achieving lower LOQ values then the validated, if considered the concentration level presenting a signal to noise ratio of 10.

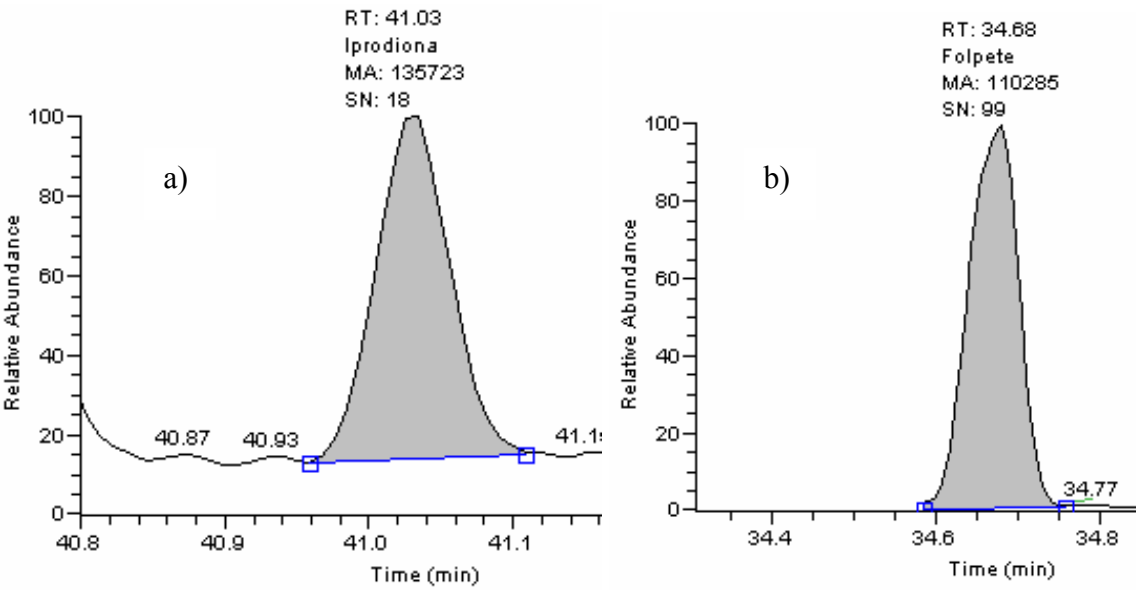

Figure 1. GC-MS-MS chromatograms obtained for: a) Iprodione, spiked at 148.11 ug/l, in FRW; b) Folpet, spiked at 198.66 ug/l, in FRW.

Table 1. Results of repeatability (*r*), intermediate precision (IP) and recovery (R) studies, for all analyzed pesticides, in FWW and FRW.

Pesticide	Fortified white wine (FWW)				Fortified red wine (FRW)			
	Level (ug/l)	IP (%RSD)	*r* (%RSD)	R (%)	Level (ug/l)	IP (%RSD)	*r* (%RSD)	R (%)
Diazinon	1.59	6.8	15.7	72.7	1.58	27.8	17.9	102.7
	2.65	8.6	12.5	80.0	2.63	17.4	14.6	94.7
	9.92	11.0	2.7	84.1	9.87	16.7	12.3	83.5
Chlorthalonil	14.52	7.0	20.7	92.2	14.77	19.5	4.2	88.2
	24.20	4.8	8.3	99.6	24.62	16.4	4.4	97.9
	90.75	10.2	6.9	100.4	92.33	16.3	11.6	93.9
Chlorpyriphos-methyl	0.46	13.0	16.6	92.0	0.64	13.2	5.2	95.1
	1.07	5.6	8.9	98.6	1.06	9.0	1.4	101.8
	4.01	5.1	6.2	98.6	3.98	13.5	6.5	94.1
Vinclozoline	17.50	3.5	14.5	104.1	18.07	7.4	7.4	101.7
	29.16	6.5	4.1	102.8	30.11	7.1	1.8	101.2
	109.36	3.7	4.6	99.4	112.92	10.1	2.4	105.5
Metalaxyl	42.30	15.1	10.7	121.3	70.33	24.3	5.0	89.8
	70.50	6.9	5.7	104.0	105.50	15.8	9.9	104.3
	264.39	9.3	3.9	94.5	263.74	12.1	5.3	115.3
Fenitrothion	0.95	10.0	17.7	104.7	1.58	12.4	4.4	101.5
	1.59	14.3	6.4	100.8	2.38	19.6	2.8	98.2
	5.95	11.0	5.2	97.8	5.94	10.2	2.1	111.7
Malathion	8.14	9.7	13.3	112.6	13.12	14.2	12.7	114.1
	13.57	13.9	6.5	108.1	19.68	14.1	6.7	113.6
	27.14	13.6	2.7	99.6	49.19	7.0	6.1	102.6
Chlorpyriphos	1.27	12.5	7.8	95.7	1.27	11.8	6.4	99.0
	2.11	3.6	5.2	101.2	2.11	7.0	2.1	98.3
	7.92	3.5	6.0	99.2	7.92	9.1	8.6	103.3
Fenthion	0.16	11.1	13.4	105.5	0.16	6.0	15.7	97.9
	0.27	7.4	10.8	97.5	0.27	7.1	6.2	98.9
	1.01	6.4	6.3	98.9	1.01	10.2	6.1	103.1
Dicofol	6.08	13.3	7.3	89.9	6.04	18.7	7.0	100.3
	10.13	12.9	6.6	85.2	10.07	11.9	8.9	85.6
	37.99	7.5	5.2	89.4	37.76	11.3	2.1	93.5
Cyprodinil	7.11	16.3	9.4	98.9	7.11	19.0	6.5	94.5
	11.85	5.7	6.5	98.5	11.84	15.9	4.8	100.2
	44.45	5.3	2.8	98.5	44.41	14.5	14.5	95.3
Procymidone	22.40	18.2	13.3	87.1	22.41	15.3	10.2	92.7
	37.33	9.4	8.0	101.2	37.35	11.5	7.0	98.8
	139.97	4.9	2.5	97.0	140.05	5.5	1.1	102.5

Pesticide	Fortified white wine (FWW)				Fortified red wine (FRW)			
	Level (ug/l)	IP (%RSD)	*r* (%RSD)	R (%)	Level (ug/l)	IP (%RSD)	*r* (%RSD)	R (%)
Folpet	119.58	6.1	18.7	91.9	119.19	12.7	27.3	77.8
	199.30	7.7	15.3	80.4	198.66	15.1	19.4	76.2
	747.38	7.3	19.0	69.9	744.96	13.4	14.3	83.7
Flusilazole	6.94	12.8	7.8	96.2	11.29	18.5	4.1	102.0
	11.57	8.6	7.6	94.6	16.94	20.2	2.5	98.1
	43.40	2.6	2.5	98.4	42.35	13.3	6.1	103.6
Kresoxim-methyl	10.18	7.3	12.2	92.1	10.19	8.3	1.4	99.1
	16.97	4.9	7.0	97.1	16.98	8.7	2.5	92.7
	63.64	8.1	4.2	100.5	63.68	13.0	6.4	106.6
Trifloxystrobin	11.30	16.4	7.0	96.0	11.40	14.8	6.0	108.7
	18.84	8.0	6.8	97.7	19.00	15.9	0.8	100.7
	70.63	3.8	4.6	96.02	71.24	13.8	5.6	111.9
Fenhexamid	103.31	20.3	10.9	99.0	172.41	17.2	3.4	104.2
	172.18	17.3	9.1	114.0	258.61	20.7	3.3	95.2
	645.67	7.5	8.2	101.7	646.53	12.4	1.1	114.7
Tebuconazole	92.25	19.6	24.5	91.3	92.47	20.6	5.1	98.1
	138.38	15.8	11.9	91.9	138.71	23.8	1.1	127.5
	345.94	9.9	5.9	100.4	346.77	18.3	2.9	117.7
Iprodione	147.00	9.9	4.5	105.9	148.11	17.7	4.8	103.0
	245.00	6.2	2.8	106.0	246.84	13.2	0.8	94.2
	918.74	5.5	1.0	101.1	925.66	13.2	5.3	100.5
Fenoxycarb	41.75	11.3	8.7	87.5	40.60	10.6	6.3	93.0
	69.59	3.2	4.0	96.7	67.67	8.9	11.0	87.6
	260.96	11.9	9.9	85.5	253.75	13.6	16.0	82.4
Lambda-cyhalothrin	5.79	17.3	15.4	102.8	5.84	19.2	8.2	98.7
	9.64	7.1	11.7	100.2	9.74	10.4	3.3	108.4
	36.15	4.8	11.0	94.6	36.53	5.3	10.8	96.6
Beta-cyfluthrin	13.42	13.9	12.9	108.0	13.31	13.2	7.3	98.8
	22.37	6.3	10.1	102.2	22.19	15.1	5.7	97.2
	83.88	7.9	8.1	93.3	83.20	11.8	8.4	103.1
Cypermethrin	6.307	12.5	15.4	99.4	6.25	10.0	9.7	105.5
	10.50	5.0	10.3	97.3	10.42	16.2	9.2	97.2
	39.39	6.9	6.1	94.7	39.08	12.1	10.8	100.9
Deltamethrin	6.76	19.0	4.8	111.5	6.52	17.6	3.2	107.6
	11.27	1.1	2.6	96.8	10.86	14.3	5.3	97.7
	42.25	8.9	1.8	102.6	40.73	10.5	9.7	98.5
Azoxystrobine	131.83	18.0	14.1	103.7	131.40	5.1	5.0	115.4
	219.72	9.2	10.6	96.9	219.23	5.0	3.9	91.0
	823.96	7.3	14.1	89.5	822.11	7.2	5.7	99.8

Table 2. Estimated LOD and LOQ (expressed in ug/l) for the analized pesticides, in FWW and FRW, and respective MRL and MRL/10 (expressed in ug/kg).

Pesticides	Fortified white wine (FWW)		Fortified red wine (FRW)		MRL (ug/kg)	MRL/10 (ug/kg)
	LOD (ug/l)	LOQ (ug/l)	LOD (ug/l)	LOQ (ug/l)		
Diazinon	0.9	2.7	0.9	2.6	10	1
Chlorthalonil	8.0	24.2	8.1	24.6	3000	300
Chlorpyriphos-methyl	0.2	0.6	0.4	1.1	200	20
Vinclozoline	5.8	17.5	6.0	18.1	5000	500
Metalaxyl	23.3	70.5	34.8	105.5	1000	100
Fenitrothion	0.3	1.0	0.5	1.6	10	1
Malathion	2.7	8.1	4.3	13.1	5000	500
Chlorpyriphos	0.4	1.3	0.4	1.3	500	50
Fenthion	0.1	0.2	0.1	0.2	10	1
Dicofol	3.3	10.1	2.0	6.0	2000	200
Cyprodinil	2.4	7.1	3.9	11.8	5000	500
Procymidone	7.4	22.4	7.4	22.4	5000	500
Folpet	39.5	119.6	65.6	198.7	5000	500
Flusilazole	2.3	6.9	3.7	11.3	200	20
Kresoxim-methyl	3.4	10.2	3.4	10.2	1000	100
Trifloxystrobin	3.7	11.3	6.3	19.0	5000	500
Fenhexamid	56.8	172.2	56.9	172.4	5000	500
Tebuconazole	18.3	55.4	30.5	92.5	2000	200
Iprodione	48.5	147.0	48.9	148.1	10000	1000
Fenoxycarb	13.8	41.8	13.4	40.6	1000	100
λ-cyhalothrin	1.9	5.8	3.2	9.7	200	20
β-cyfluthrin	4.4	13.4	7.3	22.2	300	30
Cypermethrin	2.1	6.3	3.4	10.4	500	50
Deltamethrin	3.7	11.3	3.6	10.9	200	20
Azoxystrobine	43.5	131.8	72.4	219.2	2000	200

ANALISYS OF WINE SAMPLES

The presented methodology was applied to seventeen fortified wines and residues of analyzed pesticides were not detected.

CONCLUSIONS

The proposed multiresidue method allows a simple, rapid and automated determination of 25 pesticides in fortified red wine and fortified white wine. The method yields recoveries between 69.9 – 127.5%. Limits of detection (LOD) and quantification (LOQ) values in the range 0.1 –

72.4 ug/l and 0.2 – 219.2 ug/l, respectively, were obtained. These values are significantly lower than MRL established by European Regulation, for grapes, and consequently lower than the limits for wine (MRL/10) suggested by Otteneder and Majerus.

BIBLIOGRAPHY

Cabras P., Angioni A., Garau V.L., Minelli E. V., Melis M., Pirisi F. M., 1997. Pesticides in the distilled Spirits of Wine and Its Byproducts. *J. Agric. Food Chem*., **45**, 2248-2251.

Correia M., Delerue-Matos C., Alves A., 2000. Multi-residue methodology for pesticide screening in wines. *Journal of Chromatography A*, **889**, 59-67.

Cunha S. C., Fernandes J. O., Alves A., Oliveira M. B. P. P., 2009. Fast low-pressure gas chromatography-mass spectrometry method for the determination of multiple pesticides in grapes, musts and wines. *Journal of Chromatography A*, **1216**, 119-126.

Economou A., Botitsi H., Antoniou S., Tsipi D., 2009. Determination of multi-class pesticides in wines by solid-phase extraction and liquid chromatography-tandem mass spectrometry. Journal of Chromatography A, **1216**, 5856-5867.

Flamini R., Panighel A., 2006. Mass spectrometry in grape and wine chemistry. Part II: The consumer protection, *Mass Spectrometry Reviews by Wiley Periodicals*, **25**, 741-774.

NF T 90-210 : Qualité de léau – Protocole d'évaluation initiale des performances d'une méthode dans un laboratoire. Mai, 2009.

Patil S. H., Banerjee K., Dasgupta S., Oulkar D. P., Patil S. B., Jadhav M. R., Savant R. H., Adsule P. G., Deshmukh M. B., 2009. Multiresidue analysis of 83 pesticides and 12 dioxin-like polychlorinated biphenyls in wine by gas chromatography-time-of-flight mass spectrometry. Journal of Chromatography A, 1216, 2307-2319.

Kataoka H., Lord H., Pawliszyn, 2000. Applications of solid-phase microextraction in food analysis. Journal of Chromatography A, 880, 35-62.

Otteneder H., Majerus P., 2005. Pesticide residues in wine, transfer from grapes. Bulletin O.I.V., **78**, 889-890, pp. 173-181.

Pawliszyn J., Arthur C., 1990. Solid Phase Microextraction with Thermal Desorption Using Fused Silica Optical Fibers. *Analytical Chemistry*, **62**, 2154-2148.

Regulation (EC) No 396/2005 of the European Parliament and of the Council, European Union, Brussels: - http://ec.europa.eu/food/plant/protection/pesticides/index_en.htm (accessed September 2009).

SANCO/2009/10684- Method validation and quality control procedures for pesticide residues analyses in food and feed, http:// http://www.crl-pesticides.eu/library/docs/fv/guidefinalversion.pdf (accessed on February 2010).

Vitali M., Guidotti M., Giovinazzo R., Cedrone O., 1998. Determination of pesticide residues in wine by SPME and GC/MS for consumer risk assessment. *Food Additives and Contaminants*, **15**, 280-287.

CHEMICAL AND SENSORY CHARACTERIZATION OF COMMERCIAL XAREL.LO WHITE WINES FROM THE PENEDES REGION

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RESUMEN

Con el fin de caracterizar los vinos producidos a partir de la variedad de uva blanca Xarel.lo, la más cultivada en la región del Penedés (Cataluña, España), se realizó un estudio global sobre veinticinco vinos blancos monovarietales (*var*. Xarel.lo) y comerciales procedentes de diferentes añadas y de las bodegas más representativas de la región del Penedés, basado en la aplicación del análisis sensorial descriptivo y químico. El análisis sensorial mostró dos grupos principales o estilos de vinos, los vinos jóvenes, que caracterizados por un sabor fresco y un marcado aroma a fruta y floral y los vinos envejecidos que fueron sometidos a crianza, caracterizados por atributos sensoriales más complejos y que presentaban notas de tostado, picante y compota. Las diferencias en las características sensoriales parecieron estar relacionadas con un alto contenido de acetatos de alcoholes superiores, etil y metil ésteres de ácidos grasos en los vinos pertenecientes al primer grupo mientras que los del segundo grupo mostraron baja concentración de ésteres pero alta de compuestos como el furfural, furaldehído y vitispirano.

ABSTRACT

In order to characterize wines produced from the white grape variety Xarel.lo, the most cultivated in the Penedés (Catalonia, Spain), a comprehensive study based on the application of descriptive sensory and chemical analyses of twenty-five commercial monovarietal white wines (*var. Xarel.lo*) from different vintages and from representative wine cellars along the Penedés region has been performed. The sensory analysis showed two main groups of wines, younger wines characterised by a marked fruity and floral aroma and fresh taste and older wines that underwent *crianza*, characterised by more complex odour attributes such as *toasted*, *spicy* and *compote*. The differences in sensory characteristics were related to a higher content of higher alcohol acetates and ethyl and methyl esters of fatty acids in wines from the first group, while the second group was characterized by a lower concentration of esters, but higher of compounds such as furfural, furaldehyde and vitispiranes.

INTRODUCTION

Currently, Xarel.lo is the white grape variety most cultivated in the Penedés (Catalonia, Spain) and it is allowed in the production of wines commercialized under the Origen Denomination (O.D) Penedés. Traditionally, this variety has been used, generally in mixtures, for the production of Cava wines (Spanish sparkling wines). Therefore, most of the scientific studies have been focused on knowing its technological aptitude for the production of this type of wines. However, in recent years the number of monovarietal Xarelo.lo wines produced by using different winemaking technologies has highly increased. This fact shows the necessity of studies focused on the chemical and sensory characterisation of these wines.

Although some previous works have been done on the free and bound volatile composition of Xarel.lo musts, in the case of wines, only De la Presa and Noble (1995) and De la Presa et al., (1995) performed, respectively, the sensory and chemical characterization of wines from this variety. In the latter works, the authors showed sensory and chemical differences in wines from this variety compared to white wines from other typical grapes from Penedés such as Macabeo and Parellada also used for Cava wines production. However, the rather small number of samples employed in these previous studies (two wines of each variety) may not be enough to represent the sensory and chemical characteristics of the Xarel.lo wines from different winemaking technologies currently in the market.

The aim of the present study is therefore, to characterize representative monovarietal Xarel.lo wines that are being commercialised under the O.D. Penedés using both descriptive sensory and chemical analysis. The final goal will be to find relationships between the chemical and sensory characteristics, which may helps in the development of winemaking and viticultural practices that lead characteristic sensory profiles.

MATERIALS AND METHODS

Wine samples. Twenty-five commercial monovarietal white wines (*var. Xarel.lo*) from representative wine cellars from the Penedés region (Catalonia, Spain) and vintages were analyzed. These wines were selected by the Instituto Catalan de la Viña y el Vino (INCAVI) and represent the majority of the Xarel.lo wines from the Penedés region available in the market. The global composition of the wines fit in the requirements of the Spanish O.D. Penedés.

Analysis of non-volatile compounds. Free amino acids and the sum of free amino acids plus peptides were determined by colorimetric methods (Doi et al. 1981). Peptides were quantified by the difference between both determinations. The concentration of high molecular weigh nitrogen compounds (HMWN) was determined following the Bradford method (Bradford, 1976). Total phenolic compounds concentration was determined using the Folin-Ciocalteu reagent.

Analysis of major volatile compounds. Major volatile compounds were determined by direct injection of 1 µL of wine spiked with the internal standard (0.06 g L^{-1} of 3-pentanol in ethanol 10 % v:v) on an Agilent 5890 gas chromatograph. For quantification purposes calibration curves of each standard compound in synthetic wines were made and analysed in the same conditions than the samples.

Analysis of minor volatile compounds. Minor volatile compounds were analysed by HS-SPME-GCMS. The extraction was performed with the exposure of a 85 μm CAR-PDMS fibre to the headspace of the wine for 20 minutes at 40ºC and constantly stirring (500 rpm). The fibre was desorbed in the GC injector port in splitless mode for 10 minutes. A mass spectrometer Agilent 5973N was used for qualitative purposes. Quantitative data were obtained by calculating the relative peak area in relation to that of the internal standard (methyl-nonanoate). For quantification, calibration curves of each standard compound in synthetic wines were made and analysed in the same conditions than the samples. A semi-quantitative analysis assuming that component response factors were the same as the response factor of the internal standard was performed for those compounds for which none reference compound was available.

Sensory descriptive analysis (DA). The sensory panel was composed of 12 trained judges with extensive experience in sensory analysis. Specific training sessions were previously carried out with the aim to look for the most adequate descriptive terms to be used with the wines. From these preliminary tests 16 terms related to the odour (*white flower, white fruit, stone fruit, citric, tropical, fresh grass, dry grass, compote, spicy, toasting* and *lactic*), taste (*fresh* taste, texture, persistence) and color (intensity and tonality) were selected. Wine samples were evaluated in triplicated in three formal sessions that were held in different days.

Data Analysis. The statistical methods used for the data analysis were: one-way ANOVA and Scheffe test for means comparisons; principal component analysis (PCA) (from correlation matrix) was used to examine the relationship among the variables and between samples; cluster analysis (Ward's method, from standardized data) was used to discover natural groupings of the samples; and partial least square regression (PLS) was applied to predict the sensory attributes of the wines based on the chemical composition.

RESULTS AND DISCUSSION

Sensory Characterization

Principal Component Analysis was applied to the intensity data obtained from the sensory evaluation of the wines. From this analysis, two principal components, which explained 74% of the total variation on the data were obtained. The first principal component (PC1, 41.6% of the total variance) was positively correlated with the descriptors white flower, white fruit, stone fruit and fresh taste (loadings > 0.8) and negatively with compote, toasted, spicy, lactic and dry grass (loadings < -0.8). The second principal component (PC2, 32.7% of the total variance) was negatively correlated with citric and tropical aroma, persistence, colour and tonality. In **Figure 1** the scores of the wines in the four groups, and the loadings of the sensory descriptors, are plotted on the plane defined by the two first principal components. As can be seen, four groups of wines which were already noticed by cluster analysis (data not shown) can be distinguished. Wines from group 1, were better characterized by descriptors associated to the PC1, on the contrary, wines included in groups 2 and 3 were very little associated to them. The PCA also revealed a fourth group of wines (group 4) that were negatively related with PC1 (on the contrary to that happened with wines from group 1).

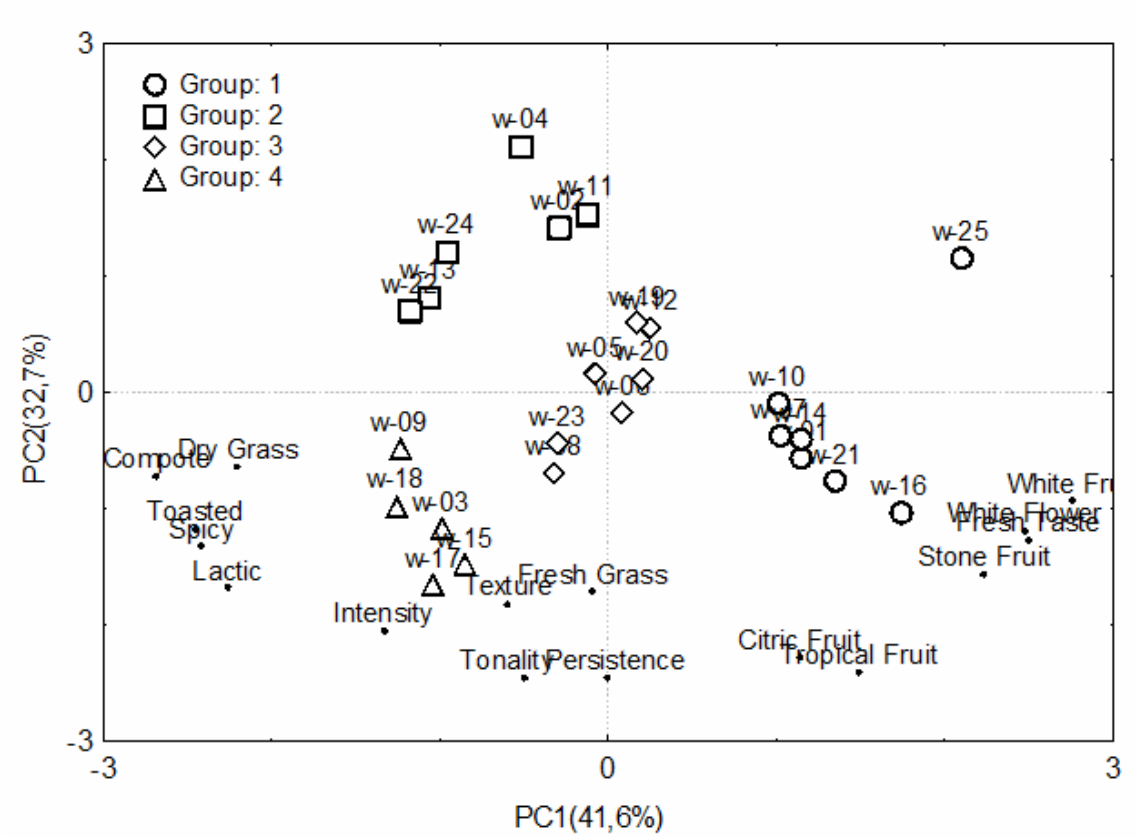

Figure 1. Plot of the wines in the four groups and the loadings of the intensity of sensory attributes on the plane defined by the first two principal components obtained from the PCA.

Chemical Characterization

The sensory differences found between wines can be related to differences in their chemical composition. Therefore, the chemical characterization of the non-volatile and volatile composition of the wines was carried out. The concentration of free amino acids, peptides and polysaccharides was in agreement with previous studies performed in Cava base wines produced from Xarel.lo grapes (Martínez-Rodríguez and Polo, 2003). The total polyphenol concentration showed a higher dispersion due to the *crianza* process that underwent some of them.

Regarding the volatile composition, 59 volatile compounds were identified. All the major volatile compounds (acetaldehyde, ethyl acetate, methanol, 1-propanol, isobutanol, isoamylic alcohols and ethyl lactate), were present in all the samples. Other minor volatile compounds also detected in most of the wines were mainly esters and specifically higher alcohols acetates and ethyl esters of fatty acids. Most of these compounds are important contributors to the fruity and flowery aroma of wines. The three medium chain volatile fatty acids hexanoic, octanoic and decanoic acids and the two alcohols 1-hexanol and 2-phenylethanol also were detected in all the wines. Limonene, linalool and α-terpineol were the only terpenic compounds detected in the wines, and in general, they appeared at very low concentration. In general, it has been indicated that their occurrence in wines can be considered as a quality factor, since they seem to supply pleasant scents to the wines such as tobacco, fruit and tea. Among them, β-damascenone was identified only in 20% of the wines at concentration above 9 μg L^{-1}

and because of the very low perception threshold of β-damascenone (45 ng L^{-1}) (Camara et al., 2007), this compound might have a great importance for wine aroma. Other important norisoprenoids that were identified in the wines, were the two vitispirane isomers and the 1,1,6-trimethylnaphthalene (TDN). The three of them were identified in more than 80 % of the wines. Other compounds identified in the wines were some furfuryl compounds such as furfural, 5-methyl-furfural, ethyl-2-furancarboxylate and acetyl-furan, which are carbohydrate degradation products that can increase with aging bottle (Rapp and Mandery, 1986).

Correlation between sensory characteristics and chemical composition of the wines

Partial least squares regression (PLS) was applied to predict the sensory attributes of the wines based on the instrumental variables (global composition, volatile and non volatile compounds). The size and sign of the values of the regression coefficients in the model for standardized predictor variables can be used to know the variables that most contribute (positively or negatively) to the prediction of the sensory attributes. The PLS results, regression coefficients for the variables that most contribute in the prediction of specific sensory attributes, number of selected components and the determination coefficient (R^2), are shown in **Table 1.** In addition, the table is showing (in brackets), the values of the correlation coefficients, significantly different from zero ($p<0,05$), between the instrumental variables and the sensory attributes. In general, the selected variables that were positively related to *white fruit* and *white flower* were higher alcohols acetates (3-hexen-1-ol acetate, phenyl ethyl acetate, hexyl acetate) and ethyl and methyl esters of fatty acids (methyl octanoate, methyl decanoate and ethy decanoate). This is in agreement with the high involvement of these compounds in the characteristic fruity and flowery aroma of some young white wines. In addition, *white flower* and *white fruit* attributes showed a relative high correlation (0.77 and 0.78) (**Table 1)** with hexyl acetate. On the other hand, and as it is shown in **table 1**, compounds such as diethyl succinate, vitispirane and TDN, were negatively associated to all the fruity attributes. These compounds have been shown to increase during wine aging (Pozo-Bayon et al., 2003). In addition, it is interesting to underline that the attribute *fresh taste* followed a similar trend (**Table 1)** than that observed by the *fruity* and *floral* characteristics and it was also associated to the higher alcohols esters and higher alcohols acetates. In general, esters did not show a contribution to the *spicy* and *toasted* sensory characteristics and even some of them such as 3-hexen-1-ol-acetate and isoamyl acetate were negatively correlated to both sensory attributes (**Table 1).** Interestingly, the only volatile compounds that seemed to contribute the most to both sensory characteristics were furfural to the *toasted* and furaldehyde to both of them (**Table 1**). These compounds are carbohydrates degradation products and it has been shown they can increase with aging bottle (Rapp and Mandery, 1986). In addition, they may have been released into the wines that underwent *crianza* process, since both volatiles may be produced by degradation of polysaccharides during oak wood toasting. It was also interesting the positive contribution showed by other non volatile variables, such as color intensity (CI), polyphenols and alcoholic degree to the *toasty* or *spicy* attributes. This positive association seems to be linked to older wines or to wines that underwent *crianza*, as accounted for all the wines from group 4, in which both sensory attributes were rated the highest as compared to the wines from the three others groups.

Table 1. Regression coefficients from PLS model, for the variables that most contribute in the prediction of specific sensory attributes, and (the correlation coefficient, significantly different from zero).

	Sensory Atributes						
Instrumental variables	**White Flower**	**White fruit**	**Stone fruit**	**Tropical fruit**	**Spicy**	**Toasted**	**Fresh taste**
CI (color intensity)	-0.045 (-0.66)	-0.047 (-0.71)			0.14 (0.69)	0.13 (0.54)	-0.048 (-0.65)
Diethyl succinate	-0.045 (-0.66)	-0.049 (-0.74)	-0.044 (-0.65)	-0.035 (-0.5)			-0.042 (-0.62)
Ethyl 2 methyl butanoate	-0.040 (-0.58)	-0.046 (-0.68)	-0.044 (-0.66)	-0.034 (-0.5)			-0.042 (-0.62)
Ethyl 3 methyl butanoate	-0.039 (-0.57)	-0.041 (-0.61)	-0.036 (-0.54)				
Vitispirane 1	-0.042 (-0.61)	-0.046 (-0.69)	-0.036 (-0.54)	-0.035 (-0.49)			-0.042 (0.59)
Vitispirane 2	-0.041 (-0.61)	-0.046 (-0.69)	-0.035 (-0.53)	-0.035(-0.50)			-0.040 (-0.61)
TDN		-0.040	-0.035	-0.323			
Ethyl acetate						0.11 (0.6)	
Polyphenols					0.113 (0.53)		
Isoamyl alcohols			-0.038 (-0.57)	-0.034 (-0.49)			
2-Phenyl Ethanol				-0.039 (-0.55)			
3-Hexen-1-ol acetate	0.040 (0.59)	0.051 (0.77)	0.043 (0.65)	0.047 (0.50)	-0.066 (-0-49)	-0.075 (-0.58)	0.041 (0.60)
Methyl octanoate	0.036 (0.53)						0.037 (0.54)
Isoamyl acetate		0.042 (0.63)			-0.060 (-0.44)		0.036 (0.54)
Hexyl acetate	0.053 (0.77)	0.052 (0.78)	0.038 (0.6)	0.377 (0.53)		-0.06 (-0.54)	0.044
Phenyl ethyl acetate	0.042 (0.54)	0.042 (0.62)					
Methyl decanoate	0.044 (0.64)			0.037 (0.52)			
Isoamyl decanoate			0.034 (0.52)		-0.060		
Isopentyl hexanoate			0.037 (0.55)	0.065 (0.51)			
peptides			0.036 (0.53)	0.038 (0.54)			
Ethyl decanoate	0.044 (0.65)	0.044 (0.66)	0.044 (0.66)	0.053 (0.75)			0.043 (0.64)
Decanoic acid				0.035 (0.49)			
Furfural						0.12 (0.62)	
5-Methyl furfural					0.117 (0.44)	0.11 (0.52)	
Linalool						-0.069 (-0.42)	
R^2	**0.63**	**0.70**	**0.62**	**0.57**	**0.77**	**0.74**	**0.60**
Number of components	**1**	**1**	**1**	**1**	**2**	**2**	**1**

CONCLUSIONS

Four different styles of monovarietal Xarel.lo wines were found based on their sensory characteristics. Among them, two styles were perfectly distinguishable: young wines, characterized by a marked fruity and floral odour and fresh taste and older wines that underwent *crianza*, characterized by more complex sensory attributes such as *toasted*, *spicy* and *compote* odours. The differences in the two styles were related to a higher content of higher alcohol acetates and ethyl and methyl esters of fatty acids in the case of young wines, while the second style was characterized by lower concentration of esters, but higher concentration of compounds related with wine aging, such as furfural, furaldehyde and vitispiranes. These results may contribute to the promotion of the use of autochthonous grapes varieties to produce high quality wines with distinctive sensory characteristics helping to diversify the current wine market.

ACKNOWLEDGMENTS

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REFERENCES

Bradford, M.M. 1976. Rapid and sensitive method for quantitation of microgram quantities of protein utilizing principle of protein-dye binding *Anal. Biochem.* 72: 248-254.

Camara, J.S., Alves M.A. and Marques J.C. 2007. Classification of Boal, Malvazia, Sercial and Verdelho wines based on terpenoid patterns. *Food Chem.* 101: 475-484.

De la Presa-Owens, C., Lamuela-Raventos R.M., Buxaderas S. and De La Torre-Boronat M.C. 1995. Characterization of Macabeo, Xarello, and Parellada white wines from the Penedes region .II. *Am. J. Enol. Vitic*. 46: 529-541.

De la Presa-Owens, C. and Noble A.C. 1995. Descriptive analysis of 3 white wine varieties from Penedes. *Am. J. Enol. Vitic*. 46:5-9.

Doi, E., Shibata D. and Matoba T. 1981. Modified colorimetric ninhydrin methods for peptidase assay. *Anal. Biochem.* 118: 173-184.

Martínez-Rodríguez, A.J. and Polo M.C. 2003. Effect of the addition of bentonite to the tirage solution on the nitrogen composition and sensory quality of sparkling wines. *Food Chem*. 81: 383-388.

Pozo-Bayon, M.A., Pueyo E., Martin-Alvarez P.J., Martinez-Rodriguez A.J. and Polo M.C. 2003. Influence of yeast strain, bentonite addition, and aging time on volatile compounds of sparkling wines. *Am. J. Enol. Vitic*. 54: 273-278.

Rapp, A. and Mandery H. 1986. Wine aroma. *Experientia*. 42: 873-884.

COMBINATION OF AN IMMUNOAFFINITY CLEAN-UP AND THE RIDASCREEN®FAST OCHRATOXIN A FOR THE SIMPLE QUANTIFICATION OF THE MYCOTOXINE IN WINE

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ABSTRACT

The presence of the mycotoxin Ochratoxin A on grapes and in wine is mainly due to the presence of Aspergillus sp. Since this secondary metabolite is highly toxic, a threshold level of 2 µg/L wine has been adopted by the European Union (1881/2006/EC). Besides chromatographic methods, immunochemical assays are a cost-effective alternative for screening purposes. For sample preparation, wine is diluted with buffer and cleaned by immunoaffinity columns (RIDA® Ochratoxin A). The methanolic eluate is diluted and analyzed by RIDASCREEN®FAST Ochratoxin A, a competitive and quick ELISA system, which generates results within 15 minutes. The limit of detection was determined by analyzing 19 blank wines (red and white) and resulted in a value of 0.5 µg/L. Spiking experiments with a red and a white wine were performed at levels between 1 µg/L and 4 µg/L and revealed recoveries between 81 and of 107 %. The intraassay coefficient of variation was determined in spiked white and red wine samples and resulted in a values between 6.8 and 15% depending on the concentration. The method was evaluated by participation in FAPAS proficiency testings. Overall, it is a quick and reliable alternative to the official HPLC-method of the OIV (MA-E-AS315-10-OCHRAT).

ABSTRACT

Das Vorkommen des Mykotoxins Ochratoxin A auf Weintrauben und in Wein geht zurück auf die Anwesenheit von Aspergillus sp. Aufgrund der hohen Toxizität dieses sekundären Metaboliten wurde ein Grenzwert in Höhe von 2 µg/L im Wein gesetzlich innerhalb der EU geregelt (1881/2006/EC). Neben chromatographischen Methoden stellen immunchemische Tests eine kostengünstige Alternative zum Screenen der Proben dar. Für die Probenvorbereitung wird der Wein mit Puffer verdünnt und affinitätschromatographisch unter Einsatz der RIDA® Ochratoxin A Säulen gereinigt. Das methanolische Eluat wird verdünnt und im RIDASCREEN®FAST Ochratoxin A ELISA quantifiziert. Dieser kompetitive ELISA liefert Ergebnisse innerhalb von 15 min. Die Nachweisgrenze von 0.5 µg/L wurde durch Vermessung von 19 Rot- und Weissweinen ermittelt. Für die Bestimmung der Wiederfindung wurden je ein Weiss- und ein Rotwein zwischen 1 µg/L und 4 µg/L dotiert. Es wurden Wiederfindungsraten von 81 bis 107 % ermittelt. Der Intraassay-Variationskoeffizient lag zwischen 6.8 und 15 % abhängig von der Konzentration. Die Methode wurde ferner durch Teilnahme an mehreren FAPAS-Runden geprüft und stellt somit eine kostengünstige Alternative zur offiziellen OIV HPLC-Methode (MA-E-AS315-10-OCHRAT) dar.

INTRODUCTION

The main reason for the presence of Ochratoxin A (OTA) during wine production is the infection with so called “black aspergilli” during growth of the grapes. While within the principal infection group of *Aspergillus niger* only 5 % of all strains produce OTA, all *Aspergillus carbonarius* strains are OTA producers. The main period of mycotoxin production is between veraison and ripening. The factors influencing the amount of the toxin are climatic conditions (warm and wet), grape varieties, damage of berries due to insects, fungal infection or irrigation/rainfall. During vinification only 4 % of the total OTA concentrations are found in must and later in wine. The most part remains on solids as grape pomace and lees (data summarized in Visconti et al., 2008). There are only a few methods to reduce OTA in wine for a significant part, but these methods (carbon or bentonite) also reduce the polyphenol content of the wine and therefore the aroma profile (Kurtbay et al., 2008; Visconti et al., 2008).
Due to the European Commission Regulation 1881/2006, a threshold level of 2 µg/kg was set for wine, fruit wine, grape juice, grape nectar, and grape must intended for human consumption. Furthermore, grape pomace maybe used as animal feed or is a rich source to isolate grape seed oil, anthocyanidins, and antioxidants but contains the most part of residual OTA (Solfrizzo et al., 2008). The reference method for the detection of OTA uses an immunoaffinity clean-up step prior to HPLC analysis with fluorimetric detection. Since the majority of viticulture matrices have low or undetectable levels of OTA, a simple and quick screening method for high-throughput analysis is necessary. For later confirmation, highly-sophisticated mass-spectrometric based systems such as e.g. LC-MS/MS could be used.

MATERIAL AND METHODS

Wines and spiking material

Commercially available Italian white (n=11) and red wines (n=8) from different cultivars, regions and years (2000-2005) were used for determination of the limit of detection (LOD) and for spiking experiments. The assay was further tested within the food analysis performance assessment scheme (FAPAS; rounds 1765, 1775, and 1785).

Immunoaffinity clean-up

Six mL wine are diluted with 6 mL 0.4 M sodium phosphate buffer (pH 7.5), mixed and passed through a RIDA® Ochratoxin A column (R1303). The column is rinsed with 5 mL 10 mM phosphate-buffered saline (also containing 0.138 M NaCl; 2.7 mM KCl; pH 7.4) and further rinsed with sodium phosphate buffer (20 mM, pH 7.5). The column is dried by pressing air through the column and eluted with 1 mL methanol. Before ELISA measurement, the eluent is diluted with 2 mL water.

ELISA for detection of Ochratoxin A

For quantification of OTA, the RIDASCREEN®FAST Ochratoxin A (R5402; R-Biopharm, Darmstadt, Germany) ELISA is used. The assay is calibrated with OTA standards ranging from 1 up to 8 µg/L. The antiserum specifically detects OTA. For testing, 50 µL standard or sample solutions (see above) were pipetted to the wells, which are coated with a secondary capture

antibody. In the next steps, 50 µL of an OTA-peroxidase conjugate and subsequently, 50 µL of the OTA-antibody solution were added. After an incubation time of 10 min, the wells were washed three times. For colour development, 100 µL substrate/chromogen solution was added to each well. The reaction was stopped after 5 min with 100 µL stop solution and absorbance is measured at 450 nm. The assay can be performed with single determinations for screening purposes.

RESULTS AND DISCUSSION

Limit of detection (LOD)

The limit of detection (LOD) for OTA determination was calculated from measurements of 11 OTA-free white wines and 8 OTA-free red wines. The readings were extrapolated from the curve, since they were all below standard 2. They were averaged and the threefold standard deviation was added. The estimated LOD was between 0.3 and 0.5 µg/L wine depending on the technician (table 1).

Table 1: Calculation of the limit of detection (LOD) using different red and white wines.

Blank samples	**Person 1**		**Person 2**	
	B/B0 (%)	**µg/L**	**B/B0 (%)**	**µg/L**
wine 1, white	93,4	0,117	104,6	-0,152
wine 3, white	86,7	0,249	101,4	-0,046
wine 4, white	89,9	0,185	103,9	-0,129
wine 5, white	87,3	0,237	96,7	0,054
wine 6, white	86,3	0,258	98,0	0,033
wine 7, white	78,4	0,440	96,2	0,063
wine 8, white	90,0	0,182	95,2	0,080
wine 9, white	84,0	0,308	98,0	0,032
wine 10, white	84,5	0,297	92,9	0,121
wine 11, white	89,6	0,190	99,6	0,006
wine 14, white	86,7	0,251	98,1	0,030
wine 17, red	89,8	0,187	97,4	0,042
wine 19, red	91,0	0,163	93,2	0,115
wine 20, red	91,1	0,161	100,0	0,000
wine 22, red	84,3	0,300	93,7	0,105
wine 23, red	95,5	0,080	98,3	0,027
wine 25, red	101,6	-0,028	109,1	-0,162
wine 27, red	94,6	0,096	101,1	-0,018
wine 28, red	87,4	0,235	97,2	0,046
mean	**89,1**	**0,21**	**98,7**	**0,01**
SD	5,1	0,10	4,1	0,08
LOD		**0,51**		**0,26**

Recoveries and Repeatability

Spiking white wine with OTA at a level of 1, 2, and 4 µg/L resulted in recoveries of more than 80 %. The recovery for OTA was between 87 and 107 %, when spiking a red wine in a range from 1 up to 4 µg/L (table 2). The intra-assay coefficient of variation (CV; repeatability) was determined by measuring the analyte in a spiked samples in one assay run (n=6). The measurement of spiked white wines at different concentrations revealed a CVs of 6.8 up to 9.1 %. In red wine, the intraassay CV was only higher in the sample spiked at a level of 1 µg/L.

Table 2: Determination of recoveries and repeatability of OTA from a white and a red wine

Replicate	**wine 11 (white)**			**wine 27 (red)**		
	1 µg/L	**2 µg/L**	**4 µg/L**	**1 µg/L**	**2 µg/L**	**4 µg/L**
1	1,09	1,74	4,01	1,04	2,08	3,82
2	1,10	1,74	3,63	0,81	2,17	3,36
3	1,14	1,57	3,59	0,88	2,15	3,49
4	1,08	1,61	2,98	1,16	2,42	3,74
5	0,96	1,75	3,37	1,06	2,17	3,33
6	0,95	1,45	3,40	0,82	1,81	3,18
7	1,10	1,49	3,71	0,82	2,22	3,36
mean	**1,06**	**1,62**	**3,53**	**0,94**	**2,15**	**3,47**
% recovery	**106%**	**81%**	**88%**	**94%**	**107%**	**87%**
CV	6,8	7,7	9,1	15,3	8,5	6,7

Proficiency testing: FAPAS samples

The assay was further validated by the participation within three FAPAS proficiency testing rounds (table 3). All three results show that the combination of immunoaffinity clean-up and ELISA is suitable for determination of OTA in wine. The somewhat lower recoveries compared to the assigned value are due to the documentation of uncorrected values, which means that a laboratory internal recovery rate was not taken into account.

Table 3: Results of FAPAS proficiency testings

FAPAS report No.	**Assigned value** µg/L	**Result (ELISA)** µg/L	**Recovery** %	**z-score**
1765	1.61	1.36	84	-0.7
1775	1.02	0.74	73	-1.2
1785	0.88	0.78	89	-0.5

During a worldwide interlaboratory study to characterize the capability of different HPLC, methods to determine OTA in wine, these recoveries and therefore z-scores were also found for the great majority of all HPLC-systems tested (Ratola et al., 2006).

CONCLUSION

The described assay is suitable for high throughput analysis of OTA in red and white wine. It fulfils all requirements of a screening method and is a simple, quick, and reliable alternative to the official OIV HPLC-Methode(MA-E-AS315-10-OCHRAT).

BIBLIOGRAPHY

Kurtbay H.M., Bekci Z., Merdivan M., Yurdakoc K., 2008. Reduction of Ochratoxin A levels in red wine by Bentonite, modified Bentonite, and Chitosan. J. Agric. Food Chem., 56: 2541-2545.

Ratola N., Barros P., Simoes T., Cerdeira A., Venancion A., Alves A., 2006. Worldwide interlaboratory study on the determination of ochratoxin A in different wine type samples. Talanta, 70: 720-731.

Solfrizzo M., Panzarini G., Visconti A., 2008. Determination of Ochratoxin A in grapes, dried vine fruits, and winery byproducts by High-Performance Liquid Chromatography with fluorimetric detection (HPLC-FLD) and immunaffinity cleanup. J. Agric. Food Chem., 56: 11081-11086.

Visconti A., Perrone G., Cozzi G., Solfrizzo M., 2008. Managing ochratoxin A risk in the grape-wine food chain. Food Addit. Contam., 25: 193-202.

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ClAIM

Chemical mutant of antibiotic N243 and $NaNO_2$ received by ultra-ray influence of highly-productive stums (N243-11, N243-11-197), and physiological an morphological characteristics studies determine Heir stability. It's been affirmed: from pointed antibiotic cultural liquid is grown received antibiotic. It's been affirmed: the pointed suppresses natural yeast microflora in fermentation natural half-sweet wine-material in defined doze and it becomes possible to stop the processing and its further stabilization.

Donc il est établi: le dégagement de l'antibiotique est augmenté du liquide cultural de la souche #243-11-197. Il esr établi: après I'introduction du susdit antibiotique dans les matières de vin naturellement semi-doux en cours de fermentation, il empêche le développement de toute sorte de microflore de la levure naturelle. Il est possible d'arrêter ce processus à un certain niveau et d'assurer sa stabilité subséquente.

Theme title: New products, new technologies, new output
Theme subtheme title: Traditional and modern Biotechnologies in winemaking
Type: Poster
Language: English
Title: Receipt of antibiotics and their influence study (yeasts, bacteria, fungi) in microorganisms

The necessary condition for the development of modern microbiological industry is receiving and usage of highly-productive stum-producent of biologically active substances.

The aim of our investigation was the antibiotic secretion from soil with the aim of finding among them highly-productive stum of producent of antibiotic 243 on the basis of modern methods selection usage of industrial microorganisms of active substances producents.

It has been studied the induced changability of the stum under the influence of $NaNO_2$ and ultra-rays according to the morphology of antibiotico-creation colonies with theaim of getting highly-productive stum. Spore suspension was processed by mutagens, brought in liquid nutritious miljeu, in the concentration 3-4,5%, in durable influence from 1 to 5 hours, ultra-rays were used in the dosage from 500 to 2000 units/mm^2.

After activity of $NaNO_2$ among received mutants were collected 4 more active stums, after action of ultry-rays-one highly productive stum.

In table 1 given results indicat that studied by us the stums of actinomicites mainly are characteried by similar with control spectrum antibiotic activity. However, test-culture Trichophyton gipseum differs in this respect from the rest that it is suppressed by the stum 243-11.

As for the influence of control and mutant stums on the activity, they are suppressed only by Act. scabies and the influence of mutant stums is more significant. The stum 243-11 is the exception in the case that it is provided with the posibility of suppressing Thichophyton gipseum.

During studying of cross antagonism among mutant stums It's been established that the antagonism among them is not revealed. These data give us the foundation to affirm that given stums by nature are close to each ather.

Table 1.

The Selected stum antibiotic spectrum Actinomyces Levoris (the method of agar blocks)

Test-culture	Zone of delay of grouth, mm					
	N stum					
	243-2	243-8	243-11	243-34	243-47	243-197
Staph. Aureus	0	0	0	0	0	0
Sapcina Lutea	0	0	0	0	0	0
Bact. Mycoides	0	0	0	0	0	0
Bact. Subtilis	0	0	0	0	0	0
Bact. Coli	0	0	0	0	0	0
Bact. Prodiqiusum	0	0	0	0	0	0
Mycobacterium B-5	0	0	0	0	0	0
Mycobact. Corynobact.	0	0	0	0	0	0
Mycobact. Phlei	0	0	0	0	0	0
Saccharomyces cerevisiae	5	5	7,5	4	4	4
Torula utilis	5	4	6	5	6	5
Candida albicans	4	4	4	4	3,5	4
Candida tropicalis	2	2	2	2	2	2
Aspergilus niger	3	3	3,5	2	3	3
Fusarium solani	2	3	3	1	3	2
Thichophyton gipseum	0	0	0	0	0	0
Penicill granul	0	0	0	0	0	0
Act. Albus	0	0	0	0	0	0
Act. Globispor	0	0	0	0	0	0
Act. Griseus	0	0	0	0	0	0
Act. Scabies	0,5	4,0	1,5	1,0	1,5	1,0
Act. Violaceus	0	0	0	0	0	0
Act. Flavus	0	0	0	0	0	0
Act. Lavendulae	0	0	0	0	0	0

In table 2 given results show that quantitative definition of antibiotico formation productivity of mutant stums of producent of antibiotic by the spectrophotometric method showed the high productivity of studied stums in comparison with the control. Among the pointed stums 243-11, forming 8230 units/ml and 243-11-197, the activity of which is 30300 units/ml. (Peikrishvili and others, 1976; Peikrishvili and others, 1981).

Table 2.

The Selected stum activity of the producent of the antibiotic 243

N stum	Activity of cultural liquid (units/ml)	% to initial stum
Initial 243	5000	100
243-2	6130	122
243-8	8000	160
243-11	8230	164
243-34	7084	141
243-47	5060	101
243-96	20 000	400
243-197	30 300	606

During the species definition belonging of studied mutant stums on the basis of morphologycal, cultural, physiological and antibiotic study It's been determined they all as the stum 243-2, belong to the species Actinomyces Levoris Krass. It's been determined that each of them produced fungicid antibiotic 243.

Thus, antibiotic 243 is fungicid antibiotic with wide spectrum of action, supprissing natural yeast microflora and can be used as inhibitor of alcohol fermentation (Kandelaki, Peikrishvili, 1985; Kandelaki, 1991).

The suggested method is carried out in the following way: production of the natural half-sweet grapewines according to the known technology. For this in the process of alcohol fermentation on geading winematerials to conditioning content of natural fementation spirit and sugar, the winematerial is poured in a new capacity and in the proportion of the winematerial flow introduce antibiotic 243 in the solution of 0,05 n of sodium hydroxide and 10% ethanol at a rate of 20 units per 1 mln yeast cells. The antibiotic 243 introduced in the winematerial doesn't undergo chemical conwerting, and turns into the insoluble form and falls into the sadiment. During pouring by the decantation and further lightening of winematerials the antibiotic 243 is fully removed from them togheter with the sediment. Treated by the antibiotic the winematerial is stored in the vessel at usual temperature in the conditions of the wineplant without the necessity of cold usage. Ready production maintains neither residue quantity of antibiotic 243, nor the products of its decomposition, It's subjected for cold bottling and is sent to realization with the guarantee of stability and high quality.

The usage of thesuggested method provides suddem cease of alcohol fermentation in fermentiring winematerials of natural half-sweet wines with the guarantee of stability from refermentation and duration of storage, without pinching aroma and taste harmony of wine, as the result the improvement of quality of the production increases. High activity of the antibiotic 243 and its dosage not according to the mass, but fungicid activity and its content in winematerials of yeast cells provides its rational usage, that in its turn lowers the production cost price.

Bibliography

1. Peikrishvili I.P., Kandelaki N.D., Mosashvili V.A., 1976, Author certificate on the invention: "Stum actinomicate Act. Levoris 243-11", N 550423 (USSR)-Discoveries, Bulletin N10, p. 68.
2. Peikrishvili I.P., Kandelaki N.D. and others, 1981, Author certificate on the invention: "The method of half-sweet grape wines averting from fermentation", n 881114 (USSR) - Discoveries, Bulletin N42, p. 134-135..
3. Kandelaki N.D., Peikrishvili I.P., 1985, Natural and induced ultra-ray changability of the producent telavimicine Streptomyces Levoris, Krass, Stum 243, Journal, antibiotics and medical biotechnology, N7, p.529-532, Moscow.
4. Kandelaki N.D., 1991, The antibiotic – inhibitor of alcohol fermentation, Viticulture and winemaking, Yalta, N5, p. 60-62.

Perfection of Wine Technology Processes for Improvement of Its Quality

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ABSTRACT

A quality and a stability of the wines produced in Georgia, are among the main factors for promotion of their realization. The heterocyclic products as well as the ones received as a result of transformation of the aromatic amino-acids are researched on different stages of boiling. On the grounds of Chromatographic analysis , several classes of compounds bearing the features of phenols, alcohol, amines, and indones, are fractioned and their effect on qualitative characteristics of the wines are studied-in. The turbid complex biopolymers are received from the turbid wines and their chemical analysis is performed, proving that a summarized preparation of such biopolymers extracted from the wines, form the phenol-protein-lipo-polysaccharide set, which concentration in excess of 5gm/dm^3 causes a colloidal turbidity

L'amelioration de la stabilite et de la qualite du vin representait l'objectif principal de notre recherché, por cette raison l'experience deroulait vers deux directions: 1. perfectionner des qualites organoleptiques du vin par l'ameloiration de l'arome et du bouquet. 2. Etudier des bio-polymeres complexses provoquant la turbidite du vin et definir les voies pour l'evitier. Il a ete etudie les produits de transformation des aminoacides hetero-circoulaires et aromatiques aux differents stades de la fermentation alcoolique sur le champ alimentaire de synthese. Il a ete fractionne les classe de differents adjuvants qui montrent leur nature phenolique, alcoolique, amine, indole. les bio-polymeres complexes sont recu des vins flous. leur analyse chimique a ete effectuee. Il est etablie que la preparation totale degagee du vin rerpresente le complexe phenolique, albumineux, lipopolysaccharide. il est elabore la methode photo-spectrometrique pour la definition quantitative des bipolymeres complexse du vin. Il est etabli que dans les comcentrations au-dessus de 5 g/dm^3 ils caisent la turbidite colloidale du vin.

INTRODUCTION

In conditions of the market economy, a quality and a stability of a wine as of a marketable product, are the necessary preconditions of its realization. Therefore an improvement of both

the quality and the stability of wines, was the main task of our study. That is why we were conducting the researches in two directions: 1. Perfection of the organoleptic features of the wines through improvement of their aroma and bouquet; and 2. Study-in the biopolymers causing turbidity and determination of the ways of avoidance thereof

Formation of both the aroma and bouquet of a wine is a complex and a long process, in formation of which the nitrogenous substances, including the amino-acids, play an important role. On one hand, the amino-acids serve as the food products for yeasts, while on other hand, as a result of conversion of the amino-acids by effect of the yeasts' ferment system, the organic acids, new amino-acids, amines, aldehydes, highest alcohols, and other substances are originated in wines, which more or les determine its organoleptic indicators and a quality of the product, in general. Despite the fact that numerous scientific works were devoted to the amino-acids conversion products, the issue has not been still researched fully, up now (J-C. SAPISI et RIBEREAU – GAYON. 1969).

MATERIALS AND METHODS

A purpose of our research was to research the secondary products of conversion of the heterocyclic and aromatic amino-acids (hystidin, triptophan, tyrosin, phenyl-alanyn). For ensuring an intensive alcohol fermentation, Rider's 10% synthesis area has been boiled with imputing therein the above mentioned amino-acids in amount of 2% and the dry yeasts produced by the German firm "Diorell", separately. For the control purposes, fermentation of the same solution was carrying out without imputing therein the amino-acids, during 12 days, in the thermostat on 25°C temperature regime. Intensity of fermentation was controlled through determining the energy of the sugar content's boiling and of the content of the accumulated ethyl alcohol (Valuiko G.G. 1980).

On the basis of the chromatographic analysis, the products of amino-acids' conversion have been fractioned and the condition, size and colour of revealing glaze (Rf) determined. The (Rf)-value of the isolated compounds are given below in Tab. 1.

Table 1

Rf-value of the Identified Compounds

No	Amino-acidsi	Reagents used			
		Vanilla	Iodine	Phosphorus-Molybdenum	Paul
1	Tyrosin50	0.10	0.70	0.30	0.35
		0.40	0.53	0.45	0.20
				0.75	0.50
					0.70
2	Triptophan	0.56	0.57	0.20	0.58
		0.76		0.80	0.83
					0.30
3	Phenyl-Alanyn	0.50	0.63	0.40	0.28
		0.90		0.60	0.40
		0.40			
4	Hystidin	0.40	0.39	0.25	0.30
		0.60			

As shown in the Table, a number of classes of compounds are identified. On the basis of their qualitative analysis, these compounds have the features of phenols, alcohol, amines, and

indones. Besides, no chromatographic glazes were found in the control solution, that indicates that the fractioned compounds are the secondary products of alcohol fermentation originated as a result of releaing the relevant amino-acids from carboxiles and amines. Their synthesis follows to dissolution of the sugar.

The experimental researches revealed that the alcohol fermentation was carried out intensively in case of use of the dry yeasts – wine mud, oenoform-ruzhe, zecoferm, while an intensification of the fermentation served as a promoting factor to accumulation of the experimental compounds within the reaction area. Of the identified compounds the aromatic oxyphenyl-ethyl alcohol (Tyrosol) was dominating, which has been received in a form of an icy crystal-like transparent solid substance and characterized by a pleasant aroma and flavour of honey. This substance is represented in the composition of the plant named Rodiola, which is characterized by a stimulating and adapting properties. In the Georgian wines, this alcohol haws not been studied yet. Therefore its research seems to be actual.

We have developed a spectrophotometric method for determining the quantitative values of Tirosol. The wines of various types and ages produced from the widely spread Georgian grapes have been used as the objects of study. The samples have been taken from the cellar of unique wine collection of the Institute of Horticulture, Viticulture and Wine Making of Georgia, as well as the wineries of Kardenakhi, Gurjaani, Kvareli, and Napareuli. The quantitative values of Tirosol was determined after filtration and procession of the wine materials. The results of the experiment are given in Tab. 2.

Table 2

Tirosol Content in European- and Kakhetian-type Wines Produced in Different Regions (Mg/l)

N.	Sample name	Year	Production Technology	Tirosol Content
1	Khikhvi Kvareli	1961	European	3.00
2	Rkatsiteli Mukuzani	1961	European	4.50
3	Rkatsiteli Napareuli	1962	European	3.45
4	Rkatsioteli Shroma	1961	European	4.20
5	Rkatsioteli Gurjaani	1961	European	4.50
6	Manavis Mtsvane	1961	European	3.45
7	Goruli Mtsvane (in Tamarasheni)	1961	European	3.00
8	Goruli MTsvane (in Vashlijvari)	1964	European	4.20
9	Rkatsioteli Gurjaani	1990	European	4.20
10	Aligote Tsriakhi	1961	European	3.15
11	Tsolikauri	1986	European	3.45
12	Rkatsiteli Tsarapi	1991	Kakhetian	6.60
13	Rkatsioteli Gurjaani	1993	Kakhetian	6.75
14	Rkatsioteli Gurjaani	2000	Kakhetian	6.90
15	Kakheti	1993	Kakhetian	6.00
16	Saperavis SAmtatskaro	1987		7.50
17	Saperavi Gurjaani	1993		7.65
18	Saperavi Akhasheni	1994		13.05
19	Saperavi Gurjaani	2006		13.35

As a result of determination of the quantitative value of Tirosol in wines produced from various sorts of grape, it was revealed that in the European-type wines its content is comparatively low (3 – 4,5Mg/l), regardless the regions of production (western or eastern) of these wines. As to the Kakhetian-type wines and those produced from the red sorts of grape,

its content was higher (6 – 13,4Mg/l). This is conditioned by a factor that the technology of production of such wines envisages involvement of the pressed mass and skin of the grapes in the process of alcoholic fermentation

The experimental studies have been carried out to determine whether the identified compounds have any influence on the wine quality. For this purpose, the identified compounds were put separately in the different wine materials. The results of the wine tasting proved that input of total compounds have no significant influence on the taste qualities of wines while in case of adding the Tirosol, the wines became delicious, and their bouquet improved considerably.

For revealing and fractioning the colloidal compounds and their forms causing a turbidity of wines, we have processed the object selected for testing by use of organic solvents of different polarity Total number of preparations of the complex biopolymers existing in the wine were served as the test object, received from the main technical sorts of grapes widely spread in Georgian namely, Rkatsiteli, Mtsvane, Tsolikauri and Chinuri.

For receiving a total number of the preparations of the complex biopolymers, a filtration of the turbid wine materials and collection, drying and segregation of te rest mass thereof was performed. The received object was then processed by use of the organic solvents of different polarity – extractive petroleum, chloroform, acetone and finally – water. A process of the extraction was performed in the Socslet apparatus (N. G. Mamardashvili. 2009).

In the course of processing the polymeric compounds of different classes were extracted from the object, including the ones met in the wines in a free and independent form, while the rest solid mass, it was of the same type but representing a complex of interlinked compounds. We managed to determine this through the acid- and alkali hydrolyse, during which the links were decomposed. As a result, the complex became disintegrated and we researched its comprising substances. The results obtained are given in Chart 1.

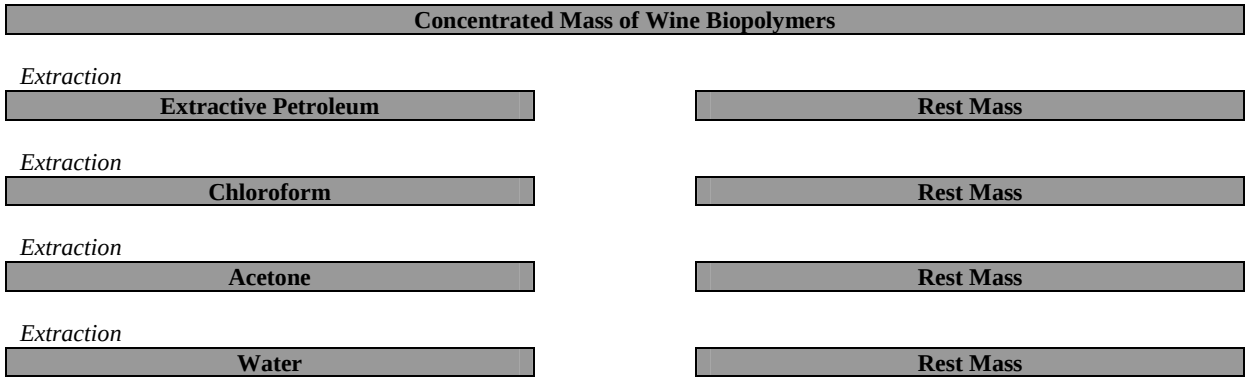


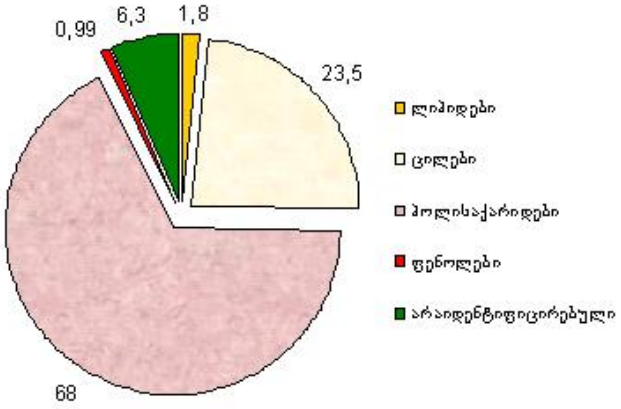
Chart 1. Fractioning of Wine Complex Biopolymers by Different Organic Solvents

We have performed the acid- and the alkali hydrolyse of the rest mass. For defining the optimal regimes of the acid- and alkali hydrolyse, we researched the factors influencing on the course of the above process, namely, the acid concentration 10% for acid, 15% for alkaline) module (1:10 for acid, 1:12 for alkaline), temperature 100°C and duration – 2-2,5 hours.

A chemical composition of the rest mass of the hydrolyse process was then researched and defined: Lipids 1,8%; Total nitrogen 3,7%, proteins 23,5%; T Total poly saccharides (easily hydrolysed 39% + hardly hydrolysed 29%) 68%; Phenols 0,99%.

We have determined and identified the following amino-acids in the received hydrolysates: cystein (in a large quantity), cystin, lysin, ornytin, argarin, aspargin acid,

aspargin, glutamine, oxyplolyne glutamine acid, treonin, β-alanyn, alanyn, tyrosin, tryptophan, isoleycine, methyonin, norvalin, β-phenylalanyn, Leycin. The results are given on the diagram.



Lipids Proteins Polysaccharides Phenols Non-identified

To prognosticate a wine's stability towards the turbidity caused by the complex biopolymers, we have developed a method of their quantitative determination (G. Kokiashvili, 2005).

One more purpose of the study was determination of the complex biopolymers in the wine materials used for producing the white table wines, because a quantitative determination of various organic compounds in the wines takes place on 540nm. Our aim was to define a share of the complex biopolymers in the quantitative data received as a result of measuring them within the ranges of this wave. For this purpose we selected the turbid wines destabilised by effect of the complex biopolymers. We defined the total data of the wine compounds by this method .

Table 3

Content of Complex Biopolymers and Differences between Spectrographic and Actual Indicators

No.	Wine Name	Method g/dm^3	Actual g/dm^3	Difference	%^
1	Gurjaani (1966)	7	4,83	2,17	31
2	Zazisubani (1998)	8	2,64	5,36	33
3	Hereti (1996)	10	3,20	6,80	32
4	Gurjaani (1966)	12	3,36	8,64	28
5	Tsinandali (1997)	11	3,19	7,81	29
6	Gurjaani (1997)	12	3,24	8,76	27
7	Hereti (1998)	9	2,34	6,66	26
8	Tsinandali (1999)	7	2,03	4,97	29
9	Hereti (Sighnaghi1995)	8	2,56	5,44	32
10	Gurjaani (1998)	6	1,86	4,14	31
11	Zazisubani (1996)	11	3,19	7,81	29
12	Hereti (1996)	12	3,6	8,4	30
Average					**32,6**

As evidenced from Tab. 3, an average difference between the obtained and actual data is 32,6%. These data were envisaged by us when developing a final method of determination of the of the complex biopolymers. Thereafter, we have defined a content of the biopolymers in both stable and turbid wines. The results are given in Tab. 4.

Table 4

Quantitative Data of Complex Biopolymers in Stable and Turbid Georgian Wines

No.	Stable Wines	Complex Biopolymers g/dm^3	No.	Turbid Wines	Complex Biopolymers g/dm^3
1	Manavi (1998)	1,1	**1**	Gurjaani (1998)	6,0
2	Vazisubani (1997)	2,0	**2**	Manavi (1997)	7,0
3	Vazisubani (1999)	4,0	**3**	Vazisubani (1999)	6,0
4	Gurjaani (1998)	3,0	**4**	Hereti (1999)	8,0
5	Gurjaani (1999)	4,0	**5**	Hereti (1998)	7,0
6	Vazisubani (1998)	3,0	**6**	Gurjaani (2002)	6,0
7	Gurjaani (2000)	2,0	**7**	Hereti (1997)	10,0
8	Manavi (1997)	1,5	**8**	Vazisubani (1998)	11,0
9	Hereti (1999)	3,5	**9**	Tsinandali(2002)	8,0
10	Tsinandali (2001)	2,0	**10**	Gurjaani (1999)	7,0
11	Kvareli (1997)	1,5	**11**	Kvareli (1997)	9,5
12	Kvareli (1998)	1,8	**12**	Tsinandali (1998)	12,0
13	Zesatafoni (1998)	1,3	**13**	Terjola (1998)	10,5
Average		**2,36**	**Average**		**9,30**

Based upon the analyses conducted, We can conclude that a total number of the complex biopolymers in Georgian white table wines does not exceed 5g g/dm^3, while the turbid wines are characterized by a higher content of such biopolymers,

Therefore, the works performed by us, will contribute to improvement of the wines stabilization and quality

Bibliography:

1. J-C. SAPISI et RIBEREAU – GAYON, Etude dans les vins du tyrozole, du tryptohol, de l'alcool phenylethylique et de la γ-butytolactone, produits secondaires de la fermentation alcoolique. Ann. Technol. agric. 1969 18 (3), 207-219.

2. Valuiko G.G. Methods of Technological and Microbiological Control in Wine-making. Food Industry. M. 1980., p.45.

3. G. Kokiashvili, N. G. Mamardashvili, L. A. Mudziiri. Srudy of Endogene Complex Biopolymers and Application of Various Sorbents for Wine Stabilization. Georgian Engineering News. #1. 2005. Tbilisi. p. 117-120.

4. N. G. Mamardashvili, M. A. Guliashvili, M. G. Kuridze, I. B. Megashvili, L. A. Mudziri, M. L. Khositashvili. Extraction of Lipids from Yeast Depositions of the Grape Wines Georgian Engineering news. #1. (vol. 49). 2009. 188.

ROLE OF GLUTATHIONE ENRICHED INACTIVE YEAST PREPARATIONS ON THE AROMA OF WINES

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RESUMEN

El efecto de preparados comerciales de levaduras secas inactivas ricos en glutatión (GSH-IDY) en la protección del aroma del vino durante su conservación ha sido investigado. Para ello, se elaboraron industrialmente dos vinos rosados a partir de uvas de la variedad Garnacha con y sin adición del preparado de GSH-IDY. La composición volátil se determinó a lo largo de la vida útil del vino (1 a 9 meses). Además en experimentos en vinos modelo, se estudió el efecto de diferentes tipos de preparados sobre compuestos volátiles representativos del vino. Los resultados mostraron que el empleo de los preparados reducen la pérdida de volátiles durante la vida útil del vino. En estos experimentos se observaron diferencias en el comportamiento de los preparados, que pueden ser debidos a la capacidad antioxidante del GSH pero también a la activación de diferentes tipos de reacciones químicas promovidas por la presencia de otros componentes en los preparados de levadura.

ABSTRACT

The effect of commercial glutathione enriched winemaking Inactive Dry Yeast preparations (GSH-IDY) to protect wine aroma during storage has been investigated. To do so, rosé Grenache wines were industrially manufactured with and without the addition of a GSH-IDY preparation. The volatile composition was determined over the shelf-life of the wines (1 to 9 months). In addition, by using experiments in model wines the effect of different types of commercial GSH-IDY preparations on representative wine aroma compounds was carried out. The results showed that these preparations reduced the lost of volatile compounds during the shelf-life of the wines. In the model wines we observed differences in the behaviour of the IDY preparations towards the aroma compounds, which may be due to the antioxidant capacity of the GSH but also on the activation effect of different types of chemical reactions promoted by other components from the IDY preparations.

INTRODUCTION

Currently, the use of winemaking Inactive Dry Yeast preparations (IDY) is gaining interest within the wine industry because of their large amount of potential applications in winemaking. Although they have been mainly used for the improvement of alcoholic and malolactic fermentation, the use of IDY for enhancing wine's sensory characteristics, is one of the most promising and interesting applications (Pozo-Bayon *et al*, 2009a).

The impact of IDY in wine's sensory properties is due to the ability of yeast components to modify wine chemical composition. As a matter of fact, it has been shown that yeast polysaccharides are able to protect wine colour, because of the interaction of yeast mannoproteins with tannins and anthocyanins, therefore, avoiding or minimising polyphenol aggregation and precipitation (Escot *et al*., 2001; Doco *et al*., 2003). In addition, we have shown that some yeast macromolecules released from IDY may affect the volatility of important wine aroma compounds (Pozo-Bayon *et al*., 2009c), which could be related to the sensory differences observed in wines supplemented with these preparations compared to control wines (Comuzzo *et al*., 2006). Moreover, the ability of IDY to release nitrogen heterocyclic volatile compounds, likely formed as a consequence of the thermal reactions accounted for in the last steps during their production has been also shown (Pozo-Bayón *et al.,* 2009b).

Besides of the above mentioned effects of IDY on wine aroma, there are currently in the market, other types of IDYs, which have been claimed to preserve aroma composition during wine storage. The protective effect of these preparations has been associated to the presence of a large amount of glutathione (GSH). This compound is a yeast intracellular tripeptide from non proteic origin, of known antioxidant properties (Penninckx, 2002). Although the use of free glutathione has been shown may avoid the oxidation of some volatile compounds in white wines (Lavigne-Cruege *et al*., 2003), the use of commercial IDY preparations enriched in GSH and their effect on wine aroma composition during wine shelf-life has not been investigated so far.

Therefore, the **objectives** of this work were firstly to study the effect of a commercial GSH enriched IDY preparation on the volatile composition of industrially manufactured rosé wines from Grenache grape variety, and secondly, by using experiments in model wines, to compare the effect of different types of commercial GSH enriched IDY preparations on representative wine aroma compounds.

MATERIAL AND METHODS

Wines and winemaking conditions

Two Rosé wines were made from the same Grenache must in 10000 L tanks in a wine Cellar from *Navarra* O.D. Both wines were from the 2008 vintage. One of the tanks was supplemented with a glutathione enriched yeast preparation (IDY-G) whereas the other tank corresponded to the control wine without IDY (IDY-C). IDY was added to the wines at the concentration recommended by the provider (20 g/Hl), before the alcoholic fermentation took place. Fermentation was carried out by using the same active dry yeast in both tanks. Once alcoholic fermentation was completed, wines were stabilized, clarified and bottled in the own vinery. The wines were storage at 12 ºC during their whole shelf-life.

Model wines

Model wines were prepared by mixing 12% ethanol (v/v) and 4 g/L of tartaric acid. The pH was adjusted to 3.5 with NaOH . Fifty mL of model wine were supplemented with 100 µL of the <3000 Da water fraction previously isolated from 4 g of an IDY preparation. In total three different model wines supplemented with the above

mentioned fractions from IDY were prepared: Two of them with two commercial GSH enriched preparations G1 and G2 (MW-IDY-G1 and MW-IDY-G2) and the third one, was prepared using an IDY usually recommended as a fermentative nutrient (MW-IDY-N1). Control model wines were prepared in the same way but without the addition of the <3000 Da water fraction from the IDY (MW-IDY-C). An aroma solution (0.1 mL) containing typical wine aroma compounds was added into the three types of model wines to obtain the following concentrations of each aroma: geraniol (0.25 mg/L), β-citronellol (0.25 mg/L), ethyl butyrate (0.25 mg/L), ethyl hexanoate (0.15 mg/L), ethyl octanoate (0.15 mg/L), ethyl-decanoate (0.08 mg/L), isobutyl acetate (0.25 mg/L), isoamyl acetate (0.25 mg/L), hexyl acetate (0.5 mg/L) and β-phenyl ethyl acetate (0.25 mg/L). The flasks containing each of the model wines were immediately sealed and kept at 30ºC in stirring conditions until the moment of their analysis to promote their oxidation. The analysis of the aroma compounds was performed after 14 days. All the experiments were performed in duplicated in independent flasks for each type of model wine.

Analysis of volatile compounds by HS-SPME-GC-MS

HS-SPME was used to study the effect of IDY in both, industrially manufactured and model wines, following the method described in Andujar *et al*., (2009) with some modifications. To do so, 8 mL of real or model wines were placed in a 20 mL headspace vial sealed with a PTFE/Silicon septum. For real and model wines methyl nonanoate was used as internal standard. Samples were allowed to reach equilibrium at 40 ºC. The extraction was performed with an automatic autosampler by the exposure of an 85 µm Carboxen-PDMS fiber to the headspace of the sample for 20 minutes at 40 ºC. After the extraction, the fiber was removed from the sample vial and desorbed in splitless mode in the GC injector port for 10 minutes. All the analyses were performed in duplicate.

Separation was performed on a Carbowax 10 M column (30 m x 0.25 mm i.d. x 0.5 µm). The oven temperature was programmed as follows: 40 ºC as initial temperature, held for 5 minutes. In a first ramp the temperature increased to 60 ºC at 1 ºC/min and in the second at 5 ºC/min to 160 ºC then held for 1 minute. In a third ramp the temperature increased to 180 ºC at 20 ºC/min, then held for 2 minutes.

Calibration curves with each of the reference compounds (5 levels of concentration x 2 repetitions) were used for quantitative purposes. The TIC signal of each aroma compound in the headspace of the samples compared to that of the internal standard was used to extrapolate in the calibration curves.

RESULTS

Effect of the addition of glutathione enriched IDY preparations on the volatile composition of wines industrially manufactured.

By using HS-SPME-GCMS, 33 volatile compounds were identified in the wines. Most of them belonged to the esters (ethyl esters of fatty acids and higher alcohol acetates), alcohols, terpenes, and terpenes derivatives, volatile fatty acids and other compounds such as the norisoprenoids β-damascenone and TDN and the aldehyde furfural. Most of them have a fermentative origin, although some terpenes identified in the wines (α-terpinene, linalool, etc) came from the grape. All of them also showed concentration values within the normal values for Grenache rosé wines

To know if there was a natural grouping of the wine samples based on the addition of GSH enriched IDY into the wines, a cluster analysis was performed with the data corresponding to the concentration of volatile compounds in both types of wines during their shelf-life (1,2,3 and 9 months wines). The results are shown in **Figure 1**. As can be seen in this figure, the dendrogram is showing two very well separated groups of wines. The first one corresponded to wines of 3 and less than 3 months, and the second one, included all the wines of 9 months. In addition, within each of these two large groups of samples, the figure, is revealing a clear separation between wines depending on the addition or not of the IDY preparation. These results are showing a major influence of the aging time on wine volatile composition, but also an effect of the addition of the IDY preparation on these compounds.

Taking into consideration these results, an ANOVA analysis to check the effect of both factors (aging time and addition of IDY) and their interactions was performed with the 33 volatile compounds quantified in the samples. The results showed that most of them were influenced by both factors. **Table 1** lists the volatile compounds which were statistically influenced by one of the two factors (or by the interaction between them). To better understand the effect of the IDY preparation over the time, **Table 1** is showing the ratios (in percentage) corresponding to the change in the concentration of a volatile compound in the 9 months wine compared to the concentration in the 1 month sample. This ratio allowed us to know the effect of the IDY preparation on the volatile composition over the shelf-life of the wine.

The results showed that in general, control wines showed a larger decrease in most of the volatile compounds compared to the wines supplemented with the IDY preparations. This was particularly evident for the compounds belonging to the ester group as it is shown in **Table 1.** The lost of this group of compounds during wine aging, has been associated to their slow hydrolysis at wine pH (Rapp and Mandery, 1986). In addition, some alcohols such as 1-butanol and 1-hexanol also decreased in a greater extend in the control wine than in the wine supplemented with IDY. In addition, the concentration of some terpenes remained unchanged or even showed a slight increased during the aging of control wines. However, this increase was much larger in wines supplemented with the IDY preparation. Although during wine aging a slow oxidation of these compounds could have been accounted for, an increase in their concentration may also be possible as a consequence of spontaneous synthesis from a precursor naturally occurring in wines (Jarauta *et al.*, 2005) or, as in the case of linalool, because it can be formed from other monoterpenoids (Pedersen *et al*., 2003). The higher increased of these compounds along the shelf-life in wines supplemented with the IDY preparation compared to the control wines, may indicate a lower oxidation of these compounds in these wines compared to the control wines. Moreover, it is interesting to underline the little change in concentration experienced by some volatile compounds associated to wine oxidation such as furfural in wines supplemented with IDY compared to control wines.

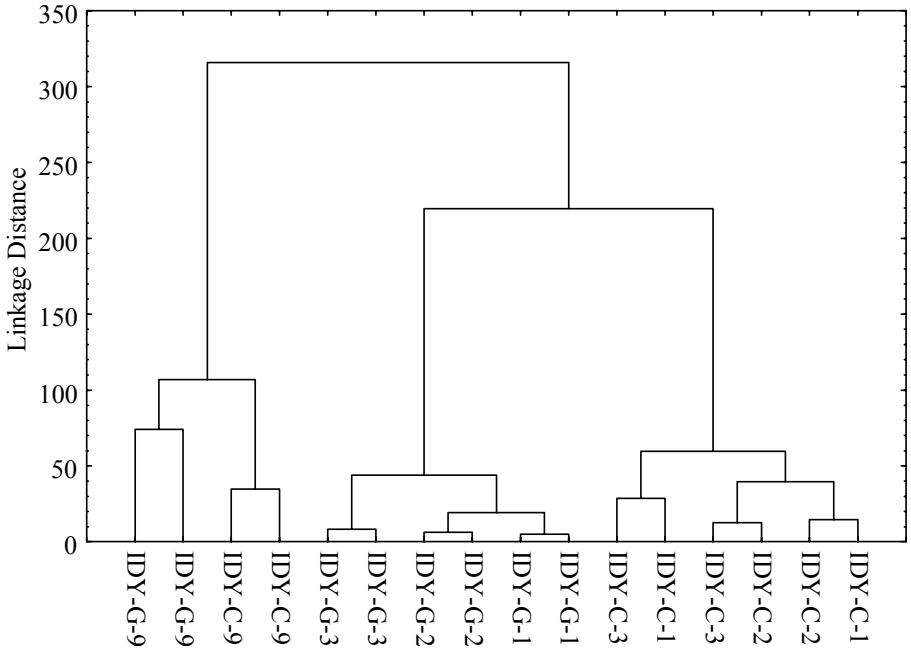

Figure 1. Dendrogram resulting from applying cluster analysis to the data corresponding to the concentration of volatile compounds determined in the wines of different aging time (1, 2, 3 and 9 months) made with or without the addition of a glutathione enriched IDY preparation (IDY-G and IDY-C respectively). (Notice that two repetitions of each wine are included).

Effect of the addition of commercial glutathione enriched IDY preparations on typical wine aroma compounds in synthetic wines under oxidation conditions

Synthetic wines aromatised with typical wine aroma compounds were supplemented with the three types of IDY preparations as was described in the M&M section. Two of them with two commercial GSH enriched preparations (MW-IDY-G1 and MW-IDY-G2) and the third one with a nutrient-type IDY (MW-IDY-N1). A control wine without IDY addition was also prepared. Wines were submitted to oxidation conditions (30 ºC stored for 14 days). The results of this study are presented in **table 2**. In the control wine (MW-IDY-C), a decrease in the concentration of the aroma compounds ranging between 10% (isobutyl acetate) and 91% (ethyl decanoate) and due to the oxidation process was observed. To know the effect of the IDY preparations on each aroma compound, the relative peak area of a compound in the sample with the preparation was compared to the peak area of the same compound in the control sample and the results were expressed as it is shown in the footnote of table 3. Therefore, in table 2, positive ratios means an antioxidant effect, while negative values shows that this compound was more oxidized in the sample with the preparation than in the control wine. As can be seen, the influence of each IDY depended on the type of IDY but also on the physical-chemical characteristics of the volatile compounds. In the MW-IDY-N1 in which the concentration of free GSH from the IDY was lower than 0.01 mg/L, the IDY mainly promoted the oxidation of the three terpenes, which may be due to the presence in the extract of some trace compounds, such as Fe, which could facilitate the oxidation processes (Ferreira et al, 1997). However, in other two model wines, MW-IDY-G1 and MW-IDY-G2, which contained above 2.5 mg/L of free GSH from the IDYs, two

different effects were observed. While preparation IDY-G2 showed antioxidant properties for the three terpenes, IDY-G1 only showed antioxidant effect for geraniol. In the case of esters, ethyl butyrate and isobutyl, isoamyl and β-phenylethyl acetates presented higher concentrations in the three model wines supplemented with the IDY compared to the control wines. However, the change in their concentrations may be also due to hydrolysis or esterification reactions or by hydroxyl radical oxidation reactions (Escudero *et al.*, 2000).

Tabla 1. Values showing the comparison (in percentage) between the concentration of volatile compounds in the 9 months wines compared to the concentration in the 1 month wines. (IDY-C: control wines; IDY-G: wines supplemented with the GSH enriched IDY).

Compounds	Wine type	
	IDY-C	IDY-G
Esters		
Ethyl propanoate	61	100
2-Methyl propanol acetate	31	53
Ethyl butanoate	43	73
2-Methyl etanol butanoate	100	148
Isoamyl acetate	33	100
Ethyl hexanoate	44	66
Hexyl acetate	34	53
Heptyl heptanoate	50	59
Ethyl octanoate	100	50
2-Phenyl etanol acetate	86	100
Alcoholes		
1-Butanol	57	100
1-Hexanol	60	100
cis-3-Hexen-1-ol	67	100
Terpenes		
α-terpinene	146	170
Linalool	100	165
Citronelol acetate	100	53
Fatty acids		
Octanoic	142	100
Decanoic	169	100
Other compounds		
2,3-Butanedione	100	24
Furfural	359	100
γ-Butirolactone	63	100

*Only compounds that showed a significant influence ($p < 0.05$) by one of the two studied factors are included.

Table 2. Results corresponding to the effect of IDY on the aroma composition of model wines submitted to oxidation conditions. Results are expressed in % of change in the concentration of each volatile compared to the control wine

	Model Wines		
Aroma compounds	MW-IDY-N1	MW-IDY-G1	MW-IDY-G2
Terpenes			
β-Citronellol	-13	-2	15
Nerol	-23	-24	21
Geraniol	-8	5	36
Esters			
Isobutyl acetate	21	32	19
Isoamyl acetate	8	12	3
Hexyl acetate	-3	-2	-8
β-Phenylethyl acetate	-4	17	26
Ethyl butyrate	14	22	10
Ethyl hexanoate	-1	-2	-10
Ethyl octanoate	-12	-11	-26
Ethyl decanoate	-43	-49	-53

Values are calculated as: [Area compound in the sample with IDY/ Area compound in the control sample * 100]-100

CONCLUSIONS

The use of GSH-IDY preparations during the winemaking of rosé Grenache wines reduce the lost of volatile compounds along their shelf-life. The studies in synthetic wines also showed lower oxidation of some important aroma compounds such as terpenes when the <3000 Da fraction from GSH enriched IDY preparation was added into the wines. This effect seems to be related to the amount of GSH originally present in the IDY and effectively release into the wines. In addition, an effect of these fractions was also observed on other volatile compounds (esters), which was however, independent of the amount of GSH released into the wines, but that seems to be more related to the catalytic effect of some trace compounds present in the IDY. Therefore, new experiments will be necessary in order to clarify the action mechanisms of GSH-IDY preparations and their truly influence in wine's sensory characteristics.

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REFERENCES

Andujar-Ortiz, I., Moreno-arribas, M.V., Martín-Álvarez, P.J.; Pozo-Bayón, M.A. (2009). Analytical performance of three commonly used extraction methods for the gas-chromatography-mass spectrometry analysis of wine volatile compounds. *J. Chrom. A*, 1216, 7351-7357.

Comuzzo, P.; Tat, L.; Tonizzo, A.; Battistutta, F. (2006). Yeast derivatives (extracts and autolysates) in winemaking: Release of volatile compounds and effects on wine aroma volatility. *Food Chem.,* 99 217-230.

Doco, T., Patrick, V., Cheynier V., Moutonet, M. (2003). Structural modification of wine arabinogalactans during aging on lees. *Am J. Enol. Vitic*., 54, 150-157.

Escot S., Feuillat, M., Dulau, L., Charpentier, C. (2001). Release of polysaccharydes by yeasts and teh influence of the released polysaccharydes on color stability and wine astringency. *Aus. J. Grape Wine Res*., 7, 153-159.

Escudero A, Hernandez-Orte P, Cacho J, Ferreira V. Clues about the role of methional as character impact odorant of some oxidized wines. *J. Agric. Food Chem*, 48 (9), 4268-4272. 2000.

Ferreira V, Escudero A, Fernandez P, Cacho JF. Changes in the profile of volatile compounds in wines stored under oxygen and their relationship with the browning process. *Z Lebensm Unters F A*, 205 (5), 392-396. 1997.

Jarauta., I., Cacho, J., Ferreira, V. (2005) Concurrent phenomena contributing to the formation of teh aroma of wine Turing againg in oak wood: an analytical study. *J. Agric. Food Chem.,* 53, 4166-4177.

Lavigne-Cruege, V., Pons, A., Chone, X., Dubourdieu, D. (2003). Ròle du glutathion sur l'evolution aromatique des vins blancs secs. In : Œnologie VII symposium International d'œnologie. Ed. Tec&Doc, pp. 385-388.

Pedersen, D.S., Capone, D.L., Skouroumounis, G.K., Pollnitz, A.P., Sefton, M.A. (2003). Quantitative analysis of geraniol, nerol, linalool and alpha terpineol in wine. *Anal Bional. Chem*., 375, 517-522.

Penninckx, M.J. (2002). An overview on glutathione in saccharomyces versus non-conventional yeasts. *FEMS Yeast Res.* 2, 295-305.

Pozo-Bayón, M.A., Andujar-Ortíz, I, Moreno-Arribas M.V. (2009a). Scientific evidences beyond the application of inactive dry preparations in winemaking. *Food Res. Int*. 42, 754-761

Pozo-Bayón, M.A, Andujar-Ortíz, I, Moreno-Arribas M.V. (2009b). Volatile profile and potential of inactive dry yeast based winemaking additives to modify the volatile composition of wines *J. Sci. Food Agric.,* 89, 1665-1673.

Pozo-Bayón, M.A., Andujar-Ortíz. I., Alcaide-Hidalgo, JM, Moreno-Arribas, MV. (2009c). Characterization of commercial inactive dry yeast preparations for oenological use based on their ability to release soluble compounds and on their behavior towards aroma compounds in model wines *J. Agric. Food Chem.,*57, 10784-10792.

Rapp, A., Mandery, H. (1986) Wine aroma. *Experientia*, 42, 873-884.

THE AROMAGRAMS DATABASE OF SLOVAK WINES

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ABSTRACT

The aroma compounds analysis could be used for wine classification, quality control or study of sensorial properties. The aroma compounds are responsible for final aromatic impression of wine. Research Institute of Viticulture and Enology successfully developed a special method on wine aroma characterisation, which significantly enriched established database. The database contained data in aromagrams, table and figure form. The database includes more than 10 000 data, which were considered to be statistically significant.

Introduction

Volatile esters are the product of an enzyme-catalyzed condensation reaction between acyl-CoA and a higher alcohol. One of the most important factors affecting ester production is certainly the yeast strain. Not only the average ester production, but also the relative proportion of each individual ester produced, differs dramatically from strain to strain. Volatile esters are only trace compounds in fermented beverages such as beer and wine, but they are extremely important for the flavour profile of these drinks. Aroma-active esters are formed intracellularly by fermenting yeast cells. Being lipid-soluble, acetate esters rapidly diffuse through the cellular membrane into the fermenting medium (Verstrepen *et al*., 2003).
The most common way to classify varietal wines is by monitoring the content of volatile aroma compounds mainly by employing gas chromatography and a subsequent application of various statistical methods (Kružlicová *et al*., 2009). Kružlicová *et al*. (2007) quantitatively analysed wines by gas chromatography using with preceding solid-phase microextraction

performed in a glass microcolumn inserted into the GC inlet. The volatile compounds, creating the wine aroma, were extracted from the headspace into a Tenax microcolumn. The microcolumn was then transferred into a modified GC injection port for thermal desorption and the compounds were released and analyzed. This method was developed by Research Institute of Viticulture and Enology and subsequently the database of aromagrams of Slovak wines was created. Samples are analysed on special treated gas chromatograph, which allows analysing substances in ppb concentration. The analysis is performed on two columns, polar and nonpolar, as from both columns are statistically significant or oenological interesting data (Lakatošová *et al*., 2010).

Material and Methods

The several wine samples of different wine varieties, vintages, categories were studied. Analyses were carried out on a GC 8000 Top Series, CE Instruments (Rodano-Milan, Italy) equipped with a modified split-splitless inlet and flame ionization detector. The inlet was modified so that it was possible to insert a glass microcolumn. The microcolumn was packed with 5.0 mg of 60 - 80 mesh Tenax TA (Alltech, Deerfield, Illinois, USA). The outlet of the microcolumn afforded a tight connection with the capillary column. The fused silica capillary column VF-WAXms, 30 m * 0,25 mm * 0,25 μm film thickness (Supelco, Bellefonte, Pennsylvania, USA), VF-WAXms 30 m * 0,25 mm * 0,5 μm film thickness (Supelco, Bellefonte, Pennsylvania, USA) and VF-5ms 30 m * 0,25 mm * 0,25 μm film thickness (Supelco, Bellefonte, Pennsylvania, USA) were used. The GC inlet and the detector temperatures were 230 °C and the initial column temperature was maintained at 30 °C. Thermal desorption was performed at a pressure of 10 kPa for 1 min, then the pressure was increased to 60 kPa and the column temperature was programmed at a rate of 5 °C/min up to 210 °C and maintained at 210 °C for 5 min. Also temperature program was used with isothermal at 30 °C (4min. or 9min.), then increased at a rate of 5 ºC/min to 200 ºC and hold 10 min. Helium was used as the carrier gas.

A volume of 100 ml of the wine sample plus 20 g NaCl was transferred into a 500 ml volumetric flask and the flask was vigorously shaken for 2 min at ambient temperature. Immediately after shaking, an appropriate volume of headspace (5 or 10 ml) was taken through the microcolumn using a glass syringe with a glass plunger lauer (Poulten and Graf, Wertheim, Germany). The distance between the microcolumn and the surface of the liquid

was about 1 cm. The loaded microcolumn, with the volatile compounds sorbed, was transferred into the GC inlet at 10 kPa carrier gas pressure and the compounds desorbed were analysed as described earlier. Analysis of each wine sample was repeated twice. A computer program Class-VP 7.2 SP1 (Shimadzu, Columbia, Maryland, USA) was used for data acquisition.

Results and Discussion

More than 100 compounds were identified in aromagram, of which 20 were determined as significantly important sensorial active compounds. From alcohols – propanol, butanol, characteristic by alcoholic perception, hexanol evoking windrow grass, important esters recognised especially by fruity aroma. The database was created from acquired aromagrams. The database included aromagrams with description of method (fig. 1), identified compounds in table with odour threshold, sensory characterisation and concentration in measured wine (tab. 1). And also aromatic profile of wine illustrated by figure (fig. 2.).

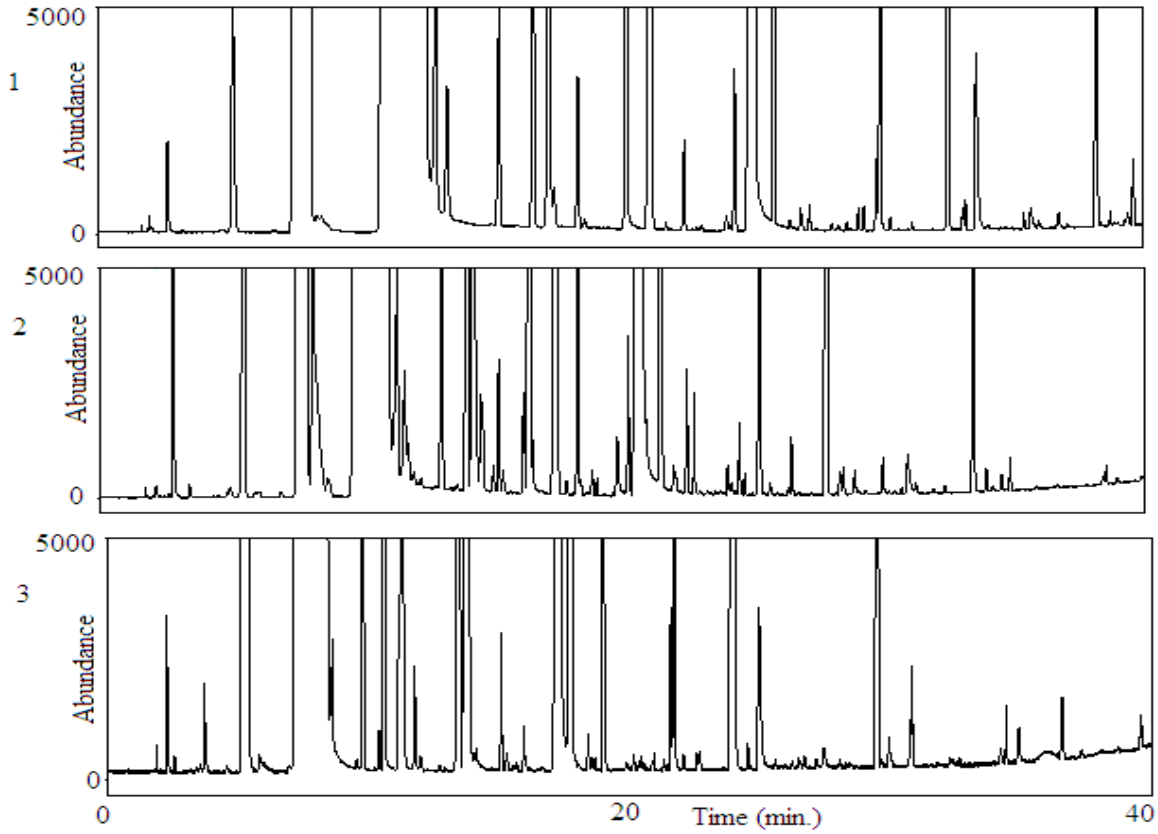

Fig. 1: Aromagram of

1. Veltliner with 10 min.isothermal program, Headspace 5 ml

2. Blaufrankisch with 5 min. isothermal program, Headspace 10 ml

3. Chardonnay without isothermal program, Headspace 10 ml

Tab. 1: Identify compounds with odour threshold, sensory characterisation and concentration in measured wine

peak	compounds	aroma	Odour threshold or Area
1	acetaldehyd	alcohol, orange, sharp	11 – 493 mg/l
2	acetone	ethereally, apple	25-40 mg/l
12	etylacetate	pineapple, anise	40 – 82 mg/l
14	2-butanol	alcoholic, apricot	9 – 37 mg/l
16	propanol	alcoholic	16 – 65 µg/l
22	amylacetate	banana	100-150 mg/l
33	linalool	rose	10-40 µg /l
35	etyl dekanoate	alcoholic	40-60 µg/l
64	2-fenyl ethanol	rose	20 – 40 µg /l

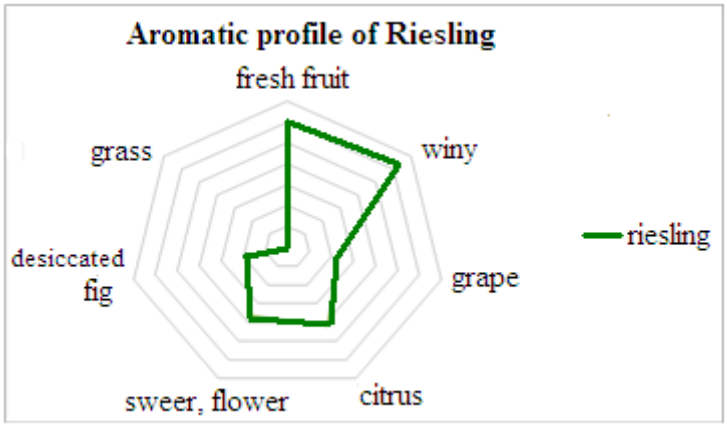

Fig. 2: Aromatic profile of Riesling

Conclusions

More than 60 aromatic compounds are measured in one aromagram, providing better classification of wines. The database is important not only for classification of wines, because included more than 10 000 of data, but it can help to prevent wine adulteration. Using SPMCE method can significantly reduce negative economic impact on honest wine producers.

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Bibliography

Kružlicová D., Mocák J., Balla B., Petka J., Farková M., Havel J., 2009. Classification of Slovak white wines using artificial neural networks and discriminant techniques. *Food Chem*., 112: 1046-1052.

Kružlicová D., Mocák J., Durčeková T., Hrivňák J., Pátková J., 10.-16. june 2007. Authentification of varietal wines by multivariate analysis of capillary GC data. *30th World Congress of Vine and Wine, 5th General Assembly of the OIV*.

Lakatošová J., Hrivňák J., Nemcová K., Kaňuchová Pátková J., 17.-19. february 2010. Analýza aromatických látok vo víne. *Viticulture and Viniculture forum – Slovakia 2010.*

Verstrepen K. J., Derdelinckx G., Dufour J.-P., Winderickx J., Thevelein J. M., Pretorius I. S., Delvaux F. R., 2003. Active esters: adding fruitiness to beer. *J. Biosci. Bioeng*., 96: 110-118.

EFFET DES CHAMPS ELECTRIQUES PULSES SUR L'EXTRACTION DES POLYPHENOLS DU RAISIN

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ABSTRACT

During vinification, red wine must was exposed to several intensities of pulsed electric field (PEF) with variable exposure time. Optical characteristics (colour intensity (CI), hue, and index of total phenolics (ITP)), as well as anthocyanins and flavan-3-ols concentrations were evaluated. The application of PEF significantly increased the extraction of major polyphenolic compounds when compared to classic wine-making, Legal concentrations of sugar, alcohol, total acidity (TA), volatile acidity (VA), pH and temperature have not been affected. The optimisation of the operational parameters is essential for this extraction of these compounds. First results have shown that extraction of the flavan-3-ols was more influenced by treatment duration time than by PEF intensity, and in contrast to the anthocyanins. Extraction optimum was achieved applying PEF of 700V/cm for 200 ms. However, this modality had the highest (35KJ/Kg) energy consumption.

RÉSUMÉ

Durant la vinification, les vins rouges ont été exposés à différentes intensités de champ électrique pulsé (CEP) avec des temps d'expositions variables. Les caractéristiques optiques (intensité colorante (IC), teinte et indice de polyphénols totaux (IPT)), ainsi que les concentrations en anthocyanines et en flavan-3-ols ont été mesurées. L'application de CEP a augmenté significativement l'extraction de la plupart des composés phénoliques en comparaison avec la vinification classique. Les concentrations en sucres, alcool, acidité totale (AT), acidité volatile (AV), pH et température n'ont pas été affectées. L'optimisation des paramètres opérationnels est essentielle pour l'extraction de ces composés. Les premiers résultats ont montré que l'extraction des tannins était plus influencée par la durée du traitement que par l'intensité du CEP, et inversement pour les anthocyanes. L'extraction optimale a été déterminée en appliquant un CEP de 700V/cm pendant 200ms. Cependant, cette modalité présente la plus haute consommation d'énergie (35kJ/Kg).

INTRODUCTION

Afin d'extraire des composés phénoliques (anthocyanes, tannins) et des arômes variétaux d'un vin rouge, on fait macérer les parties solides du raisin dans le jus en fermentation. L'extraction de ces composés dépend de nombreux facteurs qui ont été déjà étudiés et qui doivent être pris en compte : la température, la teneur en SO_2 libre, en alcool, le temps de macération, le pH, le foulage, les agitations et le non compactage du marc. Différentes techniques de vinification appliquées avant ou après les fermentations, comme les traitements thermiques et/ou sous-pression, les agitations, l'utilisation d'enzymes, de SO_2 et/ou de CO_2 ont été développées pour augmenter cette extraction. Cependant, ces techniques peuvent réduire la qualité du vin et être coûteuses en énergie et en temps. D'autre part, si le raisin n'a pas été récolté à une maturité optimale, l'extraction peut être limitée, ce qui nécessite une recherche d'alternative. L'utilisation de CEP de plusieurs kV pendant une très courte durée (quelques ms) sur la vendange éraflée et foulée peut être une solution efficace, rentable et peu coûteuse en énergie.

Des études récentes ont montré que le traitement CEP augmente l'extraction, améliore la qualité du jus et du vin (augmentation de l'IC, IPT, tannins et anthocyanes) et permet de réduire le temps de macération (Praporscic et al., 2007 ; López et al., 2009). Xin An Zeng et al. ont montré que la technologie des CEP avec un champ électrique de 600 V/cm et un temps de traitement de 3 minutes sur du vin accélère son développement tout en améliorant sa qualité (diminution des alcools supérieurs et des aldéhydes, et augmentation des esters et acides aminés libres) (Xin An Zeng et al., 2008). Les CEP n'affectent pas la proportion entre les composants de la couleur rouge du vin (la teinte et les composés jaunes, rouges et bleus) et d'autres caractéristiques de vin comme la teneur en alcool, l'AT ou le pH, mais peut réduire la concentration en sucre et l'AV (López et al., 2008). Les mécanismes mis en jeu dans les CEP sont méconnus. L'action des CEP sur les tissus de la baie de raisin n'est pas encore complètement comprise.

L'objectif de cette étude est d'évaluer si les CEP induisent un effet sur l'organisation cellulaire qui augmenterait le passage des composés phénoliques à travers les enveloppes cellulaires.

MATERIELS ET METHODES

Les raisins cv Cabernet Sauvignon utilisés pour cette étude ont été choisis pour leur représentativité du millésime 2009 des vignobles bordelais (appellation côte de Bourg). Les expériences ont été menées sur des baies éraflées et foulées, en utilisant un système de traitement de CEP de laboratoire. Les baies issues des différents traitements ont été réparties dans 5 cuves de microvinifications (30 L), correspondant à un témoin et à quatre modalités de CEP différentes (Tab.1).

Tableau 1- Paramètres des CEP pour les différents traitements.

Paramètres	*CEP1*	*CEP2*	*CEP3*	*CEP4*
Force du champs électrique E (V/cm)	4000	700	1000	700
Δt (ms)	1	200	20	20
Consommation énergétique W (kJ/kg)	4	35	10	3.5

La température, la densité, la turbidité, le pH et l'AT du moût ont été déterminés selon les directives de l'OIV (OIV, 1990). Du métabisulfite de sodium a été ajouté (6 g de SO_2 /100L) dans toutes les cuves. Des raisins ont été préparés à de la microscopie en suivant la méthode décrite par Mc Manus (1948) pour la fixation et par Thiery (1967) pour la coloration. Des coupes semi-fines (2 µm d'épaisseur) de la pellicule des raisins ont été colorées selon la méthode à l'acide périodique de Schiff pour la détection des polysaccharides (Thiery, 1967). Ces coupes ont été observées et étudiées en microscopie optique, puis photographiées à l'aide d'une caméra Olympus.

Les fermentations alcooliques (FA) et malolactique (FML) ont été déclenchées par inoculation et suivies régulièrement dans le temps. Durant l'élevage, la teneur en SO_2 libre a été contrôlée et ajustée à 25 mg/100L.

L'IC', la teinte, et l'IPT ont été mesurés durant tout le processus de vinification. A la fin de la FML, les vins ont été analysés dans le but de déterminer la teneur en anthocyanes et en tannins totaux, le pourcentage de pigments polymérisés et l'indice d'ionisation. La quantification des composés phénoliques a été déterminée par HPLC-DAD-Fluo. Toutes les analyses ont été faites en triplicata. Les méthodes de statistiques utilisées pour l'analyse des données ont été l'analyse en composantes principales et l'analyse de la variance. Le programme XLSTAT a été utilisé pour le traitement informatique des données.

RESULTATS ET DISCUSSION

Le traitement par CEP a amélioré l'extraction des composés phénoliques (anthocyanines et flavan-3-ols) et les caractéristiques visuelles des vins (IC', IPT, teinte). Les teneurs en sucre et en alcool, l'AT, l'AV, le pH et la température n'ont pas été affectées. Aussi, les moûts de raisin traités par CEP1 et CEP2 présentaient une coloration rouge framboise très intense en comparaison avec les autres moûts (figure1).

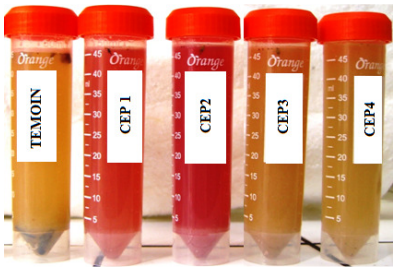

Figure 1-photographie du moût après traitement (Témoin, CEP1, CEP2, CEP3, CEP4)

L'observation des coupes semi-fines (figure 2) montre des couches cellulaires de la pellicule de CEP2 qui sont très aplaties et écrasées (surtout dans les couches les plus profondes), des parois cellulaires qui ont un aspect effrité et des vacuoles qui sont plus ou moins plasmolysées en comparaison avec les couches cellulaires de la pellicule du témoin. Les composés phénoliques ne présentent pas la même couleur : ils sont devenus, après le traitement de CEP2, bordeaux/fuchsia et n'ont plus le même aspect, ils sont sous forme de globules de diamètres différents, plus ou moins agglutinés dans les vacuoles.

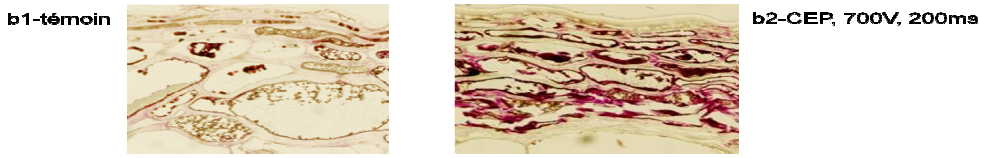

Figure 2-coupes semi-fines de la pellicule de raisin du témoin et de CEP2.

L'intensité colorante, la teinte et l'IPT des moûts et des vins a été mesuré par spectrophotométrie durant toute la vinification.

L'IC' du vin témoin a toujours été inférieure à celle des vins traités avec des CEP. Cependant l'écart avec le témoin a diminué avec le temps surtout pour les modalités de CEP1 et CEP3 à forte intensité (4000 et 1000 V/cm) qui pourraient avoir extrait des molécules plus instables. La modalité CEP2 appliquée au moût présente l'IC' la plus élevée.

La teinte des moûts et des vins a été suivie en tant qu'indicateur de la stabilité de la matière colorante. La teinte du vin témoin était supérieure à celle des vins traités avec des CEP. Cependant, l'écart avec le témoin a diminué très vite avec le temps. Les modalités de CEP1 et de CEP2 à fortes intensités électriques qui présentaient les valeurs les plus faibles dans le moût fermentant, sont devenues les plus élevées. Inversement les modalités de CEP3 et CEP4 ont leur teinte qui sont devenus les plus faibles. La couleur étant instable pendant la vinification, les mesures sur les vins vieillissants devraient nous indiquer quelles sont les modalités présentant une stabilité de la couleur extraite la plus élevée.

L'IPT dans les moûts et vins a été déterminé régulièrement pour connaître la teneur totale en anthocyanes et en tannins. L'IPT du vin témoin a été inférieur aux vins traités avec des CEP tout comme pour l'IC'. Par contre l'écart avec le témoin s'est maintenu avec le temps. Pareillement à l'IC', la modalité CEP2 appliquée au moût présente les meilleurs résultats.

De plus, d'après des mesures effectuées sur les vins, les pigments se polymérisent davantage, et sont plus présents sous leur forme cation flavylium. Des analyses complémentaires sont à réaliser pendant le vieillissement du vin pour s'assurer de la qualité des composés extraits pendant le traitement.

Plusieurs molécules d'anthocyanines et de flavan-3-ols ont été dosées dans les vins par HPLC. L'un des chromatogrammes du vin traité selon la modalité de CEP4 pour le dosage des anthocyanidines a été donnée comme exemple (figure 3).

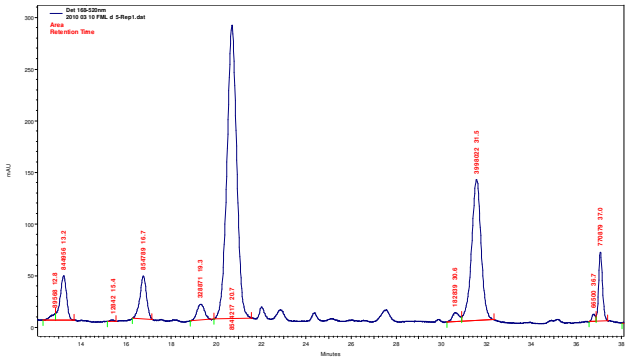

Figure 3-chromatogramme des anthocyanidines du vin traités par la modalité de CEP4.

L'extraction des anthocyanines a été significativement plus forte avec l'emploi des CEP par rapport à la vinification traditionnelle à l'exception de la malvidine-3-o-acétylé et de quelques molécules pour certaines modalités seulement. Ainsi, selon l'extraction des anthocyanines obtenus, un classement de la modalité la plus extractive à la moins extractive a été établie : CEP1, CEP3, et enfin CEP 4 et CEP2 en dernier. L'extraction obtenue par les deux modalités de CEP à 700V/cm est similaire. La variation de la durée du CEP a eu peu de conséquences. De plus, la modalité CEP3 présente une extraction des anthocyanines globalement supérieure aux vins traités par des CEP2, cela signifie qu'une forte intensité extrait davantage ces molécules qu'une longue durée de champs.

La malvidine-3-o-glucoside et la malvidine-3-o-acétylé sont les anthocyanines qui ont été extraites en quantités similaires au témoin. La delphinidine-3-o-glucoside est significativement différente du témoin pour toutes les modalités de CEP testées; nous pouvons penser que ce composé est facilement extractible avec les CEP et ceci même avec un CEP de 700V/cm, durant 20ms (CEP 4). La péonidine-3-o-coumayrollé n'a pas été plus extraite avec la modalité CEP4 (qui est la modalité la plus faible en intensité et presque en durée) qu'avec le témoin, elle serait donc extraite avec une intensité supérieure à 700V/cm ou avec une durée supérieure à 20 ms.

Les différentes concentrations en anthocyanidines des vins traités par des CEP ont été augmentées par rapport aux teneurs dans les vins témoins ; ce taux d'augmentation est donnée dans le tableaux 2.

Tableau 2- Taux d'augmentation des concentrations des anthocyanidines dans les vins traités par des CEP par comparaison aux vins témoins.

Anthocyanidines	*Taux d'augmentation des anthocyanidines par rapport au témoin (%)*			
	CEP1	*CEP2*	*CEP3*	*CEP4*
delphinidine-3-o-glucoside	34	13	32	29
pétunidine-3-o-glucoside	41	13	31	23
péonidine-3-o-glucoside	37	5	52	19
malvidine-3-o-glucoside	13	0	5	6
péonidine-3-o-acétylé	52	37	18	8
malvidine-3-o-acétylé	11	0	2	0.3
péonidine-3-o-coumayrollé	55	43	41	10
malvidine-3-o-coumayrollé	17	5	18	2

L'extraction des flavan-3-ols a été dosée par HPLC et les résultats sont donnés dans la figure 4.

Les concentrations en flavan-3-ols ont été significativement augmentées dans les vins traités par des CEP. L'application du CEP2 aux raisins a été la modalité la plus extractive en flavan-3-ols, à l'exception de la molécule B1 qui est légèrement plus extraite par la modalité CEP3. S'en suit de façon globale de la modalité la plus extractive à la moins extractive, la modalité CEP3, puis CEP1 et enfin la modalité CEP4. En effet ce classement est conservé pour les molécules catéchines, épicatéchines et B4. Une durée de champs électrique élevée a été plus extractive en tannin qu'une intensité de champs forte.

Les concentrations en catéchine, épicatéchine, B1, B2 et B4 dans les vins traités par CEP2 ont été augmentées respectivement de 34%, 55%, 12%, 52% et 32% par rapport aux teneurs dans les vins témoins.

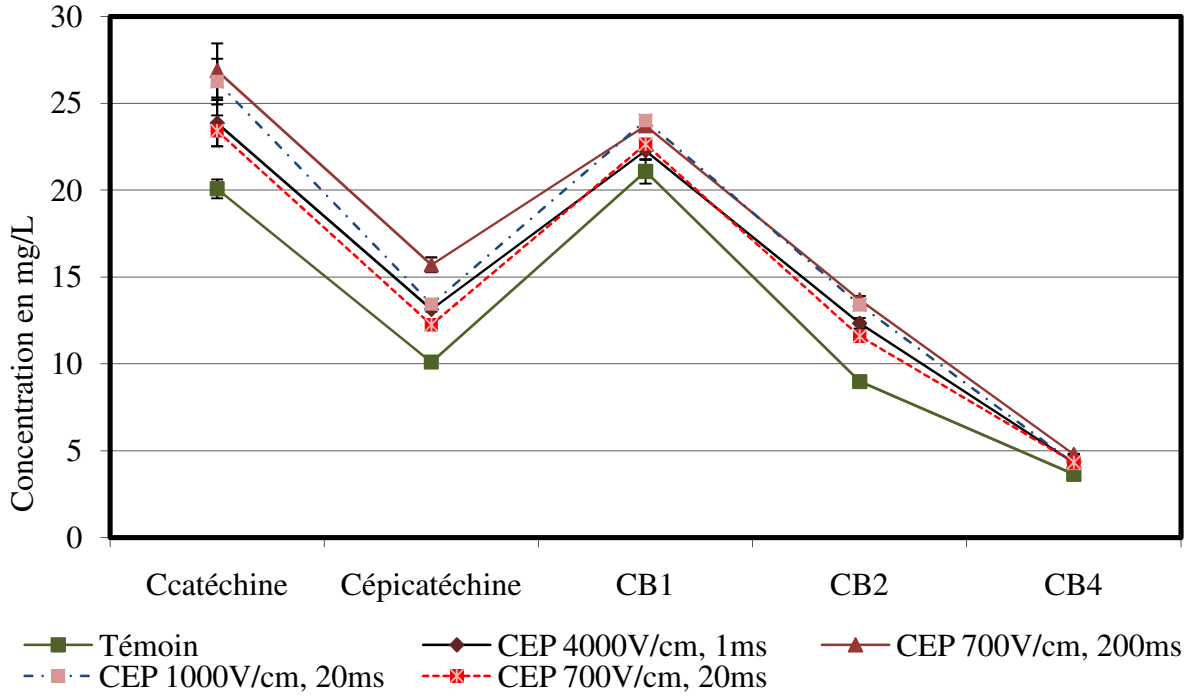

Figure 4-concentration en flavan-3-ols dans les vins témoins et les vins traités par CEP.

CONCLUSIONS

Ces résultats illustrent les effets importants des CEP sur la morphologie des cellules de la baie de raisin et sur l'extractibilité des composés phénoliques d'intérêt. Selon les observations en microscopie optique, les traitements de CEP entraînent la formation de pores au niveau des parois cellulaires, permettant ainsi la libération du contenu intracellulaire. Ce mécanisme devra être vérifié par des études complémentaires en microscopie optique et électronique. De plus, les résultats de l'IC' et de la teinte montrent que la couleur extraite par les modalités à forte intensité électrique ou de longue durée a diminué dans le temps. Des analyses devront être conduites durant le vieillissement du vin afin de s'assurer de la qualité et de la stabilité des composés extraits. Finalement, l'analyse sensorielle des vins restera l'un des critères essentiels dans le choix du traitement optimum à retenir.

Ce traitement semble être une alternative prometteuse aux technologies comme la thermovinification et à la vinification conventionnelle pour l'extraction de composés intéressants. Cependant, des études sur les paramètres opératoires (temps et fréquence) sont nécessaires pour mieux comprendre l'effet des CEP sur le vin. En effet, la qualité d'un vin peut diminuer à cause d'une extraction trop forte de tannins. Ainsi, un optimum de traitement est à définir.

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BIBLIOGRAPHIE

López N., Puértolas E., Condón S., Álvarez I., Raso J., 2008. Effects of pulsed electric fields on the extraction of phenolic compounds during the fermentation of must of trempranillo grapes. *Innovative Food Science and Emerging Technologies,* n°9: 477-482.

López N., Puértolas E., Hernández-Orte P., Álvarez I., Raso J., 2009. Effect of a pulsed electric fields treatment on the anthocyanins composition and other quality parameters of Cabernet Sauvignon freshly fermented model wines obtained after different maceration times. *LWT- Food Science and Technology,* n°42: 1225-1231.

Praporscic I., Lebovka N., Vorobiev E., Mietton-Peuchot M., 2007. Pulsed electric fields enhanced expression and juice quality of white grapes. *Separation and purification technology,* n°52: 520-526.

Xin An Zeng, Shu Juan Yu, Lu Zhang, Xiao Dong Chen, 2008. The effects of AC electric field on wine maturation. *Innovative Food science and Emerging Technologies,* n°9: 463-468.

The hard part of grape cluster influence on the quality of kakhetian wine type

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Abstract

The ancient Georgian traditional wine-making method stipulates the processing of grapes and alcoholic fermentation of must with stem contact in Kvevri (earthwork amphorae) buried into the ground. The received wine material ages in Kvevri during three - five months with hard parts of grape. The aim of our work was to study stem affect on the quality of kakhetian wine. We studied phenolic and aromatic compounds of samlpes by HPLC and Gas mass spectrometry. The wine made by Kakhetian technology contained high amounts of phenolic rather than wine made by European one. Long wine material maceration prolong the phenolic compound extraction from hard parts of seed and especially from stem. In wines made in earthwork amphorae were found the following correlation: samples with stem contact consisted less quantity of phenolic carbo acids, total polyphenols (1600 - 1490 mgL^{-1}) and total flavonoids (1710- 1360 mgL^{-1}). The reduction of phenolic compound by stem contact in wine materials may be caused by correlation of phenolic compound extracted from stem (by coagulation and sedimentation) or by their adsorption by stem.

La technologie de la fabrication du vin géorgien traditionnel prévoit le traitement du raisin et la fermentation alcoolique du marc avec les rafles dans les Qvevri (les cruches, installées dans la terre). La matière du vin obtenue subit la formation avec les parties solides de la grappe pendant trois-cinq mois dans les Qvevri. L'objectif de l'examen consistait à examiner l'influence de la rafle sur la qualité du vin kakhétien. Il a été examiné dans les spécimens les composés phénoliques, composants aromatiques et par l'analyse à l'aide de spectromètre de masse liquide et gazeuse. Dans les matières du vin fabriquées de technologie kakhétienne les composés phénoliques étaient partout augmentés que dans les matières européennes. Avec la temporisation durable de la matière du vin sur le marc il est prolongé l'extraction des composés phénoliques depuis des pièces solides de la grappe, surtout depuis des rafles. Ces modeles, lequels a arrêté avec la rafle il a raccourcit pour sentir les acides carbon de phenol, les polyphenols general (1600 -1490 mgl^{-1} mde) et les flavavonoides (1710 - 1360 mgl^{-1} mde) dans la matière du vin raccourcir les matieres phenols en contact avec la rafle, il est provoquée de l'action réciproque des composés phénoliques provenus de la rafle (avec la coagulation et la sédimentation) ou de l'adsorption par la rafle.

Introduction

The main subject of various published works about wine compounds, is the study of phenolic and volatile ones, because they are very important contributors for wine sensory properties — colour, flavour, astringency, and bitterness (Arnold and Noble 1978,Arnold et al.1980, Cocito et al. 1995, Kotseridis and Baumes 2000, Powers et al. 1988, Singleton 1992). It is well known that for given grape varieties, the kind of winemaking technology can notably affect the levels of phenolic and volatile compounds of wine. Wines made by skin fermentation with stem-contact contained much more polymeric phenols than those made by skin fermentation without stem-contact wine, and extending pomace contact time increased both total and polymeric phenol levels in wines (Kantz and Singleton 1991). Stem contact wines contained much higher concentration of proanthocyanidins than non-stem contact wines (Bourzeix et al. 1986, Spranger et al. 2004, Sun et al. 2001), due to the important transference of this compounds from stems to wines during fermentation (Sun et al. 1999).The aroma of white and red wines is the product of a biochemical and technological succession and it is decisively influenced by the alcoholic fermentation procedure (Bayonove et al. 1998, Rapp 1988). The complexity of wine aroma is defined by all technical factors. As many viticulture and oenological factors greatly influence the types and concentrations of flavour components (Zhou et al. 1996), the ability to define each individual component would provide an approach to optimize the operational conditions, mainly the winemaking technologies.

Viticulture and wine making in Georgia have been widely practiced since ancient time (Olmo 1976, Zohary and Hopf 1993, Zohary and Spiegel-Roy 1975).Continuous and intensive selection of grape varieties that favoured the production of desired wine styles led over the centuries to a plenty of native cultivars, which possess various distinct oenological characteristics and organoleptic properties. Good example of variety of Georgian wine-making is local type Kakhetian wine, which is made in earthwork amphorae (Beridze 1965) by using all parts of grape during fermentation and the first step of wine aging (Marjanishvili 1983, Nanitashvili 1978, Valero et al. 2002). Because of this, wine is characterized with varietal aroma, high extract and polyphenolic consistence. For the production of this type of white wine are always used the following grape varieties: Rkatsiteli (*V. vinifera L.)* and Kakhuri Mtsvane (*V. vinifera L.*).

The object of our work was to study characteristic consistence of Kakhetian wine type made in earthwork amphorae using different grape varieties from diverse areas and prepared in different way.

Materials and methods

Standards

Used materials: (+)-catechin, (-)-epicatechin, hexyl acetate, ethyl hexanoate, ethyl decanoate, ethyl lactate, ethyl octanoate, (Sigma, USA), procyanidin B1 (Extrasynthèse, France), caffeic acid , geraniol, nerol, linalool, benzyl alcohol, ethyl 3-OH-butyrate (Fluka, Switzerland), α-terpineol, gallic acid, 2-phenyl ethanol, isoamyl acetate, hexanoic, octanoic and decanoic acids (Merck-Schuchardt, Germany*)*, 2-phenyl ethyl acetate, diethylsuccinate (Carlo Erba, Italy).

Winemaking technologies

Grapes were harvested in September 2007 at a technological maturation. All parts of grapes were processed in earthwork amphorae: Kakhuri Mtsvane from Akhmeta region, Rkatsiteli from Kardenakhi region and the same variety from Gurjaani region – all without stem contact and also Rkatsiteli from Kardenakhi region with stem contact. Fermentation

proceeded 10-12 days, with stirring every 6 hours and approximate temperature – 20°C, thanks to spontaneously cooling (which takes place by good thermoconductivity of earthwork amphorae in the ground); at the end of fermentation and filling-up, SO_2 (30 mgL^{-1}) was added; wines were left in earthwork amphorae until March of next year, then transferred to stainless steel tanks and stored at room temperature before analyses.

Chemical and physical parameters of wines

Alcohol content, pH, total acidity, volatile acidity and chromatic characteristic were made according to the official methods of O.I.V. (1990).

Spectrophotometric analysis

Total polyphenols, flavans and flavonoids were determined according to Di Stefano et. al. (1989).

HPLC analyses

Catechins and hydroxycinnamic acids were performed according to Ummarino et al. (2001). Instrument was as follows: degasser, quaternary pump, thermostated column compartment (Agilent Technologies, 1100 series, USa), autosampler, DAD detector (Agilent Technologies, 1200 series, USA); guard column ODS-Hypersil (C18) 20 x 2.1 mm-5µm (CPS Analitica, Italy); column ODS Hypersil 200 x 2.1 mm – 5µm (CPS Analitica, Italy) for catechins and hydroxycinnamic acid. Quantities were expressed as mgL^{-1} for wine; particularly, hydroxycinnamic acids were expressed as mgL^{-1} of caffeic acid.

Volatile compounds

Volatile compounds were determined by gas chromatography as described in literature (Gianotti and Di Stefano 1991). Chromatographic conditions were: instrument GC 8000 (Fisons Instruments, Italy), detector FID, helium flow 2.9 $mLmin^{-1}$, column HP- FFAP 50 m x 0.32 mm x 0.50 µm (Agilent Technologies, USA), injection temperature 220 °C, oven temperature 33 °C held for 5 min, then 3 °C min^{-1} to 200 °C, then isotherm for 10 min, then 10 °C min^{-1} till 220°C and isotherm 220°C for 15 min. Detector temperature was 245 °C, and injection volume - 3 µL. Commercial standards were used in order to identify and quantify volatile compounds in sample, added with 1-heptanol as internal standard.

Varietal compounds

Glycosidically bound varietal compounds from wines were determined according to Di Stefano (1996). For enzymatic hydrolysis, overnight at 40°C, Cytolase (Genecor) was used. The analyses were carried out by GC HP 5890 Serie II and MS HP 5972, column HP-Innowax 30 m x 0.25 mm x 0.25 µm, splitless injection 2 µL, helium flow 1 $mLmin^{-1}$; starting temperature 30°C for 2 min, oven temperature 30°C min^{-1} from 30°C to 60°C, 2°C min^{-1} up to 160°C, 3°C min^{-1} up to 230°C, and then held for 10 min. Commercial compounds were used for calibration, with internal standard method. 200 µL of 1-heptanol (67,6 mgL^{-1} in methanol/H_2O 20% v/v) were added as internal standard. The concentration of each varietal compound was expressed in μgL^{-1}, and in µg of linalool per litre of wine, when commercial standard was not available.

Results

The general compositions of different wines are shown in Tab.1. From this table, it can be seen, that between Rkatsiteli Kardenakhi samples fermented with and without stem contact, there is not difference in all analysed factors, thus according to Ribereau-Gayon et al. (2000) wines fermented without destemming must contain less volumes of alcohol and acidity. It can be underlined a little difference in chromatic characterization, that comes among wines with

stem contact and all wines – without. Rkatsiteli from Kardenakhi fermented and aged with stem contact, had less purity and less brightness than wines vinified without stems.

Table 1 General composition of amphorae wines

Names	alcohol (% v/v)	tot. acidity (gL^{-1} tartaric acid)	volatile acidity (gL^{-1} acetic acid)	pH	purity	brightness	Dom. wave length
Kakhuri Mtsvane	12,7	5,1	0,48	3,47	16,02	91,22	575,85
Rkatsiteli Kardenakhi	11,9	5,2	0,44	3,49	12,29	92,86	576,07
Rkatsiteli Gurjaani	12,6	5,7	0,41	3,22	13,15	92,43	576,22
Rkatsiteli Kardenakhi with stems	11,8	5,0	0,54	3,45	6,67	85,75	577,19

The amounts of volatile compounds determined by gas-chromatography in Rkatsiteli and Mtsvane grape wines are presented in Tab. 2.

The reported metabolites, even if they were expression of a particular vinification process, did not let a punctual analysis about their origin because of the unknown development of microflora during fermentation.

Higher alcohols and esters, produced during alcoholic fermentation, play an important role in the flavour of the wines, depending on the types of compounds and their concentrations (Valero et al. 2002). The most plentiful compounds were the higher alcohols, in agreement with the literature (Baumes et al. 1986). At concentrations below 300 mgL^{-1} they certainly contribute to the desirable complexity of wine; when their concentrations exceed 400 mgL^{-1}, higher alcohols are regarded as a negative quality factor (Mateo et al. 2001). The total concentration of higher alcohols in experimental wines was below 300 mgL^{-1}.

It can be mentioned, that there was not any difference among wines, except of isoamyl alcohol content, which was higher in stem contact wine. Also this last wine characterized with highest amount of 1-hexanol, which indicated that extended the pomace and especially the stem contact time can raise the formation of this compound, which agrees with results obtained by other authors (Cordonnier and Bayonove 1979, Rankine and Pocock 1969). It is significant, that there was notable difference between stem and non-stem contact wines in the content of ethyl lactate, ethyl 3-OH-butyrate, ethyl 4-OH-butyrate and monoethylsuccinate, which were much higher in first one. Wine made by Kakhuri Mtsvane grape variety was distinguished with higher concentration of 2-phenyl ethanol, 2-phenyl ethyl acetate.

Table 2 Volatile Compounds in amphorae wines (μgL^{-1})

Compounds	Kakhuri Mtsvane	Rkatsiteli Kardenakhi	Rkatsiteli Gurjaani	Rkatsiteli Kardenakhi with stems
isoamyl acetate	265	241	119	144
hexyl acetate	88	64	64	68
2-phenyl ethyl acetate	83	n.d.	12	11
Σ acetates	436	305	195	223
acetic acid (gL^{-1})	0.48	0.44	0.41	0.54
Σ acetates/ acetic acid (gL^{-1})$x10^{-3}$	0.91	0.69	0.47	0.41
ethyl hexanoate	334	235	390	232
ethyl octanoate	294	207	278	198
ethyl decanoate	37	n.d.	39	25
Σ ethyl esters	665	442	707	455
hexanoic acid	2385	1662	2708	1872
octanoic acid	2741	1917	2629	1724
decanoic acid	744	363	419	311
Σ fatty acids	5870	3942	5756	3907
Σ ethyl esters./ Σ fatty acids	0.113	0.112	0.123	0.116
n-pentanol	18	18	20	35
ethyl lactate	26	n.d.	38	263
n-hexanol	1236	853	992	1787
ethyl 3-OH-butyrate	17	41	43	71
isoamyl alcohol	62226	63550	66785	101450
diethylsuccinate	2294	1770	3142	1832
ethyl 4-OH-butyrate	281	255	221	480
N(3-methybutylacetamide)	5	n.d.	13	9
benzyl alcohol	46	12	24	20
2-phenyl ethanol	37715	24792	24245	23102
Monoethylsuccinate	1580	560	2629	3782

Tab. 3 presents the results from HPLC and spectrophotometer analyses. There was not fixed any important difference among non stem contact wines. But it can be noted, that stem contact wine had less contents, in all parameters, to the contrary of wine made by the same variety, without stems. The possible explanation of this can be - extending the maceration time and especially stem contact, might extract more of other compounds, which would absorb or interact with various polyphenols and thus reduce their levels in wine (Spranger et al. 2004).

Table 3 Polyphenolic compounds in amphorae wines (mgL^{-1})

	caftaric acid	cis-p-coumtaric acid	trans-p coumtaric acid	procyani-dine B1	(+)-catechin	(-)-epi-catechin	flavans	total poly-phenols	total flavonoids
Kakhuri Mtsvane	20,8	2,1	4,7	30,0	36,7	10,0	1380	1670	1680
Rkatsiteli Kardenakhi	23,0	3,0	7,6	33,2	30,1	8,0	1530	1600	1710
Rkatsiteli Gurjaani	20,7	2,9	7,4	27,2	21,9	4,5	1320	1760	1760
Rkatsiteli Kardenakhi with stems	15,9	2,8	5,9	25,7	25,0	7,1	1200	1490	1360

The results obtained in the analyses of the volatile compounds released by aroma precursors after enzymatic hydrolysis are shown in the Tab. 4. Values of some terpenol compounds, such as linalool, α-terpineol, cis-furanlinalool oxide, of Kakhuri Mtsvane grape wine are more higher in comparison with three different Rkatsiteli wines. Differences among wines from the same varieties and between wines from different varieties are better explained when we look to the ratio among specific compounds rather than to each compound by itself. So, the relations among linalool/nerol and linalool+nerol+geraniol/α-terpineol are different in

Kakhuri Mtsvane and both Rkatsiteli from Kardenakhi wines (with and without stem contact), which, in turn, are different from another region Rkatsiteli (Kardenakhi) wine.

Table 4 Varietal compounds in amphorae wines (μgL^{-1})

Compounds	Kakhuri Mtsvane	Rkatsiteli Kardenakhi	Rkatsiteli Gurjaani	Rkatsiteli Kardenakhi with stems
trans-furan linalool oxide	9,3	6,1	7,8	6,5
cis-furan linalool oxide	19,9	11,9	13,8	11,3
linalool	25,8	12,6	4,3	12,1
α-terpineol	11,2	6,0	6,6	5,7
trans-pyran linalool oxide	9,8	4,1	6,7	4,7
nerol	6,9	15,8	14,3	19,3
geraniol	30,9	48,3	35,5	61,0
trans-8-OH-linalool	54,4	40,8	33,0	53,0
cis-8-OH-linalool	65,8	116,4	36,2	44,4
trans/cis furan linalool oxide	0,5	0,5	0,6	0,6
trans/cis pyran linalool oxide	0,9	0,7	0,8	0,9
trans/cis 8-OH-linalool	0,8	0,4	0,9	1,2
linalool/geraniol	0,8	0,3	0,1	0,2
nerol/geraniol	0,2	0,3	0,4	0,3
linalool/nerol	3,7	0,8	0,3	0,6
[linalool+nerol+geraniol]/ α – terpineol	5,7	12,8	8,2	16,2

Conclusion

Wines in earthwork amphorae were vinified using ancient, traditional method of winemaking, which can be fined as good quality wine in regards of all standards. Were studied their polyphenolic and volatile composition. Main differences were notified among wines made with and without stem contact. It was distinguished that stem contact wine consists less quantities of various polyphenols and more – n-hexanol and isoamyl alcohol. About varietal content can be said that by using this technology no difference was found among wines.

References

1. ARNOLD R.M., NOBLE A.C. (1978). Bitterness and astringency of grape seed phenolics in a model wine solution. Am. J. Enol. Vitic., (29): 150-152.
2. ARNOLD R.M., NOBLE A.C., SINGLETON V.L. (1980). Bitternes and astringency of phenolic fractions in wine.J. Agric. Food Chem., (28): 675-678.
3. BAUMES R., CORDONNIER R., NITZ S., DRAWERT F. (1986). Identificationand determination of volatile constituents in wines from different vine cultivars. Journal of the Science Food and Agriculture, (37): 927–943.
4. BAYONOVE C., BAUMES R., CROUZET J., GUNATA Z., (1998). Œnologie: Fondements Scientifiques et Technologiques. FLANZY C. (Ed.), Lavoisier, Paris, France.
5. BERIDZE G.I. (1965). Georgian wines and brendies (Вина и коньяки Грузии*)*, Tbilisi.
6. BOURZEIX M, WEYLAND D, HERADIA N. (1986). Etude des catechines et des procyanidols de la grappe de raisin, du vin et d'autres derives de la vigne. Bull OIV, (59):1171- 1254.
7. COCITO C., GAETANO G., DELFINI C. (1995). Rapid extraction of aroma compounds in must and wine by means of ultrasound. Food Chem., (52) :311-320.
8. CORDONNIER R. BAYONOVE C. (1979). Les composantes varietales et prefermentaries de l'arome de vins. Rev. Fran. d'Oenol., (16): 79-90.

9. DI STEFANO R. (1996). Metodi chimici nella caratterizzazione varietale. Riv. Vitic. Enol., (49,1): 51-56.
10. DI STEFANO R., CRAVERO M.C. GENTILINI N. (1989). Metodi per lo studio dei polifenoli dei vini. L'Enotecnico., (5): 83-89.
11. GIANOTTI S.; DI STEFANO R. (1991). Metodo per la determinazione dei composti volatili di fermentazione. L'Enotecnico., (10): 61-64.
12. KANTZ K., SINGLETON V.L., (1991). Isolation and determination of polymeric polyphenols in wines using Sephadex LH-20, Am. J. Enol. Vitic., (42): 309.
13. KOTSERIDIS Y., BAUMES R., (2000). Identification of impact in Bordeaux red Grape juice, in the commercial yeast used for its fermentation, and in the produced wine. J. Agric. Food Chem., (48): 400-406.
14. MARJANISHVILI D.A. (1983). Production of Kakhetian type table wines (Производство столовых вин кахетинского типа.), Vin. i Vinog., SSSR.
15. MATEO J.J., JIMENEZ M., PASTOR A., HUERTA T. (2001). *Yeast* starter cultures affecting wine fermentation and volatiles. Food Research International, (34): 307–314.
16. NANITASHVILI T.S. (1978). Improvement of technology of Kakhetian type white table wines production (Усовершенствование технологии приготовления белых столовых вин кахетинского типа). Vinodelie I Vinogradarstvo, SSSR.
17. O.I.V. (1990). Recueil des methodes internationales d'analyse des vins, Office International de la Vigne et du Vin, Paris.
18. OLMO H.P. (1976). Grapes. In: SIMMOUNDS N.W. (ed.), Evolution of crop plants. Longman, London.
19. POWERS J.R., NAGEL C.W., WELLER K. (1988). Proteins removal from a wine by immobilized frappe proanthocyanidins. Am. J. Enol. Vitic., (39): 117.
20. RANKINE B.C., POCOCK K.F. (1969). Beta-Phenethanol and n-hexanol in wines: influence of yeast strain, grape variety and other factors; and taste thresholds. Vitis, (8): 23-37.
21. RAPP A., (1988). Wine Analysis.LINSKENS H.F., JACKSON J.F. (Eds.), Springer, Berlin, Heidelberg, New York.
22. RIBEREAU-GAYON P., DUBOURDIEU D., DONECHE B., LONVAUD A. (2000). Handbook of Enology. *Vol. 1*. John Wiley & Sons Ltd – England.
23. SINGLETON V. L., (1992). Tannins and the qualities of wines. In: Plant Polyphenols Synthesis,Properties, Significance. HAMINGWAY R. W. and LAKS P. E. (Eds.) Plenum Press, New-York. 859-880.
24. SPRANGER M.I., CLIMACO M.C., SUN B.S., EIRIZ N., FORTUNATO C., NUNES A.,LEANDRO M.C., AVELAR M.L., BELCHIOR A.P. (2004). Differentiation of red winemaking technologies by phenolic and volatile composition. Anal Chim Acta., (513):151-161.
25. SUN B.S., PINTO T., LEANDRO M.C., RICARDO-DA-SILVA J.M., SPANGER M.I.,(1999). Transfer of catechins and proacyanidins from solid parts of the grape cluster into wine. Am J Enol Vitic., (50):179-184.
26. SUN B.S., SPANGER M.I., ROQUE-DO-VALE F., LEANDRO M.C., BELCHIOR A.P. (2001). Effect of different winemaking technology on phenolic composition in Tinta Miuda red wines. J. Agric Food Chem., (49): 5809-5816.
27. UMMARINO I., FERRANDINO A., CRAVERO M.C., DI STEFANO R. (2001). Evoluzione dei polifenoli di uve di biotipi di Pinot nero durante la maturazione. L'Enologo, (4): 71-82.

28. VALERO E., MOYANO L., MILLAN M. C., MEDINA M., ORTEGA J. M. (2002). Higher alcohols, and esters production by Saccharomyces cerevisiae. Influence of the initial oxygenation of the grape must. Food Chemistry, (78): 57–61.
29. VALUIKO G.G. (1978). Grape wines (Виноградные вина), Moscow.
30. ZHOU Y., RIESEN R., GILPIN C.S., (1996). Comparison of Amberlite XAD-2/Freon 11 extraction with liquid/liquid extraction for the determination of wine flavor components. J. Agric. Food Chem., (44): 818-822.
31. ZOHARY D. HOPF M. (1993). Domestication of plants in the Old world. Clarendon Press, Oxford.
32. ZOHARY D., SPIEGEL-ROY P. (1975). Beginnings of fruit growing in the old world. Science (187): 319-327.

The varietal effect of Georgian red grape cultivar Saperavi (*vitis vinifera L.*) and Cabernet Sauvignon (*vitis vinifera L.*) on consistence of phenolic compounds and aromatic components.

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Abstract

Red grape varieties such as: native Saperavi and introduced, French Cabernet Sauvignon, spread in Kakheti are quite interesting because of their phenolic compound consistence. The aim of our research was to study the varietal effect of Saperavi and Cabernet Sauvignon on consistence of phenolic compounds and aromatic components. The Phenolic compounds were determined by HPLC and aromatic compounds by Gas Mass spectrometry in different parts of grape. The samples were harvested during the technical maturity period. The results of this experiment have shown that the grape varietal features can influence on procyanidin, catechin, epicatechin, phenolic acids and anthocyanin consistence. The mentioned compounds were nearly twice more in Saperavi grape rather than in Cabernet Sauvignon. Such relation was found also according to aromatic compounds (TFLO, CFLO, Linalool, Nerol, TPLO, CPLO, trans-8-OH-linalool, cis-8-OH-linalool). Only α terpineol and geraniol were more in cabernet sauvignon.

Les espèces des raisins rouges répandues en Kakhéti: Saperavi aborigène et le Cabernet Sauvignon, méritent une attention particulière par leur constitution de composés phénoliques. L'objectif de recherche consiste à examiner l'effet génétique de Saperavi et de Cabernet sur la constitution de composés phénoliques et de composants aromatiques. Il a été défini les composés phénoliques par les méthodes de la chromatographie liquide et les composants aromatiques par les méthodes de la spectrométrie de masse gazeuse dans de différentes parties du grain de la grappe. Les spécimens ont été pris lors de la période de maturité technique du raisin. Les résultats de l'examen ont montré que l'espèce du raisin conditionne surtout la constitution des procyadinins, catéchols, épicatéchines, acides phénoliques et anthocyanines. La quantité des composants susdits est presque deux fois plus dans le raisin de Saperavi que dans celui de Cabernet. Un rapport analogique a été relevé d'après les composants aromatiques génétiques (TFLO, CFLO, linalool, TPLO, CPLO, nerol, trans-8 OH-Linalool, cis-8-OH- Linalool) sauf le terpinol alfa et le géraniol.

Introduction

Phenolic compounds are abundant in grapes and play an important role in the quality of wines. Anthocyanins are found in the skin tissue and are responsible for the colour of red wine. Flavan-3-ols are found in the skin and seed tissue (Cheynier and Rigaud 1986, Kovac et. al. 1992, Mazza 1995, Mistry et. al. 1991, Souquet et. al. 1996) and are responsible for the bitter and astringent properties of red wine (Gawel 1998). Many factors can affect phenolic accumulation in the grape, including maturity (Kennedy et. al. 2002, 2000), temperature (Spayd et. al. 2002), light (Doloozlian and Kliewer 1996, Keller and Hradzina 1998) and vine water status (Bellincontro et. al. 2004, Kennedy et. al. 2002, Ojeda et. al. 2002). Aroma compounds are also important in wine quality and are influenced by several factors such as grape cultivar and maturity, growing climate, alcoholic fermentation and wine age (Bueno et. al. 2003, Gomez et. al. 1994, Nyka¨nen 1986, Oliveira et. al. 2006).

Georgian and Caucasian region is the one of the most ancient vine-growing and wine-making region in the world (Olmo 1976, Zohary and Hopf 1993, Zohary and Spiegel-Roy 1975). There are about 500 autochthonous grape cultivar. Our work was about most important cultivar, which is wide spread in eastern part of Georgia - Kakhetia. Saperavi (*V. vinifera L.*) is unique and ancient Georgian red grape cultivar (Kishkovski, Merjanian 1984), which gives high quality, many types of wine in different areas, and there are 5 DOC wines in Georgia made by this cultivar.

In this work we tried to characterize this grape and wine made with this grape. For comparison, we analysed and described even Cabernet sauvignon grapes grown in the same region.

Materials and methods

Standards

Used materials: (+)-catechin, (-)-epicatechin, hexyl acetate, ethyl hexanoate, ethyl decanoate, ethyl lactate, ethyl octanoate, (Sigma, USA), procyanidin B1 (Extrasynthèse, France), caffeic acid, geraniol, nerol, linalool, benzyl alcohol (Fluka, Switzerland), α-terpineol, gallic acid, 2-phenyl ethanol, isoamyl acetate, hexanoic, octanoic and decanoic acids (Merck-Schuchardt, Germany), 2-phenyl ethyl acetate, diethylsuccinate (Carlo Erba, Italy).

Winemaking technologies

Grapes were randomly harvested from Napareuli (Denomination of Origin) vineyards in September 2008. One part was frozen and stored at -20°C until analysed. Another part was fermented at 25°C by classical method of winemaking. After ending of alcohol fermentation, SO2 (30 mgL^{-1}) was added and the wine was stored at room temperature before analyses.

Chemical and physical parameters of wines

Alcohol content, pH, total acidity, volatile acidity and chromatic characteristic were made according to the official methods of O.I.V. (1990).

Spectrophotometric analysis

Total polyphenols, flavans and flavonoids were determined according to Di Stefano et. al. (1989).

HPLC analyses

Catechins, anthocyanins, and hydroxycinnamic acids were performed according to Ummarino et al. (2001). Instrument was as follows: degasser, quaternary pump, thermostated column compartment (Agilent Technologies, 1100 series, USA), autosampler, DAD detector (Agilent Technologie, 1200 series, USA); guard column ODS-Hypersil (C18) 20 x 2.1 mm-5µm (CPS Analitica, Italy); column ODS Hypersil 200 x 2.1 mm – 5µm (CPS Analitica, Italy) for catechins and hydroxycinnamic acid, column ODS Hypersil 100 x 2.1 mm – 5µm

(CPS Analitica, Italy) for anthocyanins. Commercial standards for calibration curves were used. Quantities were expressed as mg per kg of grape (mgL^{-1} for wine); particularly, hydroxycinnamic acids were expressed as mg of caffeic acid per kg of grape (mgL^{-1} for wine). Anthocyanins were expressed as percentage of the totality of the identified compounds.

Volatile compounds

Volatile compounds were determined by gas chromatography as described in literature (Gianotti and Di Stefano 1991). Chromatographic conditions were: instrument GC 8000 (Fisons Instruments, MI-Italy), detector FID, helium flow 2.9 $mLmin^{-1}$, column HP- FFAP 50 m x 0.32 mm x 0.50 μm (Agilent Technologies, USA), injection temperature 220 °C, oven temperature 33 °C held for 5 min, then 3 °C min^{-1} to 200 °C, then isotherm for 10 min, then 10 °C min^{-1} till 220°C and isotherm 220°C for 15 min. Detector temperature 245 °C, and injection volume 3 μL. Commercial standards were used in order to identify and quantify volatile compounds in sample added with 1-heptanol as internal standard.

Varietal compounds

The skins, separated from pulp and grape seeds, were weighed and immersed in 25 mL of methanol overnight at room temperature. Glycosidically bound varietal compounds from wine and methanol extract of skin were determined according to Di Stefano (1996). For enzymatic hydrolysis, overnight at 40°C, Cytolase (Genecor, France) was used. The analyses were carried out by GC HP 5890 Series II and MS HP 5972, column HP-Innowax 30 m x 0.25 mm x 0.25 μm, splitless injection 2 μL, helium flow 1 mLmin-1; starting temperature 30°C for 2 min., oven temperature 30°Cmin^{-1} from 30°C to 60°C, 2°Cmin^{-1} up to 160°C, 3°Cmin^{-1}. up to 230°C, and then held for 10 min. Commercial compounds were used for calibration with internal standard method. 200 μL of 1-heptanol (67,6 mgL^{-1} in methanol/H2O 20% v/v) were added as internal standard. The concentration of each varietal compound was expressed in μgL^{-1} or μgkg^{-1} and in μg of linalool per litre of wine or μg per kilogram of grape, when commercial standard was not available.

Results

The general physical and chemical compounds of received wine was as follows: alcohol - 13% v/v; total acidity - 7,7 gL^{-1} expressed as tartaric acid, volatile acidity – 0,38 gL^{-1} expressed as acetic acid and pH=3,53. Chromatic characterizations were: purity - 99,83; brightness - 1,01; dominant wave length – 642,67; intensity - 15,8; Glories index – 69,6 and tonality – 0,44.

The general polyphenolic compound measurements are shown in the Tab. 1. In all parameters Saperavi skins and seeds were prevalent to Cabernet sauvignon ones.

Table 1 Spectrophotometric analyses (for wine – g/L ; for seeds and skins – g/kg of grape)

Name	Type	Flavans	total polyphenols	Total flavonoids	total anthocyans
Saperavi	skins	0,6	4,0	6,4	2,9
Saperavi	seeds	3,1	3,5	2,7	-
Cabernet sauvignon	skins	0,5	2,1	3,4	1,3
Cabernet sauvignon	seeds	2,8	2,9	2,4	-
Saperavi	wine	0,7	2,2	0,8	0,9

Hydroxycinnamic acids and some catechins were determined in the grape skins and seeds apart and in the wine. Although considerable amounts of catechins and procyanidins have been reported in solid parts of grape clusters, especially in grape seeds (Bourzeix et. al. 1986, Ricardo-Da-Silva et. al. 1992, 1991, Singleton 1992), only part of these compounds could be extracted during red wine fermentation. This is probably due to their different localization in

grape solid tissues and to the existence of various linkages between catechins or procyanidins and other compounds (proteins, polysaccharides) (Amrani 1993, Amrani et. al. 1994). Exception was much more consistence of procyanidin B1 in the wine itself, rather than in the grape skins and seeds. It is notable that all defined components were much more in Saperavi grape skins and seeds than in Cabernet sauvignon.

Table 2 Catechins and hydroxycinnamyltartaric acids (for wine – g/L ; for seeds and skins – g/kg of grape)

Name	Type	procyanidin B1	(+)-catechin	(-)-epicatechin	caftaric acid	cis-p-coumtaric acid	trans-p coumtaric acid
Saperavi	Skin	14,6	4,1	2,8	0,3	n.d.	0,1
Saperavi	Seed	16,1	34,9	25,7		-	-
Cabernet sauvignon	Skin	2,8	1,4	0,6	0,2	n.d.	n.d.
Cabernet sauvignon	Seed	7,4	14,3	11,7		-	-
Saperavi	Wine	41,5	12,0	12,5	5,5	0,5	1,6

n.d. - not determined

Percentage proportion of main anthocyanins of Saperavi skins and wine and Cabernet sauvignon skins determined by HPLC are shown in the Tab 3. The malvidine derived anthocyanins from determined anthocyanins were higher in Cabernet sauvignon grape skins (>65% of total anthocyanin pigments), than in Saperavi grapes (>42%). But this parameter was more (69%) in Saperavi wine. Second prevalent anthocyanins were petunidine (more than 9%) and peonidine derivates (>17%) in Cabernet sauvignon and Saperavi skins, respectively. The cyanidine derivatives were less (<1%) in Saperavi wine as in Saperavi skins (<6%) and the fewer in Cabernet sauvignon skins (<3%). The 3-monoglucosides represented 78,3% of total pigments of Saperavi skins and 65,6% - of Cabernet sauvignon skins. In wine this parameter was more higher – 84,5%. 3-monoglucoside-acetates were higher in Cabernet sauvignon (25%) and cinnamoyl-3-monoglucoside - in Saperavi (16,5%). All parameters in Cabernet sauvignon skins were very close to those determined by Wulf and Nagel (1978). Wine was characterized by only 6,3% of 3-monoglucoside-acetates and 9,2% of cinnamoyl-3-monoglucoside.

Table 3 Anthocyanins of Saperavi skins and wine
and Cabernet sauvignon skins (% from total anthocyanin pigments)

Compounds	Saperavi Skins	Cabernet sauvignon Skins	Saperavi Wine
delphinidine-3-G	6,7	5,4	5,1
cyanidine-3-G	4,0	1,0	0,3
petunidine-3-G	12,1	7,3	8,2
peonidine-3-G	16,2	6,2	6,4
malvidine-3-G	39,3	45,7	64,5
Σ 3-monoglucosides	78,3	65,6	84,5
delphinidine-3-G acetate	0,7	2,2	0,4
cyanidine-3-G acetate	0,2	0,2	0
petunidine-3-G acetate	0,7	2,1	0,3
peonidine-3-G acetate	0,8	1,5	0,8
malvidine-3-G acetate	2,8	19,0	4,8
Σ acetyl-3-monoglucosides	5,2	25,0	6,3
Σ cinnamoyl-3-monoglucosides	16,5	9,5	9,2

Gas-chromatographic determination of volatile compounds of Saperavi wine are shown in Tab. 4. The reported metabolities, even if they were expression of a particular vinification process, did not let a punctual analysis about their origin because of the unknown development of microflora during fermentation. Higher alcohols and esters, produced during alcoholic fermentation, play an important role in the flavour of the wines, depending on the types of compounds and their concentrations (Valero et. al. 2002). The most plentiful compounds were the higher alcohols, in agreement with the literature (Baumes et. al. 1986). At concentrations below 300 mgL^{-1} they certainly contribute to the desirable complexity of wine; when their concentrations exceed 400 mgL^{-1}, higher alcohols are regarded as a negative quality factor (Mateo et. al. 2001). The total concentration of higher alcohols in Saperavi wine was below 300 mgL^{-1}. Other compounds were in common amounts. It can be mentioned only high amount of isoamyl acetate and because of low production level of acetic acid (0,38 gL^{-1}), ratio between acetates and acetic acid was – 2,8.

Table 4 Volatile compounds in Saperavi wine μg/L

isoamyl acetate	906
hexyl acetate	76
2 phenyl acetate	89
Σ acetates	1071
acetic acid (g/L)	0,38
(Σ acetates/acetic acid) x 10-3	2,82
ethyl hexanoate	301
ethyl octanoate	228
ethyl decanoate	31
Σ ethyl esters	560
hexanoic acid	1883
octanoic acid	1727
decanoic acid	503
Σ fatty acids	4113
Σ ethyl esters/ fatty acids	0,14
N(3-methybutylacetamide)	1488
isoamyl alcohol	57988
n-hexanol	1786
diethylsuccinate	62
ethyl 4-OH-butyrate	188
acetoin	8
2-phenyl ethanol	24898

The results obtained by analyses of the volatile compounds released by aroma precursors after enzymatic hydrolysis are shown in the Tab. 5. Linalool was in very few amount in Saperavi grape and wine. The other prevalent terpens in wine were: nerol (22,1 μgL^{-1}), trans-8-OH-linalool (36,0 μgL^{-1}) and geraniol (42,4 μgL^{-1}).

Table 5 Varietal compounds in Saperavi grape and wine and Cabernet sauvignon grape (for grapes μg/kg and for wine μgL^{-1})

Compounds	Saperavi Skins	Cabernet sauvignon skins	Saperavi wine
TFLO	9,2	3,6	5,1
CFLO	9,9	4,6	4,9
linalool	1,8	n.d.	1,6
α -terpineol	4,1	5,5	4,0
TPLO	7,2	4,1	10,7
CPLO	5,3	3,1	7,4
nerol	9,4	n.d.	22,1
geraniol	14,5	33,0	42,4
trans-8-OH-linalool	23,4	14,0	36,0
cis-8-OH-linalool	13,0	4,1	13,6

Conclusion

Most important Georgian red grape cultivar Saperavi (*V. vinifera L.*) was studied and performed its polyphenolic and aromatic characteristics. In comparison to Cabernet sauvignon this cultivar contained more quantity of: flavans, total polyphenols, total flavonoids, total anthocyanins, some catechins and procyanidin B1. Particularly, Saperavi was distinguished by more consistence of 3-monoglucosides and cinnamoyl-3-monoglucosides and Cabernet sauvignon was prevalent in acetyl-3-monoglucosides forms. Wine contained volatile and varietal compounds in quantity like those obtained from non aromatic cultivar. It is first report about Saperavi grape and wine and more research is needed for the deeper study and especially to know the quality of the microbial flora of the fermentative processes.

References

1. AMRANI JOUTEI K. (1993). Localisation des antocyanes et des tanins dans le raisin; etude de leur extractibilite. Thesis, Universite de Bordeaux II.
2. AMRANI JOUTEI K., GLORIES Y., MERCIER M. (1994). Localisation des tanins dans la pellicule de baie de raisin. Vitis, (33): 133-138.
3. BAUMES R., CORDONNIER R., NITZ S., DRAWERT F. (1986). Identification and determination of volatile constituents in wines from different vine cultivars. Journal of the Science Food and Agriculture, (37): 927–943.
4. BELLINCONTRO A., DE SANTIS D., BOTONDI R., VILLA I., MENCARELLI F. (2004). Different postharvest dehydration rates affect quality characteristics and volatile compounds of Malvasia, Trebbiano and Sangiovese grapes for wine production. Journal of the Science of Food and Agriculture, (84): 1791–1800.
5. BOURZEIX M., WEYLAND D., HEREDIA N. (1986). Etude des catechines et des procyanidols de la grappe de raisin, du vin et d'autres derives de la vigne. Bull OIV, (59): 1171-1254.
6. BUENO J. E., PEINADO R., MORENO J., MEDINA M., MOYANO L., ZEA L. (2003). Selection of volatile aroma compounds by statisti-cal and enological criteria for analytical differentiation of musts and wines of two grape varieties. Journal of Food Science, (68):158-163.
7. CHEYNIER. V., RIGAUD J. (1986). HPLC separation and characterization of flavonols in the skin of Vitis vinifera var. Cinsault. American Journal of Enology and Viticulture, (37): 248–252.
8. DI STEFANO R. (1996). Metodi chimici nella caratterizzazione varietale. Riv. Vitic. Enol, (49,1): 51-56.
9. DI STEFANO R., CRAVERO M.C. GENTILINI N. (1989). Metodi per lo studio dei polifenoli dei vini. L'Enotecnico, (5): 83-89.
10. DOKOOZLIAN N. K., KLIEWER W. M. (1996). Influence of light on grape berry growth and composition varies during fruit development. Journal of the American Society for Horticulture Science, (121): 869–874.
11. GAWEL R. (1998). Red wine astringency: A review. Australian Journal of Grape and Wine Research, (4): 74–95.
12. GIANOTTI S.; DI STEFANO R. (1991). Metodo per la determinazione dei composti volatili di fermentazione. L'Enotecnico, (10): 61-64.
13. GOMEZ E., LAENCINA J., MARTINEZ A. (1994). Vinification effect on changes in volatile compounds of wine. Journal of Food Science, (59): 406–409.

14. KELLER M., HRADZINA G. (1998). Interaction of nitrogen availability during bloom and light intensity during veraison. II. Effects on anthocyanin and phenolic development during grape ripening. American Journal of Enology and Viticulture, (49): 341–349.
15. KENNEDY J. A., MATTEWS M. A., WATERHOUSE A. L. (2002). Effect of maturity and vine water status on grape skin and wine flavonoids. American Journal of Enology and Viticulture, (53): 268–274.
16. KENNEDY J. A., MATTHEWS M. A., WTERHOUSE A. L. (2000). Changes in grape seed polyphenols during fruit ripening. Phytochemistry, (55): 77–85.
17. KISHKOVSKI Z. N., MERJANIAN A. A.. (1984). Technology of Wine. Moscow.
18. KOVAC V. E., ALONSO E., BOURZEIX M., REVILLA E. (1992). Effect of several enological practices on the content of catechins and proanthocyanidins of red wines. Journal of Agricultural and Food Chemistry, (40): 1953–1957.
19. MATEO J.J., JIMENEZ M., PASTOR A., HUERTA T. (2001). Yeast starter cultures affecting wine fermentation and volatiles. Food Research International, (34): 307–314.
20. MAZZA G. (1995). Anthocyanins in grapes and grape products. Critical Reviews in Food science and Nutrition, (35): 341–371.
21. MISTRY T. V., CAI Y., LILLEY T. H., HASLAM E.(1991). Polyphenol interactions. Part 5. Antho- cyanin co-pigmentation. Journal of the Chemical Society, Perkin Transactions (2): 1287–1296.
22. NYKA¨NEN L. (1986). Formation and occurrence of flavor compounds in wine and distilled alcohol beverages. American Journal of Enology and Viticulture, (37): 84–96.
23. O.I.V. (1990).Recueil des methodes internationales d'analyse des vins, Office International de la Vigne et du Vin, Paris.
24. OJEDA H., ANDARY C., KREAVA E., CARBONNEAU A., DELOIRE A. (2002). Influence of pre-and postveraison water deficit on synthesis and concentration of skin phenolic compound during berry growth of Vitis vinfera cv. Shiraz. American Journal of Enology and Viticulture, (53): 261–267.
25. OLIVEIRA C., BARBOSA A., SILVA FERREIRA A. C., GUERRA J., GUEDES DE PINHO P. (2006). Carotenoid profile in grapes related to aromatic compounds in wines from Douro region. Journal of Food Science, (71), S1–S7.
26. OLMO H.P. (1976). Grapes. In: SIMMOUNDS N.W. (ed.), Evolution of crop plants. Longman, London.
27. RICARDO-DA-SILVA J. M., BELCHIOR A. P., SPRANGER M.I., BOURZEIX M., LEANDRO M.C. CARVALHOE.C., HEREDIA N. (1992). Oligomeric procyanidins of three grapevine varieties and wines from Portugal. Sciences des Aliments, (12): 223-237.
28. RICARDO-DA-SILVA J. M., BOURZEIX M., CHEYNIER V., MOUTOUNET M. (1991). Procyanidin composition of Chardonnay, Mauzac and Grenache blanc grapes. Vitis, (30): 245-252.
29. SINGLETON V. L., (1992). Tannins and the qualities of wines. In: Plant Polyphenols Synthesis, Properties, Significance. HAMINGWAY R. W. and LAKS P. E. (Eds.) Plenum Press, New-York. 859-880.
30. SOUQUET J. M., CHEYNER V., BROSSAUD F., MOUTONET M. (1996). Polymeric proanthocyanidin from grape skin. Phytochemistry, (43): 509–512.
31. SPAYD S. E., TARARA J. M., MEE D. L., FERGUSON J. C. (2002). Separation of sunlight and temperature effects on the composition of Vitis vinifera cv. Merlot berries. American Journal of Enology and Viticulture, (53): 171–182.

32. UMMARINO I., FERRANDINO A., CRAVERO M.C., DI STEFANO R. (2001). Evoluzione dei polifenoli di uve di biotipi di Pinot nero durante la maturazione. L'Enologo, (4): 71-82.
33. VALERO E., MOYANO L., MILLAN M. C., MEDINA M., ORTEGA J. M. (2002). Higher alcohols, and esters production by Saccharomyces cerevisiae. Influence of the initial oxygenation of the grape must. Food Chemistry, (78): 57–61.
34. WULF L., NAGEL Ch.(1978). High-pressure liquid chromatographic separation of anthocyanins of Vitis vinifera. American Journal of Enology and Viticulture, (29):42-49.
35. ZOHARY D. HOPF M. (1993). Domestication of plants in the Old world. Clarendon Press, Oxford.
36. ZOHARY D., SPIEGEL-ROY P. (1975). Beginnings of fruit growing in the old world. Science (187): 319-327.

Table Grape Variety Assembly Line

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ABSTRACT

The present paper reviews the nutritional and medicinal properties of table grape and the potential of table grape production in Georgia. It also proposes the table grape variety assembly lines for eastern and western parts of Georgia individually. Introduction of variety assembly lines will significantly increase fresh grape supply to the population of Georgia.

Dans un article il a exprime les principes de convoyeur genetique de la production du raisin de table a les conditions de georgie, quels prolonge le terme garantir de marche par le nouveau raisin de table.

INTRODUCTION

Of all viticultural products table grapes are most widely consumed. Their nutritional, medicinal and dietary properties have been well known for a long time.

Grapes contain the following chemical compounds: carbohydrates, pectic substances, glucosides, organic acids and organic acid salts, minerals, non-nitrogenous compounds (tannins, colouring matter and aroma compounds, fat, wax, etc) and nitrogenous compounds (proteins, peptides, amino acids, amines, organic bases, nitrates); enzymes, vitamins (A, C, P, B_1, B_2).

Grape juice contains 16-32% of sugars. The sugar contents of 1 kg of grapes provide 440-840 calories. Grape sugar or glucose makes up 50% of the total sugar content in grapes. The level of fructose is equal to that of glucose (Pasenko P. et al, 1967, “Grapes and Human Health”, Krasnodar).

Due to its simple chemical composition and high solubility glucose is quickly absorbed by human body, converted into glycogen and stored in liver and muscles.

The curative dose of grapes is 2 kg. 1.5 litre of grape juice with 20% sugar contents contains 300 gr. sugar, 10 gr. organic acids, absorbable colloids (proteins, amino acids, phosphorous-containing organic acids, etc) and provides 1250 calories of energy, i.e. one third of an adult’s daily energy consumption. Consequently, supplementing the usual human diet with grapes helps to gain weight in a relatively short period of time.

The positive influence of grape sugars on the human nervous system is explained by their vasodilator effect.

As it is known brain tissues contain more sugar than any other human organ. Grape sugar has beneficial effect on the cardiac performance. It increases the dilation capacity of coronary arteries, thus improving cardiac muscle performance. Grape consumption contributes to cardiac muscle nourishment, improves blood circulation and digestive tract performance.

Organic acids, especially tartaric and malic acids have vital importance for human health. They improve stomach microflora, regulate gastric acidity and diuresis.

Organic acid salts are converted in the human body into carbonates. Notwithstanding its sour taste grape juice has alkaline reaction after digestion. 1 litre of grape juice has the same effect as 6 gr. of soda (Prostoserdov N. 1955, "Grape Uvology", Moscow).

Grapes and grape juice have diverse positive effects on human health and therefore they are widely used as therapeutic and food products.

Georgia has a long history of high-quality winemaking. However it is also abundant in agroecological resources for expansion of table grape production. Centuries long history of winemaking in Georgia should not hamper production of table grapes and grape juice. The determining factors should be the market demand and rational use of agroecological resources, which would ensure production profitability. For this purpose development of the viticulture-wine cadastre is a matter of great urgency. The cadastre will ensure inventorying, certification and mapping of those zones and districts where high-quality, controlled wines bearing the historical-geographical names of the specific zones are produced. The cadastre will also scientifically determine the raw material sources for various types and brands of wine as well as systematize the potential of table grape and grape juice production. Development of the viticulture-wine cadastre is a topic in its own right, which we have discussed in one of our articles. (Gotsiridze V. 1993, "Development of the Viticulture-Wine Cadastre", Issue No2 of "Vine and Wine" Magazine, pg. 25).

As the aforementioned Cadastre, which is a universal regulatory document for the sector, has not yet been developed we should use the available literary sources and experimental data.

Prof. D. Tabidze stated: "There are a lot of district in Georgia where high-quality table grapes can be produced, e.g. in Gardabani and Bolnisi Districts" (Tabidze D. 1964, Bulletin of the Academy of Sciences of Georgia, Vol. XVI).

According to the modern standards low places with hot climate and mechanically cultivable soil are most suitable for commercial growing of table grapes. In East Georgia such areas are Gardabani, Marneuli, Bolnisi districts, and suburbs of Tbilisi, Zilicha-Taribana Valleys and Samukhi Valley in Dedoplistskaro district of Kakheti Region. Unlike Zilicha and Taribana Valleys where grapes varieties for table and port type wines and cereal crops are grown, Samukhi Valley (about 20,000 ha according to provisional data) is a virgin land to be developed and cultivated.

In West Georgia the most favourable places for table grape growing are Imereti low places, Guria-Samegrelo and Ajara lowlands and mountain slopes, which are unsuitable for tea and citrus. The best conditions for table grape growing are in Ochamchire, Sokhumi, Gudauta and Gagra districts of Abkhazia. Restoration and expansion of viticulture, specifically table grape production, will significantly contribute to the revival of this region. It is well known that vines give the quickest return on investment as compared with the other perennials (fruit, citrus, and tea). In the regions with sufficient moisture vines give their first harvest in the third year after planting and reach fruit bearing age in the fourth and fifth years. The current development of Georgia's resort and tourism potential will provide impetus to table grape production. Scientifically based recommendations on the vine production zones, varieties and effective agricultural practices are needed for expansion of table grape and grape juice production. The present paper reviews promising varieties of table grapes and proposes

the variety assembly lines based on the agrobiologic and economic-technological studies of vine varieties and the data available at the IHVO and its branches.

According to the current agronomical regulations (Viticulture Regulations, Tbilisi, 1975) three table grape varieties (*Kartuli Saadreo, Ganjuir, Gorula*) are recommended for East Georgia and five varieties (*Chasselas*, *Tskhenis Dzudzu*, *Apkhazuri*, Karaburnu) – for West Georgia.

The Law "On Vine and Wine" of Georgia (Adopted by the Parliament in 1998) envisages cultivation of only 14 table grape varieties. Due to the incomplete and scarce assortment of the table grape varieties the great agroecological potential of Georgia is still unutilized. In both West and East Georgia the environmental conditions and their variation in vertical and horizontal zones it is possible to produce table grapes of all ripening periods (very early, early, medium, late and very late ripening). The table grape variety assembly lines for East and West Georgia are shown in tables No 1 and 2 below.

Table No1
Table Grape Variety Assembly Line for East Georgia

No	Vine Variety	July				August						September					
		VII				VIII						IX					
		15	20	25	30	5	10	15	20	25	30	5	10	15	20	25	30
1	*Favorit*																
2	*Kartuli Saadreo*																
3	*Sentatives mushkotay2*																
4	*Khalili*																
5	*Naranchizi*																
6	*Early Malingre*																
7	*Sentatives mushkotay1*																
8	*Kechkemeturi 159*																
9	*Portugieser*																
10	*Koroleva Vinogradnikov*																
11	*Royal Vineyard*																
12	*Bestashvili Tetra*																
13	*Tsiteli Budeshuri (Budeshuri Red)*																
14	*Poloshkeummuskotal*																
15	*Rkatsiteli*																
16	*Senso*																
17	*Poloshkeummuskotal*																

No	Vine Variety	July				August						September					
		VII				VIII						IX					
		15	20	25	30	5	10	15	20	25	30	5	10	15	20	25	30
18	*Mahmud*																
19	*Muscat of Alexandria*																
20	*Taif of Ordubad*																
21	*Aguna*																
22	*Iveria*																
23	*Vardzia*																
24	*Sakartvelo*																
25	*Gorula*																
26	*Nimrang*																
27	*Tbilisuri*																
28	*Naranchiz 5*																
29	*Delisi*																
30	*Muscat Rkatsiteli*																
31	*Poloshkeummushkotal3*																
32	*Naranchiz 3*																
33	*Vermentino*																
34	*Tsytsa Capri*																
35	*Agadai*																
36	*Kharistvala Kolkhuri*																

Table No2
Table Grape Variety Assembly Line for West Georgia

No	Vine Variety	July				August					September					October					November			
		VII				VIII					IX					X					XI			
		5	0	5	0	0	5	0	5	0	0	5	0	5	0	0	5	0	5	0	0	5	0	
1	*Kartuli Saadreo*																							
2	*Pearl of C'saba*																							
3	*Madeleine Angevine*																							
4	*Madeleine Royale*																							
5	*Chaushi*																							
6	*Chasselas white*																							
7	*Cielo -Jielo*																							
8	*Tsiteli Budeshuri (Budeshuri Red)*																							
9	*Pukhlyakovski*																							
10	*Shabash*																							
11	*Chasselas Napoleon*																							
12	*Rishbaba*																							
13	*Muscat Hamburg*																							
1	*Muscat of*																							

4	*Alexandria*					
15	*Muscat de Madeira*					

No	Vine Variety	July				August						September						October						November					
		5	0	5	0		0	5	0	5	0		0	5	0	5	0		0	5	0	5	0		0	5	0		
16	*Karaburnu*																												
17	*Malvasia*																												
18	*Catalan winter*																												
19	*Akabil Black*																												
20	*Kharistvala Kolkhuri*																												
21	*Azhkapshi*																												
22	*Tskhenis Dzudzu Abkhazuri*																												
23	*Makhaturi*																												
24	*Mekrenchkhi*																												
25	*Kakhuri Tetri*																												
26	*Tskhenis Dzudzu Ajaruli*																												
27	*Klarjuli*																												
28	*Vaios Sapere*																												
29	*Samarkhi*																												

The variety assembly line for East Georgia comprises 36 vine varieties, including 12 local and selection varieties and 22 introduced ones. The presented variety assembly line will ensure fresh grape supply to the market for 5 months (from July 15 to November 25).

It should be taken into account that regardless their ripening period the grapes may remain on the vine for 5-10 more days or can be selectively picked 5-10 days prior to ripening. Therefore ripening periods of various grape varieties overlap, which ensures stable supply of fresh grapes to the market.

Taking into consideration the aforesaid we can make the following conclusions:

1. The agroecological resources of Georgia make it possible to grow table grapevines of various ripening periods. Due to the scarce assortment of industrial varieties of table grapevines the potential of table grape production in Georgia is not fully utilized.
2. The climatic and soil conditions of Georgia, vertical and horizontal zoning, a large number of local and introduced vine varieties make it possible to considerably expand industrial assortment of table grapevines;
3. Based on the results of our experiments as well as other data of vine variety studies we have developed the first variety assembly lines for Georgia ensuring fresh grape supply to the market from June 5-10 to November 5-10 (i.e. for 4 months) in East Georgia and from July 10-15 to November 10-15 (i.e. for 5 months) in West Georgia;
4. Over the past few years we have been testing some table grapevine varieties, which we introduced in the environment of Georgia. The varieties demonstrating the best agricultural and technological properties are Victoria, Regina, Italia, Matilda, Michael Poliero, Cardinal, Black Magic, Sultanina and Manikur. Together with our newly developed hybrids they will enrich the table grape assortment in Georgia.

Bibliography

1. Gotsiridze V. 1993, “Development of the Viticulture-Wine Cadastre”, Issue No2 of “Vine and Wine” Magazine, pg. 25.
2. Pasenko P. et al, 1967, “Grapes and Human Health”, Krasnodar
3. Prostoserdov N. 1955, “Grape Uvology”, Moscow
4. Tabidze D. 1964, Bulletin of the Academy of Sciences of Georgia, Vol. XVI.

The technology of getting the concentration of juice of grapes (liquid and powder)

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ABSTRACT

A technology of receiving dry extracts proved to be very effective, in the periods of embargo on an excessive harvest of grapes and of the processed products thereof. In the main viticulture regions of Georgia, the juices from grapes and their concentrates are produced. The dry concentrates from the grape juice is received with adding the nutritive fillers thereto. For this purpose the flour of corn and wheat with 1:1 rate and the high content of the protein was chosen as an additive, so that an initial concentration of the dry substances in the mixture was 35%. For drying the mixture a special apparatus is used, with 150°C determined as the optimal working temperature.

La technologie est très efficace lors de la récolte excessive du raisin et de l'embargo des produits traités. Les micro-zones écologiquement pures et les espèces industrielles vignobles Dans les régions principales de la viticulture de la Georgie a été produit le jus de raisin et son concentrât. La réception du concentrât sec du jus de raisin se fait par le supplément du produit d'apport alimentaire pris d'après les qualités mécaniques-structurelles et la valeur alimentaire. La farine du maïs et du blé ayant une haute composition albumineuse a été choisie comme le supplément du jus de raisin avec une compatibilité 1 : 1, d'une telle manière que dans le mélange la concentration initiale de la matière sèche est 35%. La température optimale de 150°C a été choisie pour sécher le mélange dans un appareil spécial.

INTRODUCTION

Currently, a high attention is paid to the products introduced in a form of powders produced on the basis of the grape juices having a high biological value, This is conditioned by a number of positive features of such products. These powders (water soluble) are widely used for producing various types of soft drinks, tonics, baby- and diet foods, which, with their chemical

content and biological qualities stand closer with the initial raw materials (A. Kacharava et al., 1990; Verkn B.I. at al., 1986; Durmishidze at al., 1986). An economic efficacy of the technology for producing the dry concentrates, is predetermined by its simplicity and a transportability.

The grape must obtained from the experimental winery of the Horticulture, Viticulture and Wine Making Institute was used as the main object of the study. The preliminary experimental works have been performed on the must of the industrial sort of the wine "Rkatsiteli", of which the following products were produced:

The grape's must concentrate and the grape vacuum must, with using the instructions applied in the production. The following materials are used for producing the powder-type products: icing sugar, potable water, liquid sugar, citric acid, protein concentrates, corn flour, wheat flour, koller, etc.

The dry substances in the powder-like mixture were determined from a total mass of the raw material, in the grape must and other single liquids – according to the Refractometric Method, while a total sugar content – according to the Bertran Method.

A study of the mechanical-and-structural characteristic of the grape must, kinetics of drying, correlations of conditions of spraying and water losing of its single drops, as well as preparation of different compositions on its basis have been conducted *in vitro*, through the spraying device. For the industrial tests for receiving the powders, the device "Niro Atomaizer" with 500l/h capacity, of the Danish origin, was used.

Currently, a method of drying through sprays is applied widely, since it ensures a receipt of high-quality powders from the liquid raw materials. The products manufactured by help of this technology are used in the fields of food, medical microbiology, etc.

An advantage of drying through the spray is as follows: both the drying and spraying processes are implemented in one apparatus and, a homogeneity of the powder (which preserves all the valuable features of basic components of the raw material and is almost soluble) is achieved in and a process of drying lasts for several minutes, only (Malertskaya, 1973, Dolinsky et al., 1987) .

Proceeding from the above mentioned, for the purposes of receiving the powder from the grape juice, we have used the method of drying through the spray, requiring to research an appropriateness of the kinetic of drying as well as the structural-and-mechanical properties of the received powder.

MATERIALS AND METHODS

The tests have been performed on the concentrate of a pure grape must, in which a content of the dry substances varied within 20-45% limits, the air temperature – within 50-170°C. The results of the said test have shown that no boiling takes place below 145°C. After starting evaporation the drop's temperature is increasing slowly up to a certain point (KP_2), while thereafter the linear changes during a certain period of time are observed in the limits from point KP_1 to point KP_2. This specificity is related to both the process of plastic deformation of dry particles of the grape must and the hygroscopic properties thereof. A complex composition the grape juice, which serves as a determinant of its thermal plasticity, indicates that no process of dehydration of the juice drop takes place on higher temperatures, the particle remains soft and floating in the hot vapour, while increasing the temperature above 150°C causes a total burning of the product.

Therefore, the results of drying tests of the pure grape must have shown that the powder cannot be received from it, because of thermal plasticity of the grape juice. So, to receive the powder, a selected nutritive filler should be added to the grape juice.

We have selected the filler for putting into the grape juice based upon the following principles: structural, mechanical, physical, chemical properties and nutritive qualities of the raw material, with which they differ from the grape must. A due selection of such ingredients ensures receipt

of a high-quality powder, increases and widens its nutritive values as well as the spectrum of its bioactivity.

Taking into account the fact that in line with the grapes juice the corn flour and the wheat flour were also used in Georgia in the past times for preparing the national products (Tatara, Pelamushi, Churchkhela, ertc.), we used the aforementioned corn flour and wheat flour, as the fillers to the grape juice.

We have conducted the experiment on the mixture of the grape juice and the flour, with the initial concentration of the dry substances C=35% and within the temperature ranges t= 110°C,150°C and 180°C.

RESULTS AND DISCUSSION

As a result of the experiment, the optimal temperature was fixed as 150°C, a lower temperature 110°C makes the process longer in duration, while a higher temperature like 180°C makes the process shorter, but at the same time causes burning of the product and decrease a solubility of the powder.

The results of visual and cinemagraphic observations on the evaporation and drying processes involving the composites of various contents, has shown that boiling of the mixture of the grape must and the corn/wheat flours is reported on 100-150°C, but with a lower intensity of evaporation, neither spraying nor disintegration of the mixture is detected. Therefore, we may conclude that for the raw materials giving the powder, it has no sense to increase their density.

For receiving the dry soft drinks, we were using as the basic ingredient the grape juices of various quantities with adding thereto the protein concentrates, extracts of the green parts of the Apple-tree, the tartaric acid and the granulated sugar (see Tab.1), determining their optimal concentrations in the composition through the organoleptic indicators with taking into account their qualities, physical-chemical and mechanical properties, researching different versions thereof and defining their drying temperature and duration, as well fixing their basic chemical content.

Table 1

Ingredient composition varieties for receiving non-alcoholic beverages

№	composition	Version, % a. c. m.							
1		I	II	III	IV	V	VI	VII	VIII
2	Vacuum- juice	30	35	40	45	50	55	60	65
3	Albumenous concentrate	40	35	30	25	25	20	20	20
4	apple-tree leaf extract	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
5	Vinous acid	3	3	3	3	3	3	3	3
6	sugar	25	20	15	20	15	-	-	-
7	orange essence	0.6	0.6	0.6	0.6	0.6	0.6	0.6	0.6

Due to the fact that the grape juice contains a large number of biologically active substances able to become disintegrated on higher temperatures, we have conducted the drying process on the temperature not exceeding 200°C.

Both chemical and organoleptic characteristics of the received compositions have shown that as a result of lowering the rate of the grape juice and increasing the rate of the protein concentrates in the mixture, the qualitative properties of the product are lowered. Thereafter we determined an optimal content of the composition of the grape juice and protein concentrates (1:1) These data have served as the basis of development of the dry soft drinks production technology.

On the next stage, we have conducted the tests for determining the optimal quantity of the tartaric cid. For this purpose we took the basic ingredients of the concentrate and the different doses of the tartaric acid. The received mixture was then dried and the basic components, namely – saccharose, glucose, fructose, organic acids, the "C" vitamin, lipids and polysaccharides determined. The results of the above mentioned tests are given in Tab. 2.

Table 2

Physical-chemical data of dry, non-alcoholic beverage composition varieties

№	Version	Index		
		Humidity, %	Mass volume, g/cm^3	Porosities, %
1	Version I	8.5	0.31	60.98
2	Version II	7.6	0.43	60.51
3	Version III	7.3	0.47	60.18
4	Version IV	7.0	0.52	59.85
5	Version V	6.8	0.58	59.47
6	Version VI	6.4	0.61	59.11
7	Version VII	6.1	0.64	58.82
8	Version VIII	5.7	0.71	58.61

As evidenced from the data provided in the Table, the quantities of the main chemical compounds are partly reduced, in case of increase of the temperature.

Taking into account the obtained results, an optimal version of receipt of the composition of dry concentrate of the grape was achieved, with the following contents: grape juice – 37%, protein concentrate 35%, tartaric acid – 3,5%, sugar – 20-23%, and essence – 0,8%. These data were taken as the basis for developing the technological scheme of production of the dry concentrate of the grape juice.

The scheme envisages the following technological processes: grape juice is placed into the mixing reactor (1), from which the mixture moves to the vacuum – evaporator (2). Then the mixture is concentrated up to 25-30% content of dry substances. The thickened concentrate is then carried to the homogeniser (3) for receiving the homogenous mass. This homogenised mass through the vacuum-pump (4) is moved to the drying disc (5) of the dryer with the rotation rate of 7000-7500RPM. From this device the sprayed mass moves to the drying chamber (6) where the air stream from the thermal generator (7) is heated up through the ventilator (8) and mixed to the clouds of liquid. The dry concentrate is then filled up with the chamber and settled as a powder and directed through the heat carrier to the cyclone (9) for dividing.

The concentrate is evaporated in advance, up to 6-8% moisture content value. The cooling system (10) is used in the course of drying, that ensures a filling of the powder. The mixture from the Cyclone (11) pneumatically moves to the unloading cyclone (12) through the pipe. The used-up heat carrier passes through the filter (13) and the product dried and cooled by the ventilator (14) is supplied to the hopper (15) and then directed for sorting and packing. 20kg polyethylene bags are used as the packing material. The used-up air is released into the atmosphere through passing the ventilator (16).

Bibliography

1., Kacharava L.T. Mudziri L.A. et al. Powder-type mixtures on the base of the grape juice. Food Industry. 1990 2. p.35-36

2., Verkn B.I., Maximenko G.I., t al. Development of the powder-type mixtures for soft drinks, through the cryogen technology. M. CSR T Food Industry. 1986. V.2 – p.18-20

3. Durmishidze S.V., Khachidze O.T. Biochemistry of the grape plants – Tbilisi, 1986

Research of Scopolamine in Oak wood and Brandy Alcohol and their Influence on Product Quality

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ABSTRACT

It's known from literature, that alcohol drinks contain cumarins and their productions – scopoletine, which might be important in forming brandy spirits aroma. The research confirmed the bark of the oak contains cumarins –scopoletine.It has been established, that scopoletine is in the composition of oak tkechi and by the time it going out in brandy spirits. Therefore, we can say, that tone of antiquity of spirits is partly caused by containing scopoletines in it. Containing of scopoletine in brandy spirits with the amount of 2,5-5,0 mg/ L^{-1} is one of the typical sign of its natural character.

D'après la littérature de différentes boissons alcooliques contiennent de coumarines et leur produit – scopolétine qui doit assurer le rôle positif pour la formation du bouquet des alcools de la Brinze. L'examen de l'extrait éthylacétate du ligneux du chêne a confirmé la présence des coumarines, y compris la scopolétine. Il est confirmé que la scopolétine entre dans la constitution des ligneux du chêne et avec le temps elle déborde au fur à mesure dans les alcools de la Brinze. Dans les alcools de la Brinze composition de scopolétine du 2,5 au 5,0 mg/dm^3 peut être considérée comme une des particularités pour définir son caractère naturelle.

INTRODUCTION

Classical technology of brandy production is that new distillated brandy spirits must be outdated in the oak barrels. During this period unique aroma and taste is formed which distinguishes brandy from other alcohol drinks.

It's known that in brandy production oak barrels and extract of oak are used to accelerate outdating process, which contain cumarins together with other components.

In brandies , brandy spirits and clapboard of oak Flavonoids and Catechins were founded by the scientists. It has been established that brandy spirits, brandy and clapboard of oak contains triterpens, steroid components and glucozids and etc.

Together with catechins a spot of substance was fixed, which gets light blue by fluorescence and ultra-violet light. With qualitative analysis was established that fixed component belonged to cumarin derived.

According to the literature different spirit drinks contain cumarins. It was studied contents of cumarins in rum, whisky and their distillated which was caused by their outdated process in oak (Fernandez Jzquierdo et al. 2001). Cumarins might be important in forming brandy spirits aroma (Horwitr et al., 1970; Livingston, Bickoff, Yuggol, 1960;) Preparation.

MATERIALS AND METHODS

The aim of our study was to determine scopoletine in the bark of oak and brandy spirits and its effect on the quality of the products.

The object of our research was 1 kg sawdust of oak for establishing cumarins in the bark of oak, which was treated by 80 % ethyl alcohol with ratio 1:10. Received extract was thickened, after that was extracted separately with ether and ethyl acetate. Separation of extract ethyl acetate was carried out on the columns of Polyamide with water, 45 % and 90 % ethyl alcohol. By chromatographic analysis of received fractions was found that water brings the substances which has low RF, in 45% ethyl alcohol is shown almost nothing. In 90% ethyl alcohol was found the sum of substances which contains mostly tannins and also the sum of substances of ethyl acetate's extract.

As water fraction is characterized with high fluorescence that's why we carried out its hydrolysis with 5% H2so4. The hydrolizate was extracted with ethyl acetate. The extract was thickened and liquefied in 1 ml. ethyl alcohol and was studied contents of cumarin in the selected system. The spot is developed in hydrolizate by its rf 0,29 0,02, by colour (on ultraviolet light) and by alkali development it coincides with scopoletine. Scopoletine may be in bark of oak in free form but it in the sum of phenol substances is hidden. In hydrolizate it is confirmed that scopoletine is in free form in the bark of oak. The following aim of our research was to study extracts received from treated oaks and without it.

For analyzing 7 samples were taken:

1.The chloroform extract of oak's sawdust.
2. the extract of oak's bark treated without warm
3. scopoletine
4 the extract of oak's bark treated by warm
5.the derived of cumarin umbeliferone.
6. dry sawdust of oak's bark
7 the extract of oak (French)

the extraction of oak'sawdust was carried out with 70% ethyl alcohol. The whole other extracts (1;2;4;6; and 7) were thickened under vacuum (until without water) and each of them was extracted three times by chloroform. The extracts were dried with Na_2SO_4 and were thickened until 1-2 ml. The samples were transferred on the paper chromatography for studying of scopoletine and were put in the system chloroform\ formamide.

RESULTS AND DISCUSSION

From the figure is cleared up that all samples contain scopoletine the derived of cumarin. It is not confirm existence of umbipheron.

The following aim of our research was to study qualitative and quantitative analyses of cumarin in brandy spirits and their influence on the quality of the products. For analyses long outdated brandy spirits on bark of the oak were taken (1941, 1949,1956 and 1968 years). The objects were prepared beforehand for analyses. There qualitative analyses were carried out. The give reaction on cumarin.

It has been established that the samples in spite of their different ages there was nearly no difference by qualitative contents. If we don't take into account the spots before rf 0,33, which is very little in the sample of 1968 years. With the years the amount of scopoletines increases in these types of spirits.

It has been shown derived of cumarin – scopoletine in spirits outdated different time on the bark of the oaks.

It has been determined the quantities of scopoletine in outdated brandy spirits different time on the bark of the oak 1918, 1955, 1968 years, in foreign brandy spirit (Azerbaijan), and in model solution of the spirits 40% and 60%. Scopoletine and some volatile compounds were determined by chrom mass spectrometry . the results are shown in the tab. 1.

table 1

conents of scopoletine in the gamples

#	name	Scopoletine mg/ L^{-1}
1	brandy	2,0
2	New distillated spirit	0,009
3	brandy spirit 1968 years	1,9
4	brandy spirit 1955 years	2,8
5	brandy spirit 1918 years	4,1
6	Foreign brandy spirit (Azerbaijan)	0.5
7	Model solution of brandy spirit 65% +5 mg/ L^{-1} scopoletine	5,5
8	Ethyl alcohol 65% + 10 mg/ L^{-1} scopoletine	10,0
9	Model solution of brandy 40% + 5 mg/ L^{-1} scopoletine	5,0
10	Model solution of brandy 40% + 25 mg/ L^{-1}scopoletine	2,0
11	Ethyl alcohol 40%	-
12	High alcohol drink „golgen“	1.9

In the table is shown that after the years has been observed increase of scopoletine in these types of spirits. It proves that scopoletine is in the composition of the oak's bark and by the time it going out in brandy spirits. young brandy spirit contain the following amount of scopoleitine – 0,5mg L^{-1}/, in the spirits of different ages the amount of it was: 1968 – 1,3; 1955 – 1,8; 1918 – 2,1mg/L^{-1}.

From the table we can say that in the brandy spirit of 1918 years scopoletine is 4,5 % more than in new distillated spirit. Our consideration agrees with results obtained by other authors Fernander Jzquierds et al. (2001) that tone of antiquity of spirits partly caused by containing scopoletines in it. By the taste of scopoletine the intensively of smell determined with 5 point.

According to the experiment was established that the new distillated spirits did not contain scopoletine. Its amounts is increased according their outdating process.

By the dates of taste committee, the model solutions of scopoletine , in which was added 2,5-5,0 mg/ L and give the tones of outdated spirits. scopoletine, has smell, taste and perfume of specific flower and hay. Also yellowish colour. Containing of scopoletine in brandy spirits with the amount of 2,5-5,0 mg/ L^{-1} is one of the typical sign of natural character of it.

LITERATURE CITED

1. Horwitr W., Editor P., Ch. and H.Reundds. 1970. Eleqnth Edition, p.743.
2. Fernandez Jzquirdo M.E., Quesada Granador J., Villalon Mir M., Lopez Martinez M.C. 2001. Factores qui condisionanla presencia de cumarinas en bebidas aalkoholicas envejecidas en barril de robbe. Alimontaria. 38, N320, c.127-135
3. Livingston A., Bickoff E., Juggolz Y, Ray C. 1960. Anolit. Chem, 32, 1620.

The getting and using of biological active admixtures from the processed products of grapes

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ABSTRACT

Industrial processing of grapes causes accumulation of large amounts of waste. In the course of production of the wine materials, from 1t. grape about 100-140kg dry grape branch (Chacha) and 4,0 – 4,5 Dal yeast mud are formed. The said wastes are rich with bioactive substances and serve as essentially valuable raw material for a large number of products. From the wastes received as a result of grape processing (the pressed mass of the grapes and the mud) the following materials are produced mainly: the nutritive additives and paints, which, after being processed, are used in production of alcohol drinks and confectionery. The grape's seed is also a valuable material for the protein isolates.

Le traitement industriel du raisin est lié à une grande présence des restes œnologiques. Lors de la production des matières du vin il est produit d'une (1) tonne de raisin en moyenne de 100-140 kg marcs secs et 4,0 – 4,5 dal (décalitre) de la lie de levure. Les susdits restes de la production de vinification sont riches des substances biologiquement actives et représentent la source des matières de valeur d'un grand nombre des produits. Des restes du traitement du raisin (du marc et de la lie) il est obtenu en général : des suppléments alimentaires et teintures et après le traitement et la purification ces derniers sont utilisés dans l'industrie des boissons alcooliques et des confiseries. Le grain de la grappe est une matière de valeur pour la réception des isolats albumineux.

INTRODUCTION

In the course of processing the grapes, the wastes thereof are also received together with the main product, which are rich with the biologically active compounds, of which the most important ones are grape branches (both fermented and non-fermented); The grape's rod; The

must released from the mud after settling and alcoholic distillation, as well as in the course of wine maturation; The mud received as a result of pasteurization, sulphatization, cooling and production of the grape juice; Grains (distillery refuse) - as a result of distillation of the wine material.

A high content of the free fat acids and glycerine is detected in the grape branch and seed, while in their extracts a number of biologically active substances are identified (Mudziri, 1988)

The grape seed contains 22-56% of the total poly-phenols, 28-58% of leukoanthocytes, 67-68% catekhines and an important volumes of the gal and the coffee acids (Ribero-Gayon, et al., 1981)

The issues related to a complex processing of the wine secondary waste and a relevant application thereof, has not been still studied-in duly. Such the complex processing of these materials can make the wine production ecologically safe.

MATERIALS AND METHODS

A purpose of the study was to receive the biologically active compounds from the secondary products (grape rod, branch, wine mud, grape grain) and their use in different fields of the National Economy.

A task of the study was to determine a content of nitrogenous compounds in the secondary wine products and to establish their biological quality; To develop the means of separation from the secondary wastes the biologically active compounds and their products and the use thereof.

The secondary grape- and wine products, namely – the wine mud and the grape grain (distillery refuse) were selected as the objects of the study. Besides, the alcohol was isolated from the fermented grape branch. Thereafter, these secondary products underwent a process of drying and segregation and the biologically active substances were extracted from them and concentrated.

A thickened mass of the hydrolyse received by use of the fermentation apparatus for malising the processed wine mud, is a protein-vitamin concentrate containing up to 50% soluble proteins and considerable amounts of amino-acids and vitamins. According to the above mentioned results, it is possible to use the said protein-vitamin concentrate as a stimulator for plants growing.

For receiving the alcohol and the tartaric acid's lime from the liquid mud, we conducted the distillation of the same and extracted the grains from the tartaric acid-containing compounds.

As to the paste-like mud, it was first diluted in the water and mixed intensively, while the received liquid mass was used for distillation of the alcohol and extraction of the tartaric acid's compounds.

The wine mud of the "Rkatsiteli" grape sort being fully fermented, was used by us as the object of study. We have researched the wine mud until and after distillation of the alcohol and sedimentation of the tartaric acid's salts. In order to separate the protein fractions from the yeast cells it became necessary to disintegrate the cell membrane through acidic hydrolyse of the wine mud. The protein-containing components were determined by a method (Pavlenko et al. 1968), during which we received the proteins soluble in water, salt, alcohol, and alkaline, while their quantitative in single fractions of the protein were determined by the Gartre method, and the total nitrogen content – by the Keldal method.

RESULTS AND DISCUSSION

As revealed, the wine mud after distillation of alcohol and sedimentation of the tartaric acid's salts, the wine mud contains the protein substances with a total quantity up to 23%.

We have researched also the grape seed's nitrogenous substances, namely – the protein-containing and not containing nitrogen, globulins, albumins, amino-acids. The seeds of the grape

sorts of “Saperavi”, “Rkatsiteli” and “Kaberne” being in the technical maturity phase were selected as the object of research.

From the secondary wine products, namely, from the pressed mass of the grapes and the mud (both white and red) we have received the natural herbal paints containing the colourless leukoanthocyanides.

The technology of comprehensive processing of the wine products waste envisages production of the protein-enriched and biologically active food products from the dry yeast refuse, the food flour, the grape seed and the grape branch (Razuvaev et al., 1985).

Production of the protein-containing foods from the dry yeast is related to the energy-consumed and low-productive thickened yeast production processes. That is why, earlier, there were some attempts to use the yeast refuse as the food additive for animals. The content of the raw protein in the yeast refuse does not exceed 2,5% of the mass weight, while a total content of the dry substance makes 9-12% of the mass weight. A nutritive value of the yeast refuse is about 0,5 on per 1 dl (Razuvaev, 1975). In most of wineries of Georgia, the grape branch is used mainly for livestock feeding and placed in the distillatory tanks. Distillation is carried out through the apparatus of NDT-3M model. Thereafter, the alcohol-free branches are to be shipped to the mixed fodder enterprises.

We think that the primary wineries are able to produce the food products rich with bioactive compounds from the grape branches, distillery refuse of the thickened yeast and, use them for animal feeding purposes.

In order to develop the technology for production of the food products enriched with the bioactive compounds, certain experimental works became necessary to conduct, giving us an opportunity to determine an optimal composition of the food products, the regimes of preparation thereof, the parameters of the relevant processes and, to carry out a test of experimental feeding of animals.

In the course of the experimental works we used the fermented grape branches (pH 5, Tartaric Acid content – 0,1%, alcohol content – 2,5%) The grape branches were distilled on 95°C and, being heated up to 58-60°C, and re-loaded into the thermally insulated reservoir which was preliminarily filled with the weed. The food value rate of the alcohol-free grape branch made 0,15 unit.

For receiving the suspension, the dry mud of the yeast was mixed with the water up to 10% of the mass weight. The received blend was then distilled, while the distillery refuse received was delayed for 12 hours. After filtration, the received thickened refuse (pH 3,2; t=85°C) contained dry substances in amount of 10% of the mass weight and 2% tartaric acid. The nutritive value (rate) of the yeast refuse was 0,1 unit. The grape branches placed in the reservoir, were added to the hot yeast refuse in various ratios and mixed. During the whole period of storage in the reservoir, i.e. for 3 – 7 days, the temperature level was preserved constant - 58±2°C. The nutritive value of the product made 0,1 unit. A permissible period of storage of the product in natural conditions was determined after thermostating, depending upon the sample’s state. The food products unloading was organized through using the containers arranged on the trucks, with their further distribution to the livestock farms. A food made of the yeast mud and the flour with 91% moisture content, was used as the sample for the control testing purposes.

A comparison of the results of analysis of the experimental food prepared for testing and the one used for control testing, proved that the food proposed by us has much higher quality and nutritive value and is much better by view of content of the bioactive substances, than the control sample. In the experimental food, a content of the raw protein is by 12,5% higher, while a total content of the nitrogen-free extracts exceeds the control one by about 19%

Bibliography

1. Mudziri L. 1988. Niektore aspekty bezodpadovej technologie spracovania vedl'ajsich produktov pri vyrobe hrozna a vina //Kvas. prum. C.10. S.296-300
2. Ribero-Gsyon J., Peino E., Ribero-Gayon P., Sudrou P. 1981 The Wine-Making Theory and Practice. V. 4 M. Food Indistry. p.352
3. Razuvaev N. 1975. Combined Processing of the Wine Secondary Products. M. Food Industry. p. 168
4. Razuvaev N., Melyaev V., Martinenko E., Zankal T., Selezneva L., Belan V., Vasilieva L. 1985. Rational Technology of Receiving the FQI from Yeast Mud // Wine-making and Viticulture in the USSR, No. 1, p.19-21
5. Pavlenko N., Datuashvili E. 1968. On Fermenting Hydrolyse of the Grapes Protein // Wine-making and Viticulture in the USSR, No.5, p.6-8

STUDIES CONCERNING THE MACERATION-FERMENTATION TECHNOLOGIES ON CHROMATIC PARAMETERS OF BĂBEASCĂ NEAGRĂ RED WINES FROM PANCIU VINEYARD

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ABSTRACT

This study analyzed the influence of some maceration-fermentation technologies (classical maceration, ROTO-tank maceration, thermo-maceration, microwave maceration and ultrasounds maceration) on the chromatic parameters in the wine samples obtained from the black grapes of Băbească neagră grape variety from Panciu vineyard. The data shows the influence of the maceration-fermentation technology on the wines' color, a great phenolic potential being found in microwave maceration, closely followed by ultrasound maceration.

INTRODUCTION

In the Panciu vineyard pedo-climatic conditions, local grape varieties start gaining more and more value, offering in a good year, quality wines. This study wants to analyze the different factors that contribute to a wine's final quality, especially the technology used in extracting color and aroma, the maceration-fermentation procedure. Obtaining a fine colored wine, according to the climatic conditions and color accumulation, is an important issue in wine quality and analysis, therefore, proper knowledge of means of obtaining specific color parameters is very important.

MATERIAL AND METHOD

Băbească neagră grapes were used harvested in 2009 from Panciu vineyard, Ţifeşti center. The grapes were manually harvested, in wooden crates and transported to Iaşi Pilot Research Station where they were processed by different technologies.

Table 1

Compositional characteristics of grapes at harvest

No	Vineyard	Harvest date	Reductive sugars (g/L)	Total acidity (g/L) $C_4H_6O_6$
1	Panciu	19.09.2009	233,52	7,65

Wine making technologies:

In the case of **classical maceration**, selected yeasts of the *Saccharomyces cerevisiae* sort were added (30 g/100 kg), as well as pectolytic enzymes (1,5 g/100 kg). Maceration - fermentation was performed in stainless steel tanks, for 120 hours, with pumping over twice a day. At the end of the maceration process, the marc was pressed by a hydraulic press, the working pressure being no more than 2 atm. The obtained must has been kept in stainless

steel tanks for finishing its alcoholic and malo - lactic fermentation. After finishing its malo - lactic fermentation, the wine was racked and conditioned. Bottling was done after filtering.

When using thermo - maceration, the must was drawn separately from the rest of the marc and two thirds of it was heated up to 70 °C and maintained at this temperature for 20 minutes, then mixed with the rest of the marc and after another 5 minutes, cooled back to medium temperature using the left third of the unheated must. Many studies have shown that heating the must at 70 °C, for 15 – 30 minutes, ends in a better anthocyans extraction and also inactivate the oxidases (Valeriu D. Cotea, 1985). After this process, the must was subjected to the same conditions from the method above.

When macerating in ROTO - tanks, the must was kept in stainless steel tanks for 72 hours, rotating them three times a day, 3 minutes/ rotation.

In order to enhance the color intensity, the following procedures were undertaken: after obtaining and homogenization of marc and after a short pre-fermentative maceration with pectolytic enzymes (1,5 g/hL), the color intensity was concentrated; thus, from the marc mass, 10% of must was extracted, resulting in a higher color compounds concentration per volume of obtained wine. This variant of color concentration was followed by the same technological operations as in classical maceration-fermentation procedures.

In the case of ultrasounds maceration, the process took place at 2000 W in an ultrasonic cavity, with a frequency of 35 kHz for 15 minutes. After this treatment the marc undertook the same technological operations as in classical maceration-fermentation procedures.

In the case of microwave maceration the marc was irradiated at 750 W for 15 minutes. After 30 minutes, the marc was cooled at 20˚C, with a third of its unheated volume and then *Saccharomyces cerevisiae* (30 g/100 kg) and pectolytic enzymes (1,5 g/100 kg) were added.

Physical - chemical analyses were done according to international standards.

Total acidity, alcoholic strength, reductive sugars, non - reductive extract, variation of chromatic parameters, anthocyans content and phenolic content were registered.

The following abbreviations were used in this study: M =classical maceration; V1 = color intensity concentration 10 %; V2 = ROTO-tanks maceration; V3 =thermo-maceration; V4 = ultrasounds maceration 15'; V5 = microwave maceration 750 W - 15'.

RESULTS AND DISCUSSION

The main compositional characteristics of Băbească neagră wines obtained through different maceration-fermentation technologies are presented in table 2.

Table 2

Main compositional characteristics of red wines from Băbească neagră grapes harvested from Panciu vineyard

No.	Maceration type	Alcoholic concentration (% vol. alc.)	Reductive sugars (g/L)	Relative density 20ºC	Total acidity g/L $C_4H_6O_6$	Volatile Acidity g/L $C_2H_4O_2$	pH	Free SO_2 (mg/L)	Total SO_2 (mg/L)	Non-reductive extract (g/L)	Total dry extract (g/L)
1.	M	13,42	3,05	0,9924	6,35	0,46	3,74	18,17	58,27	22,45	25,5
2.	V1	13,76	2,12	0,9922	5,92	0,32	3,69	11,48	51,29	23,98	26,1
3.	V2	13,53	2,87	0,9921	6,18	0,36	3,73	16,29	58,12	22,13	25
4.	V3	14,08	2,34	0,9920	6,23	0,29	3,73	14,52	55,47	23,96	26,3
5.	V4	13,61	2,15	0,9920	5,95	0,41	3,69	15,23	54,63	22,85	25
6.	V5	13,83	3,16	0,9924	5,87	0,38	3,68	21,72	62,45	23,64	26,8

The alcoholic concentration varies from 13,42 % vol. In the control sample (M) up to 14,08% vol. in V3 (thermo-maceration) (Fig.1).

The obtained wines are dry, with a content of maximum 4g/L.

The total acidity (Fig. 2) and pH values underline the fact that the wines were fermented malo-lactic, the 3,74 pH in the M variant corresponds to a total acidity of 6,35 g/L tartaric acid, while in V5 pH is of 3,68 corresponds to a total acidity of 5,87 g/L tartaric acid.

The natural conditions of Panciu vineyard, the place of origin of the used biological material, combined with the favorable conditions of 2009, led to the obtaining of fine, equilibrated and extractive wines. Therefore, in variants M, V2 and V4 the wines have extract between 22,13 g/L in V2 and 22,85 in V4 (Tab.2). In variants V1, V3 şi V5, the extract varies from 23,64 la V5 to 23,96 in V3 (Tab.2). The values of non-reductive extract together with the values of the alcoholic concentration results in classifying the wines as wines with controlled origin denomination, harvested at full maturity for variants M, V2 and V4, and late harvest for variants V1, V3 and V5.

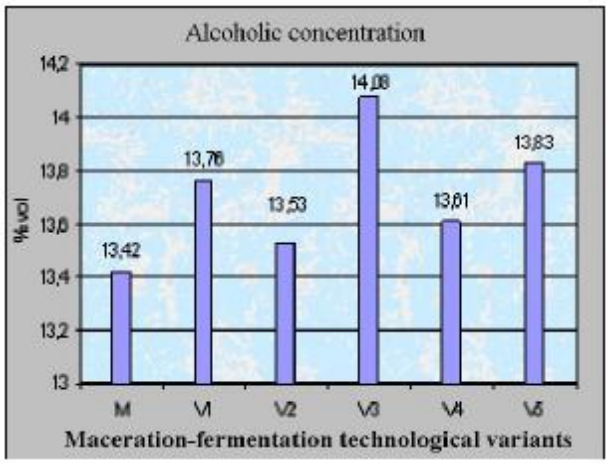

Fig.1 Graphical representation of alcoholic concentration

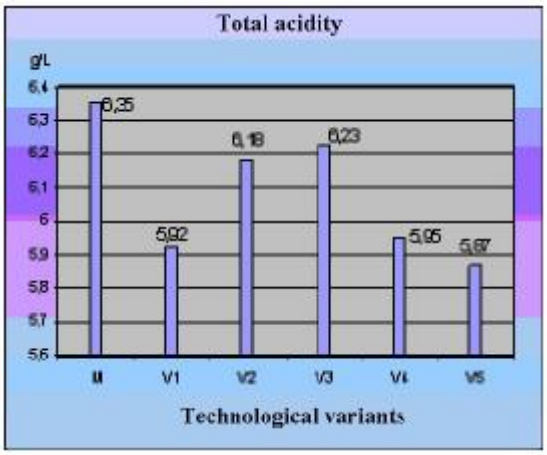

Fig. 2 Graphical representation of total acidity

The color components were calculated based on the absorbency spectrum registered in the visible domain (VIS) with a Analytik Jena S-200 spectrophotometer. The absorbency specters were numbered and analyzed with the VINCOLOR program, made within the research group, in order to obtain the chromatic parameters L, a and b, according to the CIE Lab 76 method.

With the help of the above mentioned parameters, the color of each wine (white, red, rose) can be graphically represented in a orthogonal linear system, whose coordinates are the same chromatic parameters L, a and b (Fig. 4). This system is comprised in the space named colors' solid CIE Lab 76.

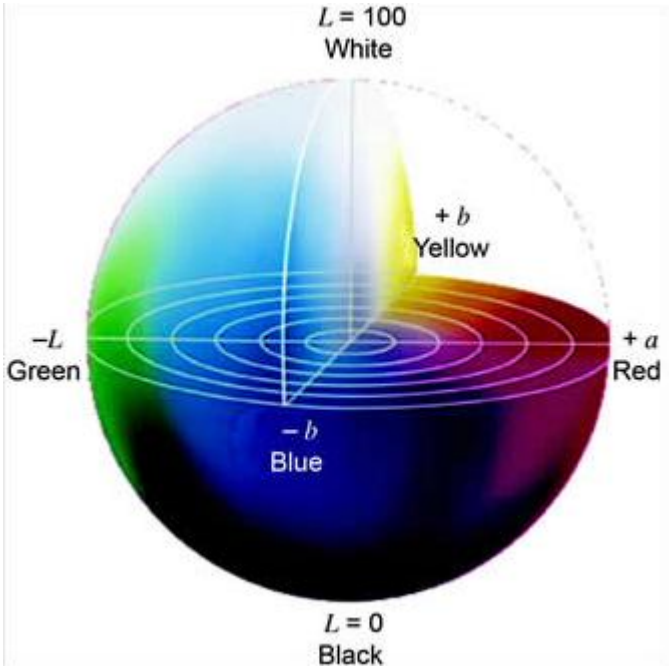

Fig. 4. CIE Lab 76 color space

In table 3, the chromatic parameter values for the different wines obtained are presented.

Table 3

Chromatic parameters of wines obtained from Băbească neagră grape variety

No.	Maceration type	Clarity L	Color coordinates		Saturation C	Tonality H	Luminosity	Hue	Δ E	Δ H
			a red(+) - green(-)	b yellow(+) - blue(-)						
1	M	50,3079	57,4584	20,3877	60,9682	19,5360	2,6102	0,6713		
2	V1	34,5516	62,8696	31,1419	70,1598	26,351	4,5791	0,5893	19,829	7,7747
3	V2	38,2343	62,4262	31,2989	69,8330	26,628	4,1162	0,6083	17,015	8,0714
4	V3	29,4582	60,6940	36,6503	70,9014	31,1258	5,4346	0,6545	26,639	13,27676
5	V4	26,7707	53,8241	27,9976	60,6704	27,482	5,0239	0,659	25,002	8,4278
6	V5	24,5384	54,5142	32,6528	62,8534	31,4254	6,1745	0,6623	28,691	12,4718

Clarity „L” characterizes the more or less „bright” visual aspect of the wine’s color and can have values between zero for an opaque black sample to 100 for transparent colorless samples. For the studied wines, the values vary between 24,53 in V5 to 50,30 in the control sample. These values prove the efficacy of microwave maceration, that leads to a better extraction and diffusion of anthocyanic pigments and other compounds found in the skin, avoiding unwanted changes in the taste and odor that can appear in the case of excessive heating specific to thermo-maceration.

The <<a>> component red-green (Tab. 3) of color represents the coordinate of complimentary colors red-green – this parameter frequently has negative values in white wines where green tonalities prevail on red hues and positive values in red wines; it varies from 53,82 in V4 to 62,86 in V1.

The <<b>> component yellow-blue of color has values that vary from 20,38 in the control sample (M) to 36,65 in V3. Therefore, the above values demonstrate the efficacy of microwave, ultrasounds and thermo-maceration in color compounds extraction. The colorimetric difference between two samples, ΔE, is the value that registers the differences between wines: V2 (17) and V5 (28) (Tab.3). Another way of evaluating chromatic characteristics from a mathematical point of view is the difference in hue in colors, ΔH, with values between (7,77) in V1 and (13,27) in V3 (Tab.3).

CONCLUSIONS

The used maceration-fermentation method has a big influence on the compositional characteristics of the wine and especially on the chromatic parameters.

A global evaluation of the analyses proves that all the wines obtained by different maceration-fermentation have values that are higher than the control sample, as seen below: V5, V4, V3, V1, and V2.

When comparing the compositional characteristics of the control sample (M) with the values from the sample obtained by color intensity concentration (V1), a difference in se observă o diferență asupra concentrației alcoolice, a extractului nereducător, dar mai ales asupra parametrilor de culoare.

BIBLIOGRAPHY

1. Cotea D.V., 1985, Tratat de Oenologie, vol. I. Bucureşti, Editura Ceres.
2. Cotea D.V., Barbu N., Grigorescu C., Cotea V.V., 2000, Podgoriile şi vinurile României, Editura Academiei Române, Bucureşti.
3. Cotea V.V., Cotea D.V., 2006, Tehnologii de producere a vinurilor, Editura Academiei Române, Bucureşti.
4. Cotea, Victoria, Cotea, V.V., 1996, Viticultură, ampelografie şi oenologie. Edit. Didactică şi Pedagogică, Bucureşti.
5. Pomohaci, N., Stoian, V., Gheorghiță, M., Sîrghi, C., Cotea, V.V., Namoloşanu, I., 2000, Oenologie, Vol. I, Prelucrarea strugurilor şi producerea vinurilor, Editura Ceres, Bucureşti.
6. Pomohaci N., Cotea V.V., Popa A., Stoian V., Sîrghi C., Năməloşanu I., Antoce Arina, 2001, Oenologie, Vol. 2, Îngrijirea, stabilizarea şi îmbutelierea vinurilor. Construcții şi echipamente vinicole, Editura Ceres, Bucureşti.
7. Țârdea C., Sârbu G., Țârdea Angela, 2000, Tratat de vinificație, Editura Ion Ionescu de la Brad, Iaşi.
8. Țârdea C., 2007, Chimia şi analiza vinului, Editura Ion Ionescu de la Brad, Iaşi.
9. Ribereau–Gayon, J., Peynaud, E., Sudrad, P., Ribereau–Gayon, P., 1982, Sciences et Tecniques du Vin, vol.I, Analyse et controle du vin, Dunod, Paris.
10. ***, International Code of Oenological Practices, 2008 - O.I.V., Paris
11. ***, International list of vine varieties and their synonyms, 2008 - O.I.V., Paris.
12. ***, International Oenological Codex, 2008 - O.I.V., Paris.

STUDY OF ORGANIC ACIDS IN STABILIZATION TREATMENTS APPLIED TO ROMANIAN WINES

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The study was conducted to assess organic acids: tartaric, malic, lactic, succinic, citric, acetic, fumaric and shikimic in the case of stabilization treatment on Romanian wines. The samples used in the experiment were blended Romanian wines, chosen specifically for their high acidity. White wine was treated with bentonite and the red wine, with gelatin. For tartaric stability appreciation, it was evaluated the effect of carboxymethylcellulose compared with various doses of arabic gum. For a sweet white wine an experiment was made using different samples as unpasteurised, pasteurized and treated with cold plasma (dielectrically barrier discharge method). Based on obtained data for certain organic acids it was remarked specific changes according to the realised treatment. Generally, a significant variability of the assessed parameters values was not found.

L'étude a été menée pour évaluer les acides organiques: tartrique, malique, lactique, succinique, citrique, acétique, fumarique et shikimique pour la mise en œuvre du traitement de stabilisation des vins roumains. Les échantillons utilisés ont été le coupage de certains vins roumains, choisis spécialement pour leur forte acidité. Il y avait ajouté de bentonite à un vin blanc et de la gélatine à un vin rouge pour évaluer leur effet. Un autres traitement a été fait avec la carboxyméthylcellulose par rapport à différentes doses de gomme arabique pour l'évaluation de la stabilité tartrique; pour un vin blanc doux on a fait une expérience avec des échantillons différents: non pasteurisé, pasteurisé et traités par du plasma froid (méthode de barrière électrique de déchargement) pour évaluer une possible variation de l'acidité. Par l'évaluation des données on a pu voir des changements spécifiques pour certains acides organiques selon le traitement. Globalement, on n'a pas constatée une grande variabilité des paramètres évalués.

INTRODUCTION

Acid analisys has a great importance for the characterization of wine composition evolution with important implication in the chemical and biochemical processes. The grape contains most of the acids involved in the glycolytic and shikimic acid pathways as well as in the Krebs and glyoxylic acid cycles, the rest remaining unmodified, being transmitted in wine [Ribereau-Gayon *et al.*, 2006].

An exhaustive study is unknown in regard to the different aspects of organic acids evolution in wines and the way in which they are influenced by various treatments.

In this paper we present preliminary data of treatments with various stabilization products. Bentonite is a deproteinization material [Ribereau-Gayon *et al.*, 2006] and may influence acids in a way that will determine a shift in the ionic equilibrium of wine. Gelatine doesn’t

alter in any way organic acids, but in regard to phenolic acids treatment is a very efficient adjutant [Villano *et al.*, 2006]. The combined use of activated charcoal, gelatine and the hydroxycinnamic acid bentonites decreased concentrations of Sherry wines by 13-34% [López *et al.*, 2001]. Carboxymethylcellulose (CMC) is a material that intervenes decisively in the wine acids stabilizing treatments by conferring an increased stability against tartaric precipitation, yet in previous experiments we have seen protection limitations in the case of lowered temperatures [Cotea *et al.*, 2007]. Using CMC for tartaric stabilization is particularly simple and economically feasible. CMC is inert and inexpensive, having a broad spectrum of use in foods. Study of auxiliary substances use in the winemaking process will ensure in the future a more versatile material against tartaric precipitation. The organic acid's evolution during the pasteurization process is monitored and presented in the final part of the study.

MATERIALS AND METHOD

In this study results from four experiments are presented in order to emphasize possible major changes in the composition of organic acids during treatments with classical oenological products and also by using nonconventional stabilization techniques.

For the analysis of the organic acids a HPLC Shimadzu Prominence series 20 was used. The sample was treated through a C18 6 mL SPE cartridge according to the methodology described in the method MA-E-AS313-04-ACIORG by O.I.V. standards. Only in the experiment with CMC we have used the classical methodology with an S-DVB column. For the cold plasma experiment, a two columns method was used, derived from the OIV standard, in order to analyze organic acids [Niculaua *et al.*, 2009]. A 10 µL volume of sample or standard (without SPE treatment) was injected through two analytical columns Prevail Organic Acid 5 µm 250x4.6 mm, at a flow rate of 0.6 mL/min. using H_2SO_4 0.065 M, as eluting solution, for bentonite and gelatine experiments.

Blended wines from Romanian varieties were used in the treatment specifically chosen for their high acidity. Table 1 presents the main material used in the oenological stabilization experiments: carboxymethylcellulose (CMC), gum Arabic (GA), a mixture of bentonite with casein-soluble oenological mix, soluble gelatine.

Tab. 1

Product type/Cod	Product nume	Treatment applied doses
CMC – A	CP Kelco Cekol 30	60, 80, 120 mg/L
CMC – B	CP Kelco Cekol 30G	
CMC – C	Wolf Walocel Crt 30	
CMC – D	Wolf Walocel Crt 30GA	
CMC – E	Akzo Akucell AF 0301	
CMC – F	Akzo Akucell AF 0305	
CMC – G	Hercules Blanose 7LF	
Arabic gum – GA	chemical pure dust	20, 50 g/hL
Oenological bentonite with soluble casein	Vinitol	0, 25, 50, 75, 100 g/hL
Soluble gelatine	Erbigel Bio	0, 3, 6, 9, 12 g/hL

Treatment with CMC and Arabic gum (Tab. 2 and 3) was used for a dry white wine blend (denoted by FA, ie Fetească regală and Fetească albă blend) and a red one (FN, ie Fetească neagră and Băbească neagră blend) blend. A volume of 1 L of the whole dose was used for the treatment. With this the necessary quantities was administered for each variant to a volume of 3 L and, after SO_2 correction for the mixed wine, the bottling procedure was made by using three bottles of 750 mL that were retained at a temperature of –5 °C for 30 days.

Treatment with bentonite and casein was used for a dry white wine made from white Feteasca albă variety. The product powder was dissolved slowly in 2 L of wine and afterwards was administered in doses presented in Table 1 to a 5 L quantity of wine form each variant and left for a period of 15 days in the cellar demijohns, away from heat and light.

The treatment with soluble gelatine was used for a dry red wine blend from several varieties, so chosen in order to obtain a high total acidity (6.8 g/L tartaric acid equivalent). Management methodology was similar to that of bentonite and only a 10 days storage period was achieved.

For the cold plasma treatment [Hnatiuc *et al.*, 2008] tests were conducted with a sweet white wine (31.89 g/L reducing sugars), for which a certain treatment schedule was proposed (Table 5), with different flow rates (Q = 1, 4, 7 L/min.) each using different passages in the reaction zone for three numbers (N = 3 times passing over, 6 times passing over, 9 times passing over). The comparison was done in laboratory conditions, and a pasteurization process at 70 °C for 10 min was conducted. Processed samples were left for 24 hours for normalization, after which the analysis was made.

RESULTS AND DISCUSSION

In Tables 2 and 3 are the results of treatments applied to stabilize the blended white and red dry wines with CMC. The evolution of two macro parameters is presented: total and volatile acidity and the variation of some important organic acids: acetic, malic, tartaric and lactic. It may be noted that after handling the wine a variation of property (both acidity and acid amount) is evident between time zero (start of the treatment) and the time of the analysis (after 30 days).

Tab. 2

Experimental variants	volatile acidity g/L acetic ac.	acetic acid g/L	total acidity g/L tartaric ac.	tartaric acid g/L	malic acid g/L	lactic acid g/L
Initial wine	0.27	0.15	7.82	3.52	2.54	0.87
Untreated wine	0.36	0.28	6.78	1.87	1.79	2.39
FA-A-60	0.35	0.28	7.01	2.00	2.06	1.75
FA-A-80	0.35	0.24	7.01	2.11	2.09	1.70
FA-A-120	0.33	0.24	7.00	2.24	2.11	1.59
FA-B-60	0.31	0.22	7.05	2.18	2.07	1.84
FA-B-80	0.30	0.22	7.08	2.19	2.02	1.90
FA-B-120	0.31	0.21	7.05	2.31	1.99	1.83
FA-C-60	0.31	0.25	7.08	2.02	1.86	2.27
FA-C-80	0.31	0.23	7.06	2.09	1.75	2.09
FA-C-120	0.31	0.21	7.04	2.25	1.88	2.08
FA-D-60	0.32	0.26	7.05	2.28	2.22	1.67
FA-D-80	0.31	0.22	7.01	2.37	2.09	1.72
FA-D-120	0.33	0.23	7.00	2.49	2.15	1.61
FA-E-60	0.31	0.26	7.05	2.67	2.41	1.09
FA-E-80	0.32	0.27	7.05	2.62	2.41	1.01
FA-E-120	0.31	0.21	7.04	2.85	2.44	0.88
FA-F-60	0.31	0.26	7.04	2.83	2.32	0.93
FA-F-80	0.31	0.23	7.00	2.82	2.40	0.90
FA-F-120	0.37	0.27	7.09	3.01	2.49	0.92
FA-G-60	0.35	0.25	7.04	2.62	2.18	1.38
FA-G-80	0.35	0.26	7.07	2.60	2.20	1.32
FA-G-120	0.36	0.26	7.02	2.72	2.27	1.22
FA-GA-20	0.32	0.20	7.00	2.64	2.12	0.98
FA-GA-50	0.31	0.24	7.12	2.79	2.42	0.92
Average	0.33	0.24	7.04	2.44	2.16	1.51
Standard deviation (s)	0.02	0.02	0.03	0.31	0.20	0.44
Coefficient of variability (s%)	6.53	9.12	0.39	12.52	9.41	28.87

Tab. 3

Experimental variants	volatile acidity g/L acetic ac.	acetic acid g/L	total acidity g/L tartaric ac.	tartaric acid g/L	malic acid g/L	lactic acid g/L
Initial wine	0.38	0.37	8.99	3.63	3.46	0.57
Untreated wine	0.51	0.41	8.48	2.76	2.95	0.86
FN-A-60	0.64	0.53	8.65	2.91	3.21	0.63
FN-A-80	0.68	0.57	8.68	2.96	3.25	0.60
FN-A-120	0.62	0.56	8.69	2.99	3.30	0.53
FN-B-60	0.71	0.64	8.52	2.95	3.24	0.65
FN-B-80	0.72	0.62	8.54	2.91	3.29	0.64
FN-B-120	0.73	0.66	8.60	2.91	3.34	0.64
FN-C-60	0.61	0.53	8.15	2.79	3.17	0.71
FN-C-80	0.62	0.55	8.20	2.80	3.15	0.63
FN-C-120	0.65	0.59	8.25	2.86	3.16	0.59
FN-D-60	0.70	0.59	8.45	2.96	3.27	0.67
FN-D-80	0.63	0.56	8.47	2.96	3.24	0.66
FN-D-120	0.68	0.57	8.50	2.99	3.34	0.61
FN-E-60	0.67	0.52	8.41	3.08	3.32	0.61
FN-E-80	0.64	0.47	8.47	3.06	3.33	0.61
FN-E-120	0.62	0.57	8.49	3.14	3.37	0.60
FN-F-60	0.67	0.53	8.28	3.10	3.30	0.66
FN-F-80	0.69	0.52	8.27	3.07	3.34	0.68
FN-F-120	0.71	0.55	8.24	3.19	3.35	0.58
FN-G-60	0.61	0.43	8.28	3.07	3.27	0.72
FN-G-80	0.63	0.42	8.29	3.20	3.34	0.68
FN-G-120	0.64	0.53	8.32	3.17	3.35	0.69
FN-GA-20	0.59	0.50	8.60	3.18	3.15	0.68
FN-GA-50	0.56	0.46	8.67	3.12	3.24	0.63
Average	0.66	0.55	8.42	3.00	3.28	0.64
Standard deviation (s)	0.04	0.06	0.16	0.12	0.07	0.05
Coefficient of variability (s%)	5.87	10.85	1.95	4.01	2.04	7.27

Variants codes are comprised of: the type of blending (eg FA, FN), used oenological material taken from Table 1 (eg A) and dose also taken from Table 1 (eg 50).

By comparison with the Arabic gum treatment, is apparent that the parameters variability is comparable between substances and both are clearly significant in comparison to the wine left over as initial (before untreated as aged or bulk material). At the end of both tables, the arithmetic average was calculated, standard deviation and coefficient of variability for all treated samples for the assessment of the treatment impact.

From the presented data, one can say that tartaric stabilization with CMC does not entail any modification of physico-chemical wine macro parameters (total and volatile acidity), regardless of dose or type of CMC applied. For this study, a significant variation of acids is observed, that appears to be random and dependent on the type of wine.

In Table 4 the evolution of key organic acids from wine after the stabilization treatment are presented. As well as in Tables 2 and 3, three statistical parameters were calculated to see if there is a significant change due to the conducted treatment. From the analysis of the two treatments one can state that, besides the untreated sample, slightly significant variations occur for tartaric, lactic and succinic acids (s% < 5). and even more significant are in the cases of other acids studied. Major variations in citric and fumaric acids (s% > 10) at white wine may be considered acceptable in comparison to the red wine and this may be due to the influence of aeration process.

Treatments with bentonite and casein fining for white wine, as well as gelatin for red wine don't have a major impact for organic acids. In the case of shikimic acid, which is considered a defining parameter for black grape varieties, fining treatments were not able to change or eliminate quantitatively this acid.

Tab. 4

Doze g/hL	tartaric acid g/L	malic acid g/L	shikimic acid g/L	lactic acid g/L	acetic acid g/L	citric acid g/L	succinic acid g/L	fumaric acid g/L
Vinitol (Oenological bentonite with soluble casein)								
0	2.75	0.45	0.028	2.87	0.52	0.12	1.76	0.0004
25	2.61	0.41	0.028	2.91	0.53	0.09	1.73	0.0003
50	2.55	0.39	0.027	2.95	0.55	0.10	1.67	0.0003
75	2.49	0.38	0.025	3.04	0.54	0.10	1.66	0.0003
100	2.42	0.36	0.023	3.15	0.59	0.10	1.59	0.0003
Average	2.56	0.40	0.026	2.98	0.55	0.10	1.68	0.0003
Standard deviation (s)	0.13	0.03	0.002	0.11	0.03	0.01	0.07	0.0000
Coefficient of variability (s%)	4.90	8.59	8.27	3.76	4.95	10.74	3.93	13.98
Soluble gelatin								
0	2.75	0.45	0.028	2.87	0.52	0.12	1.76	0.0004
3	2.68	0.42	0.026	2.89	0.56	0.11	1.78	0.0005
6	2.62	0.41	0.025	2.79	0.59	0.11	1.83	0.0005
9	2.59	0.37	0.026	2.90	0.58	0.10	1.82	0.0005
12	2.52	0.39	0.024	2.99	0.54	0.10	1.91	0.0005
Average	2.63	0.41	0.026	2.89	0.56	0.11	1.82	0.0005
Standard deviation (s)	0.09	0.03	0.001	0.07	0.03	0.01	0.06	0.0000
Coefficient of variability (s%)	3.33	7.43	5.75	2.48	5.13	7.75	3.18	9.32

Cold plasma conditioning experiment is an unconventional treatment intended to study the effect that might have on sweet wines and to learn various aspects of his chemistry. In Table 5, presented below, the results of this treatment are compared with the initial wine (untreated) sample and to a sample obtained by conventional pasteurization.

Tab. 5

Experimental variants	N - no. of passes	total acidity g/L tartaric ac.	tartaric acid g/L	malic acid g/L	lactic acid g/L	acetic acid g/L	citric acid g/L	succinic acid g/L
Initial wine	-	6.54	3.68	0.42	1.49	0.46	0.17	0.90
pasteurized wine 70 °C, 10 min.	-	6.49	3.49	0.57	1.27	0.40	0.14	0.79
Q - flow (L/min) 1	3	6.52	3.42	0.41	1.51	0.54	0.16	0.82
4	3	6.49	3.52	0.53	1.34	0.50	0.14	0.80
7	3	6.47	3.45	0.48	1.31	0.37	0.12	0.79
1	6	6.52	3.55	0.58	1.08	0.40	0.13	0.78
4	6	6.54	3.53	0.54	1.33	0.62	0.12	0.81
7	6	6.47	3.46	0.60	0.75	0.59	0.10	0.79
1	9	6.49	3.50	0.39	1.45	0.39	0.09	0.80
4	9	6.47	3.60	0.50	1.16	0.38	0.10	0.81
7	9	6.52	3.47	0.55	1.28	0.70	0.09	0.83
Average		6.50	3.50	0.51	1.25	0.50	0.12	0.80
Standard deviation (s)		0.03	0.06	0.07	0.23	0.12	0.02	0.02
Coefficient of variability (s%)		0.41	1.60	14.12	18.25	24.29	20.55	1.97

By analyzing the statistical parameters for cold plasma treatment changes in composition of organic acids is notable, but total acidity does not present a major quantitative variation. For tartaric and succinic acids no influence of treatment is observed and their level remainis almost unchanged. The highest variation occurs in the case of acetic, malic and lactic acid, presumably by destroying bacteria compared with untreated control [Neacşu *et al.*, 2010]. The citric acid variation may ascribe the change of polarization due to the complexation processes of wine.

CONCLUSIONS

Clarification and stabilization treatments applied to wines do not influence macro parameters like total acidity and volatile acidity. Composition of organic acids commonly present in wine can sometimes significantly change, with variations depending on the type of wine or degree of aeration, but overall these treatments are over efficient in acid stabilization and also for protein or microbiological being safe and so very important.

ACKNOWLEDGMENT

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BIBLIOGRAPHY

Villaño D., Fernández-Pachón M., S., Troncoso A., M., García-Parrilla M., C., 2006. Influence of enological practices on the antioxidant activity of wines, *Food Chemistry*, 95, 394–404

Hnatiuc B., Burlica R., Hnatiuc E., Brisset J.–L., Neacşu I., Niculaua M., 2008. The wine treatment by DBD discharges, *Simposium HAKONE XI*, Oleron Island, September 7-12

López S., Castro R., García E., Pazo J. A. S., Barroso C. G., 2001. The use of activated charcoal in combination with other fining agents and its influence on the organoleptic properties of Sherry wine, *European Food Research and Technology*, 212, 671–675

Niculaua M., Cotea V. V., Zamfir C. I., Odăgeriu Gh., Nechita B., Chiriță O., 2009. Assessment of Organic Acids of Wine of Grape Varieties from the Romanian Ampelography Collection of UASVM Iaşi, *Bulletin USAMV*, nr. 66 (1-2), 4 pp., electronic version - CD

Neacşu I., Cotea V. V., Zănoagă C., Niculaua M., Hnatiuc B., Colibaba L. C., 2010. Effects of the cold plasma treatment in wine, *The 9th International Symposium – Process Technology for Process Control and Alcohol Management, INTERVITIS INTERFRUCTA*, 24-28 March 2010, Stuttgart, vol. 60, p. 50-53

Ribereau-Gayon P., Dubourdieu P., Donèche D., Lonvaud A., 2006. Handbook of Enology Volume 1 – The Microbiology of Wine and Vinifications, 2nd Edition, John Wiley & Sons, West Sussex

Ribereau-Gayon P., Glories Y., Maujean A., Dubourdieu P., 2006. Handbook of Enology Volume 2 – The Microbiology of Wine and Vinifications, 2nd Edition, John Wiley & Sons, West Sussex

Cotea V. V., Niculaua M., Nechita B., Zamfir C., Experiments concerning the use of carboximetylcellulose for the tartaric stabilization of wines, *The 8th International Symposium - Innovations in Enology, INTERVITIS INTERFRUCTA*, 22-26 April 2007, Stuttgart, vol. 59, p. 36-44

REDOX ASPECTS OF CERTAIN ACIDS IN GRAPES, MUST AND WINE

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ABSTRACT

The three acids that manifest theirselves during wine-making have redox modulating qualities. The tartaric acid, representative almost exclusively for grape, induces, in its current concentration, right the optimum rH for Saccharomyces, initiating the alcoholic fermentation. In its current concentration, the malic acid modulates a close to optimum rH, so that it replaces the tartaric acid as it precipitate. On the contrary, the lactic acid that results out of the malic acid from the malo-lactic fermentation, modulates oxidizing rH, able to provoke the inactivation of Saccharomyces.

INTRODUCTION

No one is surprised by the affirmation according to which wine-making is the first, historically speaking, biotechnology, this only because the fruit alcoholic fermentation is a spontaneous process. In this context, one cannot distinguish wine-making processes in different technologies like obtaining cider, but mostly because of certain biochemical peculiarities of the grape. One of these is the high content in tartaric acid; according to [4] the grape is the only one that has it, but according to [3], in a much lower concentrations is present in other fruits, as cherries for example, but this aspect is reflected in some redox aspects which will be emphasized in the present study by subjecting three acids to a previously established experimental protocol [6]; three acids, two of these are present in high quantities in grapes - tartaric and malic acid – and another that develops itself after the alcoholic fermentation of must and during the malolactic fermentation (applied for 30% of white wines and 70% of red wines [4]).

MATERIAL AND METHOD

The work was based on watery solution of each acid with concentrations between 1/100÷1/1000000 (from this point on referred to in logarithmical form in order to ensure a symmetry of OX axis in the resulted graphics (ex: C = 1/100 – lg C = –2; C = 1/1000 – lg C = –3; C = 1/10000 – lg C = –4 etc.)), which also comprises the current acids concentrations in wine.

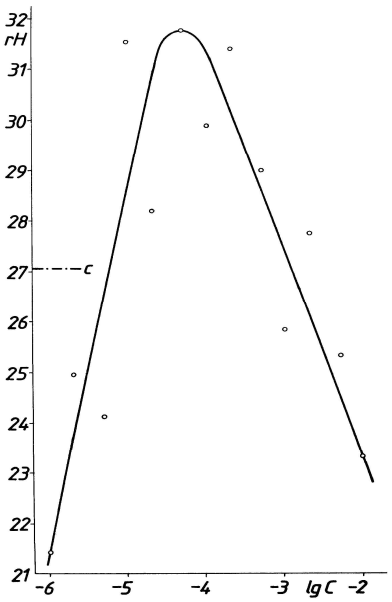

Fig. 1

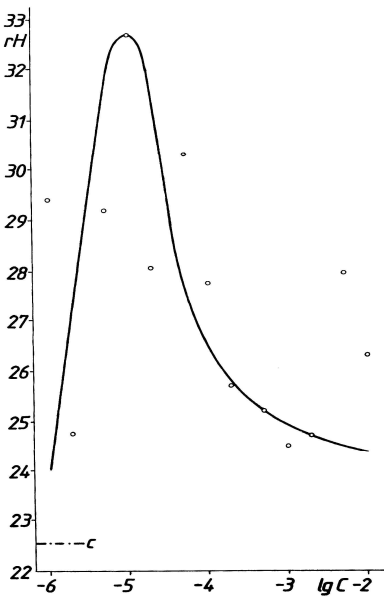

Fig. 2

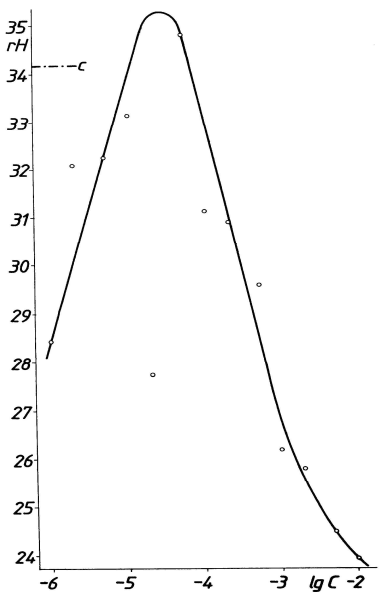

Fig. 3

RESULTS AND DISCUSSIONS

The first step was to obtain the rH concentration dependency, though an electrometric method previously described [5], for each of the solutions stated above. The obtained results are present in figures 1, 2 and 3.

One can observe that the acids appear to be ambivalent (also adding similar cases like [1]), in the sense that they develop a reductive character (lowering the rH with the concentration increase) at high concentrations (up to 1/10000÷1/100000), respectively an oxidant one (increasing the rH with concentration) at lower concentrations (below above mentioned values).

In order to achieve the aims of the present paper, we are obligated to present the rH gradient behavior of a yeast culture (*Saccharomyces cerevisiae*) previously described (fig. 4 [2]), where the peak is at rH ~24.

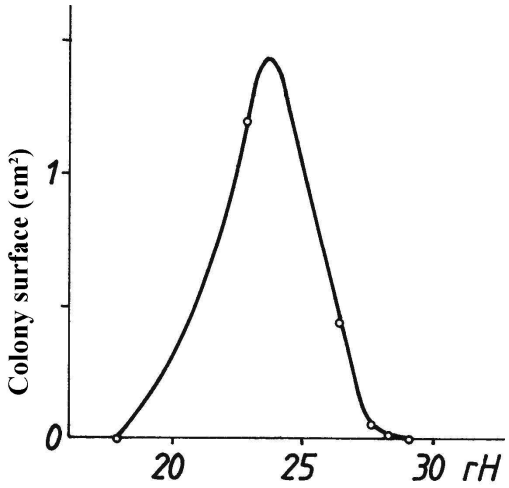

Fig. 4

In the present grape and must concentration (cca. 0,5% – lg C = –2,3), the tartaric acid is characterized by a rH values of 24,5 (fig. 1), thus present in an optimum concentration which stimulates the yeast that promotes alcoholic fermentation, marking the beginning of the transformation process in which the sugars are metabolized from CO_2 and turn into CO_2 in the end. At the end of the alcoholic fermentation, by changing the solubility of tartaric acids salts, because the solvent becomes hydroalcoholic (cca. 10% ethylic alcohol), provokes the massive precipitation of tartrates; the modified rH by the tartaric acid presence becomes oxidant,

"inviting" the yeast to "leave the stage", because the living conditions became hostile. But, because the wine-making process avoids sudden developments, as any natural process, a delay occurs due to malic acid. As it is observed in figure 2, in the present grape concentration, in must and later in wine (cca. 0,05% – lg C = –3,3) (malic acid doesn't form non-soluble salts with other elements present in wine), it determines an rH medium of 25,3, close to *Saccharomyces* optimum. The gradual yeast decrease leads to a decline in malic acid, through its transformation from a bibasic acid, with strong off-tastes, in a monobasic one (lactic acid) with valuable sensory qualities due to the following malo-lactic fermentation. In figure 3, it is noticeable that, at an equivalent concentration of malic acid from which it comes from, the lactic acid changes the medium towards a much more oxidant rH (cca. 27), capable to inactivate the yeast along with the malic acid.

The second stage of the study, the confirmation of the modular redox character highlighted in the first stage, though the influence of a certain organism (the use of the above mentioned solutions used as a growth medium (Petri dishes, in the dark and 25 ^{0}C temperature) for wheat seeds), emphasizes the following (fig. 5-7): besides the fact that at relatively low concentrations, (lg C < –3) (unused dots) one cannot observe – at either substance – but a slight stimulation simultaneous with an rH increase – normal aspect for wheat. In the case of lactic acid (fig. 7) one can observe for higher concentration (lg C = –2…–3) (full points), a beginning of redox modulation (the superiority of a Gaussian allure). In other words, besides the fact that the malolactic fermentation lowers acidity, the resulted lactic acid accentuates the taste sensation through the fact that it responds positively to the redox determinism of this sense, highlighted by [2]. N the case of tartaric acid (fig. 5) and malic acid (fig. 6), the high concentrations do not satisfy the redox modulator tendency present in the case of lactic acid; they act through a different mechanism, probably osmotic.

In order to the redox modulating effect to highlight itself in relation to an organism (as a part of the modulating substance in the environment of that organism) a sufficient time is necessary. The third stage of the project tests on small plants with a 1/10000 from the tested solution (1 mL/plant), in lighting conditions, was conducted, in order to manifest the reductive effect typical of plants. The obtained results of rH evolution are stated on figures 8-10 proving that tartaric acid (fig. 8) poses "no resistance" the reductive effect of the plants exerting itself undisturbed with the exceptions of late experimental stages where the plants seem to be allelopathic inhibited. At the same time, the tartaric acid leads to a certain wine illness (tourne or pousse) because of tartaric-propionic fermentation, where the tartaric acid becomes a nutritive medium. On the other hand,

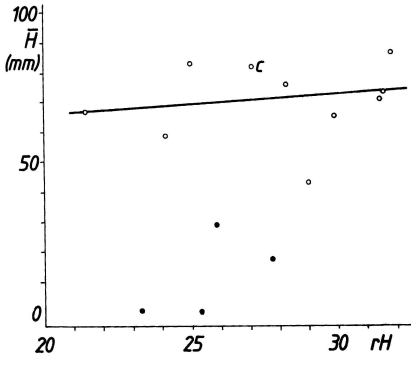

Fig. 5

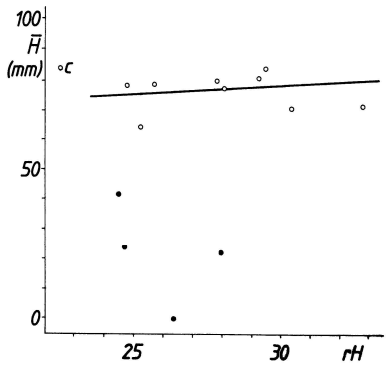

Fig. 6

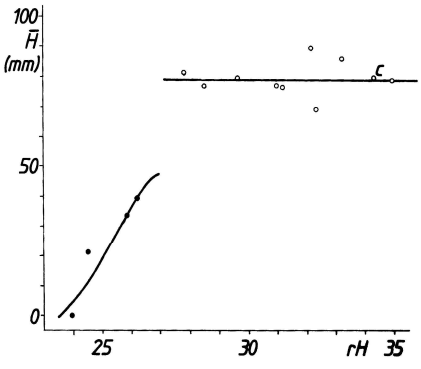

Fig. 7

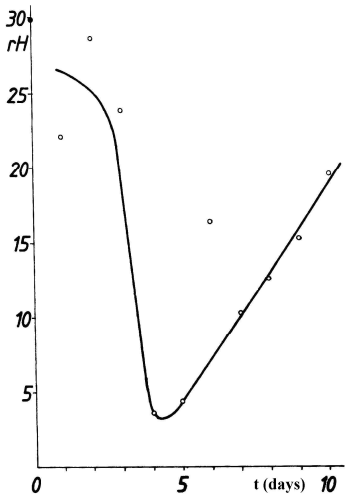

Fig. 8

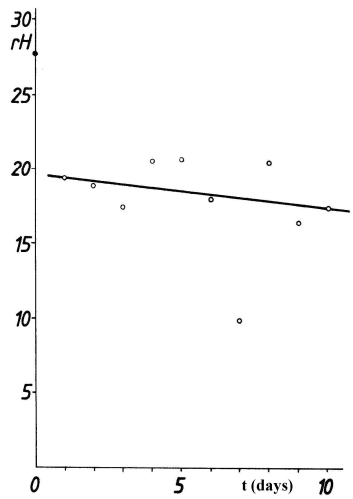

Fig. 9

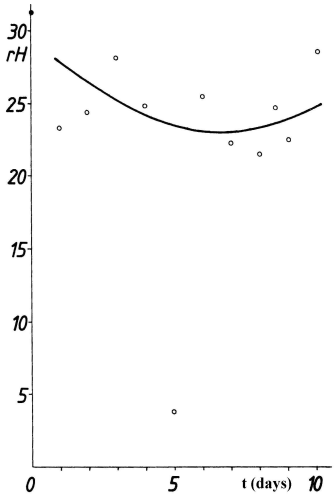

Fig. 10

The dynamism graph appears linear, characteristic to malic acid (fig. 9) and lactic acid (fig. 10), thus demonstrating a resistance in relationship with plant action, mostly in the case of heterotrophic organisms, with less energetic disponibilities. This fact can be correlated with the idea that many wines don't undergo malolactic fermentation and also with the ones that do, up until the final stages of their development.

Such a study should also be available for the food industry: all the above discussed acids are additives (E 335, 296, respectively 270) and the secondary effects, those that are redox mediable, must be well known.

CONCLUSIONS

The three acids that manifest themselves during wine-making have redox modulating qualities. The tartaric acid, almost exclusivelly representative for grapes induces, in its current concentration, the optimum rH for Saccharomyces, initiating the alcoholic fermentation. In its current concentration, the malic acid modulates a close to optimum rH, so that it replaces the tartaric acid as it precipitates in a salty form. On the contrary, the lactic acid that results out of the malic acid from the malo-lactic fermentation, modulates an oxidizing rH, able to provoke the inactivation of Saccharomyces.

BIBLIOGRAPHY

1. Cotea V. V., Oană Cristina, Zănoagă C. V., Odăgeriu G., *Aspecte redox asupra unor antiseptici de uz oenologic*, în acelaşi volum

2. Duca G., Zănoagă C. V., Duca Maria, Gladchi Viorica, 2001 – *Procese redox în mediul ambiant*, Ed. Universității de Stat din Moldova, Chişinău

3. Gherghi A., Burzo I., Mărgineanu Liana, Deneş Steliana, Dobreanu M., Pattantyus K., 1983 – *Biochimia şi fiziologia legumelor şi fructelor*, Ed. Academiei Române, București

4. Usseglio-Tomasset L., *Chimie œnologique*, 1995 – ed. 2-a, Ed. Technique & Documentation, Londres, Paris, New York

5. Zănoagă C. V., Neacşu I., Zănoagă Mădălina, 1988 – *Considerații asupra tehnicii de determinare a rH-ului unor probe biologice*, St. cerc. biochim., **31**, 1, p. 53-58

6. Zănoagă C. V., Oană C., Zănoagă Mădălina, 2004 – *Principii ale testării efectelor biologice potențiale, mediate redox, ale aditivilor alimentari*, în *Simpozionul internațional "Inventica – performanță şi ceativitate tehnică, Iaşi, 26-30 mai 2004"*, p. 459-64

Evoluzione del profilo antocianico di vini ottenuti da cloni di uve Sangiovese coltivate in Toscana

Evolution of the anthocyanin profile in wines made from Sangiovese grape clones grown in Tuscany

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RIASSUNTO

Dalla vinificazione di uve di cloni di Sangiovese coltivati in Toscana sono stati ottenuti i corrispondenti vini in purezza. Il profilo antocianico delle uve analizzate è caratteristico della cultivar Sangiovese, con una concentrazione in malvidina-3-glucoside elevata seguita dalla cianidina-3-glucoside e dalla peonidina-3-glucoside, mentre gli antociani acilati non superano il 2%. Il profilo antocianico dei vini si modifica profondamente sin dalla fermentazione e già dopo due mesi dalla vinificazione si osserva un incremento relativo della malvidina-3-glucoside ed una riduzione della cianidina-3-glucoside. Tale tendenza si accentua in affinamento e dopo un anno il tenore relativo in malvidina-3-glucoside raggiunge mediamente il 48%, mentre gli antociani acilati subiscono un decremento, con una perdita più marcata di antociani p-cumarati. Le vitisine A e B si formano rapidamente, mentre la presenza dei piranoantociani si manifesta in modo evidente dopo sei mesi e già dopo un anno la loro concentrazione supera quella delle antocianine acilate.

SUMMARY

From the winemaking of Sangiovese clone grapes grown in Tuscany, the corresponding wines were obtained. The anthocyanin profile of the grapes analyzed is typical of the Sangiovese cultivar, with a high concentration in malvidin-3-glucoside follwed by cyanidin-3-glucoside and peonidin-3-glucoside. The concentration of acylated anthocyanins never exceeds 2%. The evolution of the anthocyanin profile leads to changes that are visible since fermentation and as early as two months after the winemaking, we can observe a relative increment of malvidin-3-glucoside and a reduction of cyanidin-3-glucoside. Such trend is accentuated during the aging process and after a year the malvidin-3-glucoside amount reaches 48% on average, while the acylated anthocyanins go decreasing, with a more considerable loss of p-coumaroylated anthocyanins. The rapid creation of vitisine A and B is emphasized, while the presence of pyranoanthocyanins appears significantly after six months and as early as a year later, their concentration exceeds that of the acylated anthocyanins.

INTRODUZIONE

Lo studio dei metaboliti secondari, in particolare composti fenolici e aromatici, fornisce un contributo notevole alla caratterizzazione varietale delle cultivars.

E' ormai consolidata la tesi che il profilo antocianico sia sotto prevalente controllo genetico, poco influenzato dalle variabili ambientali e agronomiche. Attraverso il suo studio si possono raggiungere due importanti obiettivi: il primo strettamente legato alla caratterizzazione varietale delle uve e un secondo legato alla tracciabilità varietale dei vini rossi, quando le particolari caratteristiche delle uve da cui è stato ottenuto lo consentono.

Il profilo antocianico viene utilizzato a scopo tassonomico da molti anni, valutando l'abbondanza relativa dei cinque principali antociani non_esterificati (delfinina, cianina,

petunina, peonina e malvina) e dei rispettivi antociani esterificati, che comprendono la somma degli antociani esterificati con acido acetico e di quelli esterificati con acido p-cumarico.

Si arriva così a classificare le cultivars a bacca rossa in gruppi e sottogruppi di appartenenza prendendo come riferimento le caratteristiche comuni del profilo antocianico (Mattivi *et al.* 1990, 2006). I dati della letteratura collocano le uve Sangiovese tra quelle a prevalente tenore in malvina, cianina e peonina, caratterizzate da una modestissima presenza di antociani esterificati, intorno al 2% e con un rapporto tra antocianine cumarate e antocianine acetate sempre maggiore di uno (Bucelli *et. al*. 1992, 2007; Mattivi *et al*. 2006; Baldi *et al.* 1993, Calò *et al.* 1994, Di Stefano *et al.* 1994). L'evoluzione del profilo antocianico nel vino segue meccanismi complessi ma l'aspetto piu' importante da sottolineare è che il comportamento nel tempo degli antociani acilati e non e dei relativi derivati segue andamenti e tendenze simili anche per cvs diverse.

In questo studio vengono riportati i dati relativi all'evoluzione nel tempo del profilo antocianico di vini ottenuti da uve di 4 cloni Sangiovese raccolte in Toscana nel 2008.

MATERIALI E METODI

Le uve dei cloni di Sangiovese del 2008 provenivano dalla Toscana (val d'Orcia) e sono state raccolte a maturità tecnologica con un contenuto zuccherino di 241 g/L, 240 g/L, 243 g/L, 255 g/L, rispettivamente per Janus50, VCR5, VCR23 e BF30 e vinificate nella stessa azienda con lo stesso protocollo di vinificazione.

Estrazione e analisi degli antociani dell'uva e del vino

Le bucce di 10 acini, vengono separate dalla polpa e dai semi e poste in tampone tartarico a pH=3.2. Dopo circa 4 ore le bucce vengono omogeneizzate e dopo varie operazioni si ottiene un estratto che verrà poi analizzato (vedi per maggiori dettagli Di Stefano *et al*. 1994).

1 mL di vino tal quale, previamente filtrato su una membrana da 0.45 μm, viene posto in vial per l'iniezione diretta nel cromatografo.

Cromatografia in fase Liquida (HPLC-DAD) e (HPLC-MS-MS)

L'analisi degli antociani monomeri è stata condotta secondo le indicazioni descritte nel metodo OIV 2008, utilizzando un cromatografo Perkin Elmer Series 200 con rivelatore a diodi (HPLC-DAD), equipaggiato con una colonna LiChrospher 100 RP-18 (5 μm) LiChroCart 250-4 e relativa precolonna LiChroCart 4-4. L'analisi è stata effettuata in gradiente lineare da 94% di A a 6% di B in 40 min, con una fase mobile contenente acqua, acido formico e acetonitrile; solvente A 87:10:3 (v/v/v) e solvente B 40:10:50 (v/v/v); flusso 0.8 mL/min, lunghezza d'onda 518 nm. Le concentrazioni individuali vengono espresse come percentuale di ciascun composto sul totale delle aree. L'identificazione dei singoli antociani è stata effettuata valutando il tempo di ritenzione, lo spettro Uv-vis del picco cromatografico e la $\lambda_{max,}$.

I dati ottenuti per le uve ed i vini sono stati confermati per LC-MS, utilizzando un triplo quadrupolo Agilent 6410 con ESI. Lo strumento operava nelle seguenti condizioni: corrente del capillare 4000V, temperatura del gas di desolvatazione 350°C, flusso del gas a 13 L/min, nebulizzatore 35 psi. L'analisi è stata condotta in SRM, in modalità positiva, con tempi di dwell time di 50 msec. L'identificazione degli antociani è stata effettuata utilizzando il tempo di ritenzione e gli ioni molecolari *m/z* 493, *m/z* 479*, m/z* 465*, m/z* 463*, m/z* 449*,* rispettivamente per la malvidina (Mv), petunidina (Pt), delfinidina (Dp), peonidina (Pn) e cianidina (Cy) e gli ioni $(M-162)^+$ derivanti dalla perdita del glucosio; per gli antociani acetati sono stati utilizzati gli ioni molecolari e quelli derivanti dalla perdita $(M-204)^+$, mentre per gli antociani cumarati gli ioni molecolari e gli ioni $(M-308)^+$. Gli ioni molecolari utilizzati per le vitisine e le piranoantocianine sono rispettivamente *m/z* 561 (Mv-vitisina A)*, m/z* 517 (Mv-

vitisina B), *m/z* 609 (Mv-3-glucoside-4-vinilfenolo), *m/z* 625 (Mv-3-glucoside-4-vinilguaiacolo) e *m/z* 625 (Mv-3-glucoside-4-vinilcatecolo).

RISULTATI E DISCUSSIONE

La Tab. 1 mostra i dati relativi al profilo antocianico dei cloni di uve Sangiovese, Janus50, VCR5, VCR23 e BF30. Per tutti i cloni si osserva una elevata percentuale di antociani disostituiti con una maggior variabilità a carico della cianina e della malvina. Gli antociani esterificati rappresentano una minima parte degli antociani totali, con valori inferiori al 2%, tranne che per il clone Janus50 (2.5%); il rapporto tra antociani cumarati e antociani acetati risulta sempre maggiore di 1, in modo piuttosto netto. Questo profilo antocianico riscontrato nei diversi cloni rispecchia quello della popolazione varietale del Sangiovese ed è in accordo sia con i dati della letteratura, sia con quelli da noi trovati nelle ultime annate.

Tabella. 1 Profilo antocianico di cloni di uve Sangiovese coltivate in Toscana.

	UVE	2008			
	località	valle d'Orcia			
	clone	**JANUS 50**	**VCR5**	**VCR23**	**BF30**
antocianine non acilate	delfinidina-3-glucoside	11,03	10,71	9,73	10,11
	cianidina-3-glucoside	23,07	25,44	21,18	28,60
	petunidina-3-glucoside	13,09	14,17	13,80	12,84
	peonidina-3-glucoside	21,64	19,31	18,81	18,53
	malvidina-3-glucoside	28,65	28,71	34,69	28,24
antocianine acilate	delfinidina-3-acetilglucoside	0,07	0,10	0,10	0,10
	cianidina-3-acetilglucoside	0,09	0,08	0,07	0,10
	petunidina-3-acetilglucoside	0,08	0,06	0,08	0,06
	peonidina-3-acetilglucoside	0,20	0,10	0,10	0,10
	malvidina-3-acetilglucoside	0,50	0,39	0,35	0,31
	delfinidina-3-cumarilglucoside	< L.Q.	< L.Q.	< L.Q.	< L.Q.
	cianidina-3-cumarilglucoside	0,39	0,26	0,31	0,29
	petunidina-3-cumarilglucoside	0,09	0,08	0,07	0,08
	peonidina-3-cumarilglucoside	0,40	0,17	0,20	0,17
	malvidina-3-cumarilglucoside	0,71	0,41	0,53	0,42
∑ antocianine non acilate		97,48	98,34	98,22	98,32
∑ antocianine acetate		0,94	0,74	0,69	0,67
∑ antocianine cumarate		1,59	0,93	1,11	0,96

Note: La concentrazione delle singole antocianine è espressa come percentuale degli antociani totali; L.Q. = limite di quantificazione strumentale.

Un momento fondamentale per lo sviluppo del colore del vino è quello del passaggio da uva a vino, fase in cui le antocianine si diffondono dalla buccia al mosto e inizia l'azione del lievito con produzione di etanolo. In questa fase intervengono diversi fattori che determinano profonde modificazioni del profilo antocianico originario.

Le diverse cinetiche con cui le antocianine si diffondono nel mosto sono legate fondamentalmente alla loro struttura molecolare e influenzate dal tipo di sostituenti presenti sull'anello B, come osservato da diversi autori anche per le uve Sangiovese (Di Stefano *et al*. 1994). Le antocianine che diffondono prima nel mosto sono quelle più esposte all'ossidazione per azione delle polifenolossidasi e ciò spiega la forte diminuzione della cianina in tutti i campioni.

Le modificazioni indotte dall'azione del lievito sono riconducibili a due cause principali. La prima è legata all'assorbimento passivo delle antocianine da parte dei lieviti sia in fase di fermentazione, sia in affinamento quando i vini vengono lasciati a contatto con le fecce; tuttavia l'assorbimento delle antocianine non modifica sostanzialmente la concentrazione relativa delle singole antocianine come accertato per vinificazioni di vari tipi di uve tra cui quelle della cv Sangiovese (Mangani *et al*. 2008).

Una interazione indiretta lievito-antocianine si manifesta con la produzione di metaboliti microbici da parte dei lieviti, quali l'acido piruvico e l'acetaldeide, che attraverso un meccanismo di cicloaddizione formano addotti stabili con le antocianine chiamate rispettivamente vitisina A e B (Bakker *et al*. 1997; Fulcrand *et al*. 1998). La formazione delle vitisine inizia con la fermentazione e prosegue in affinamento per un certo tempo sin quando sono soddisfatte le condizioni favorevoli alla reazione (Romero *et al*. 1999; Asenstorfer *et al*. 2003; Hayasaka *et al*. 2007).

Non meno importante il ruolo dei lieviti nella formazione di piranoantocianine per l'azione da essi svolta sulla decarbossilazione di alcuni acidi idrossicinnamici durante la fermentazione (Schwarz *et al*. 2005) e la formazione di 4-vinilfenoli, di cui si dirà più avanti. Il tipo di lievito è determinante nell'orientare la formazione sia delle vitisine sia delle piranoantocianine come dimostrato nel Cabernet Sauvignon da Hayasaka *et al*. 2007.

Nei vini analizzati dopo circa un mese dalla vinificazione (Tab.2) si vede già una forte diminuzione della percentuale di cianina e un notevole aumento della malvina. Diminuiscono anche le altre antocianine ma in modo meno marcato, mentre più netta appare la diminuzione degli antociani acilati.

Al secondo campionamento nel maggio 2009, ad un incremento relativo della malvina si accompagna una diminuzione degli altri antociani, mentre compaiono nuovi derivati della malvina, quali le vitisine A e B, la cui presenza è fortemente legata alla concentrazione iniziale dei precursori.

Al terzo campionamento, dopo oltre un anno dalla vinificazione, osserviamo le stesse tendenze riscontrate in quello precedente, ma diventa manifesta la presenza di alcune piranoantocianine (Mv-3-glucoside-4-vinilcatecolo, Mv-3-glucoside-4-vinilfenolo e Mv-3-glucoside-4-vinilguaiacolo). Il contributo relativo degli antociani acilati è nettamente più basso di quanto osservato nell'uva, con decrementi superiori al 50%. Il livello più alto di antociani acilati si osserva per il clone Janus50 (1,2%), mentre il più basso per il clone VCR5 (0,3%). Questi dati confermano sostanzialmente dati precedenti sull'evoluzione nel tempo degli antociani su vini da uve Sangiovese e Nebbiolo (Mazza *et al*. 2005).

Le Fig. 1 e 2 mostrano chiaramente come dopo tredici mesi dalla vinificazione il profilo antocianico dei vini risulti profondamente modificato rispetto a quello originario delle uve, ma tendenzialmente si hanno le stesse trasformazioni in tutti i vini.

Tabella 2. Evoluzione nel tempo del profilo antocianico di vini ottenuti da cloni di uve Sangiovese.													
clone di riferimento		JANUS 50	VCR5	VCR23	BF30	JANUS 50	VCR5	VCR23	BF30	JANUS 50	VCR5	VCR23	BF30
data campionamento		**novembre 2009**				**maggio 2009**				**novembre 2008**			
antocianine non acilate	delfinidina-3-glucoside	9.41	9.00	8.85	8.70	10.01	9.34	9.48	9.14	10.20	9.36	9.56	10.11
	cianidina-3-glucoside	10.52	15.01	12.80	11.21	11.61	15.35	13.46	11.82	11.81	15.44	13.29	12.04
	petunidina-3-glucoside	11.58	11.92	10.80	12.37	11.46	12.50	11.33	12.56	12.00	13.79	11.65	12.90
	peonidina-3-glucoside	12.47	15.68	15.58	13.89	14.86	16.93	17.05	15.64	14.38	17.23	16.68	15.10
	malvidina-3-glucoside	53.63	47.41	50.49	52.51	50.50	45.00	47.51	51.02	49.80	43.62	47.83	48.93
antocianine acilate	delfinidina-3-acetilglucoside	< L.Q.	< L.Q.	< L.Q.	< L.Q.	< L.Q.	< L.Q.	< L.Q.	< L.Q.	< L.Q.	< L.Q.	< L.Q.	< L.Q.
	cianidina-3-acetilglucoside	< L.Q.	< L.Q.	< L.Q.	< L.Q.	< L.Q.	< L.Q.	< L.Q.	< L.Q.	< L.Q.	< L.Q.	< L.Q.	< L.Q.
	petunidina-3-acetilglucoside	< L.Q.	< L.Q.	< L.Q.	< L.Q.	< L.Q.	< L.Q.	< L.Q.	< L.Q.	< L.Q.	< L.Q.	< L.Q.	< L.Q.
	peonidina-3-acetilglucoside	0.12	< L.Q.	< L.Q.	< L.Q.	0.18	< L.Q.	< L.Q.	< L.Q.	0.22	< L.Q.	0.12	0.15
	malvidina-3-acetilglucoside	0.39	0.21	0.29	0.32	0.39	0.24	0.30	0.27	0.48	0.31	0.30	0.25
	delfinidina-3-cumarilglucoside	< L.Q.	< L.Q.	< L.Q.	< L.Q.	< L.Q.	< L.Q.	< L.Q.	< L.Q.	< L.Q.	< L.Q.	< L.Q.	< L.Q.
	cianidina-3-cumarilglucoside	< L.Q.	< L.Q.	< L.Q.	< L.Q.	< L.Q.	< L.Q.	< L.Q.	< L.Q.	< L.Q.	< L.Q.	< L.Q.	< L.Q.
	petunidina-3-cumarilglucoside	< L.Q.	< L.Q.	< L.Q.	< L.Q.	< L.Q.	< L.Q.	< L.Q.	< L.Q.	< L.Q.	< L.Q.	< L.Q.	< L.Q.
	peonidina-3-cumarilglucoside	0.18	< L.Q.	0.14	0.12	0.20	0.07	0.14	0.13	0.31	0.10	0.11	0.17
	malvidina-3-cumarilglucoside	0.51	0.10	0.35	0.28	0.49	0.18	0.40	0.31	0.68	0.16	0.40	0.42
vitisine + piranoantocianine	malvidina vitisina A	0.59	0.39	0.34	0.23	0.21	0.29	0.21	0.10	0.09	< L.Q.	0.10	< L.Q.
	malvidina vitisina B	0.32	0.12	0.21	0.18	0.10	0.12	0.10	< L.Q.	< L.Q.	< L.Q.	< L.Q.	< L.Q.
	malvidina-3-glucoside-4-vinilcatecolo	0.10	0.09	0.07	0.10	< L.Q.	< L.Q.	< L.Q.	< L.Q.	< L.Q.	< L.Q.	< L.Q.	< L.Q.
	malvidina-3-glucoside-4-vinilfenolo	0.12	0.11	0.08	0.12	< L.Q.	< L.Q.	< L.Q.	< L.Q.	< L.Q.	< L.Q.	< L.Q.	< L.Q.
	malvidina-3-glucoside-4-vinilguaiacolo	0.09	< L.Q.	< L..	< L.Q.	< L.Q.	< L.Q.	< L.Q.	< L.Q.	< L.Q.	< L.Q.	< L.Q.	< L.Q.
∑ **antocianine non acilate**		97.61	99.01	98.52	98.71	98.10	99.12	98.83	100.17	98.19	99.43	99.01	99.07
∑ **antocianine acetate**		0.51	0.21	0.29	0.32	0.57	0.24	0.30	0.27	0.70	0.31	0.42	0.40
∑ **antocianine cumarate**		0.69	0.10	0.48	0.40	0.69	0.25	0.54	0.44	0.99	0.26	0.51	0.59
∑ **vitisine**		0.91	0.51	0.55	0.41	0.31	0.41	0.31	0.10	0.09	0.00	0.10	0.00
∑ **piranoantocianine**		0.31	0.20	0.15	0.22	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

Note: La concentrazione delle singole antocianine è espressa come percentuale degli antociani totali; L.Q. = limite di quantificazione strumentale.

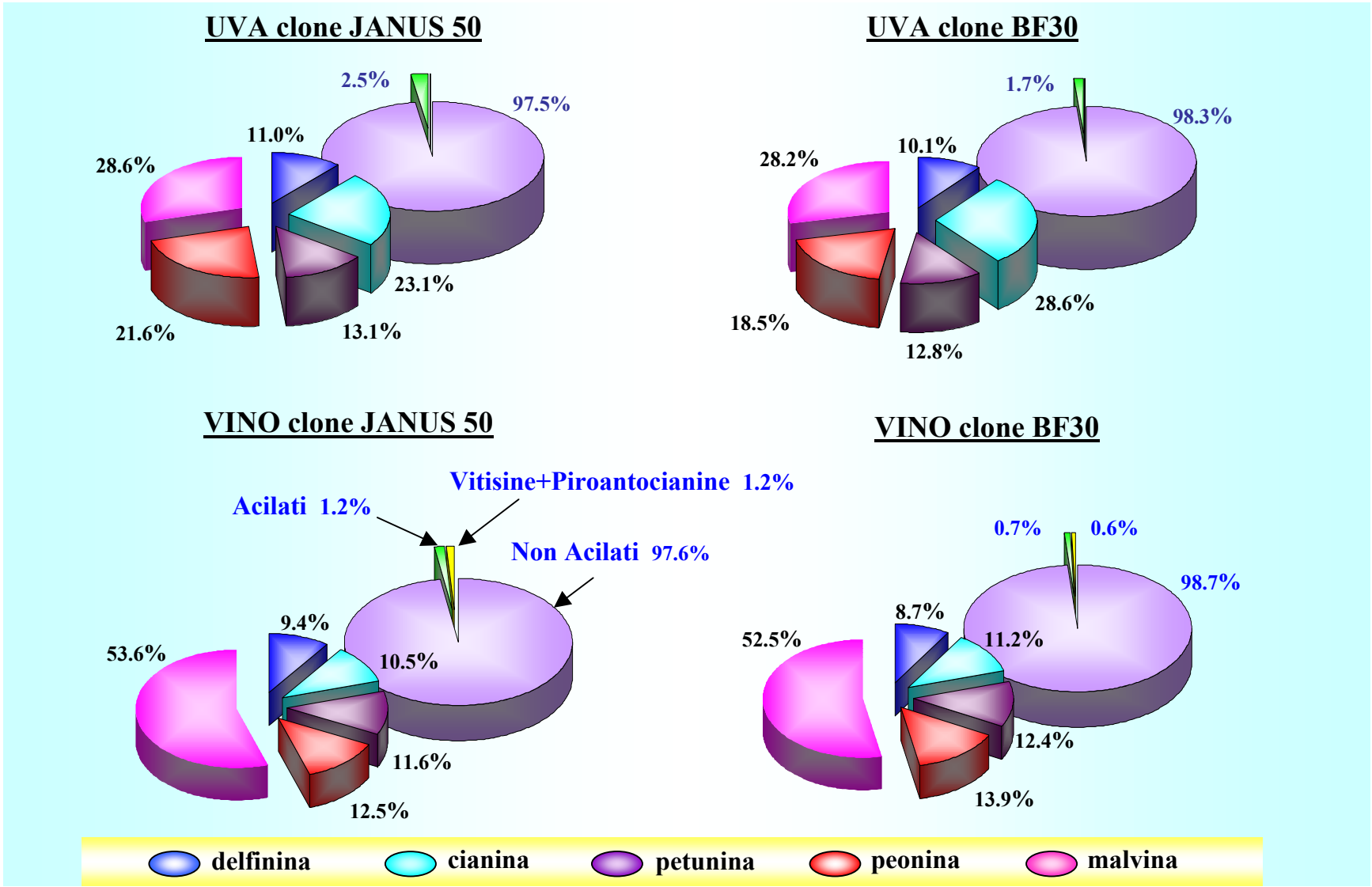

Fig.1: Rappresentazione grafica degli antociani delle uve della cv Sangiovese (cloni Janus50 e BF30) e dei corrispondenti vini.

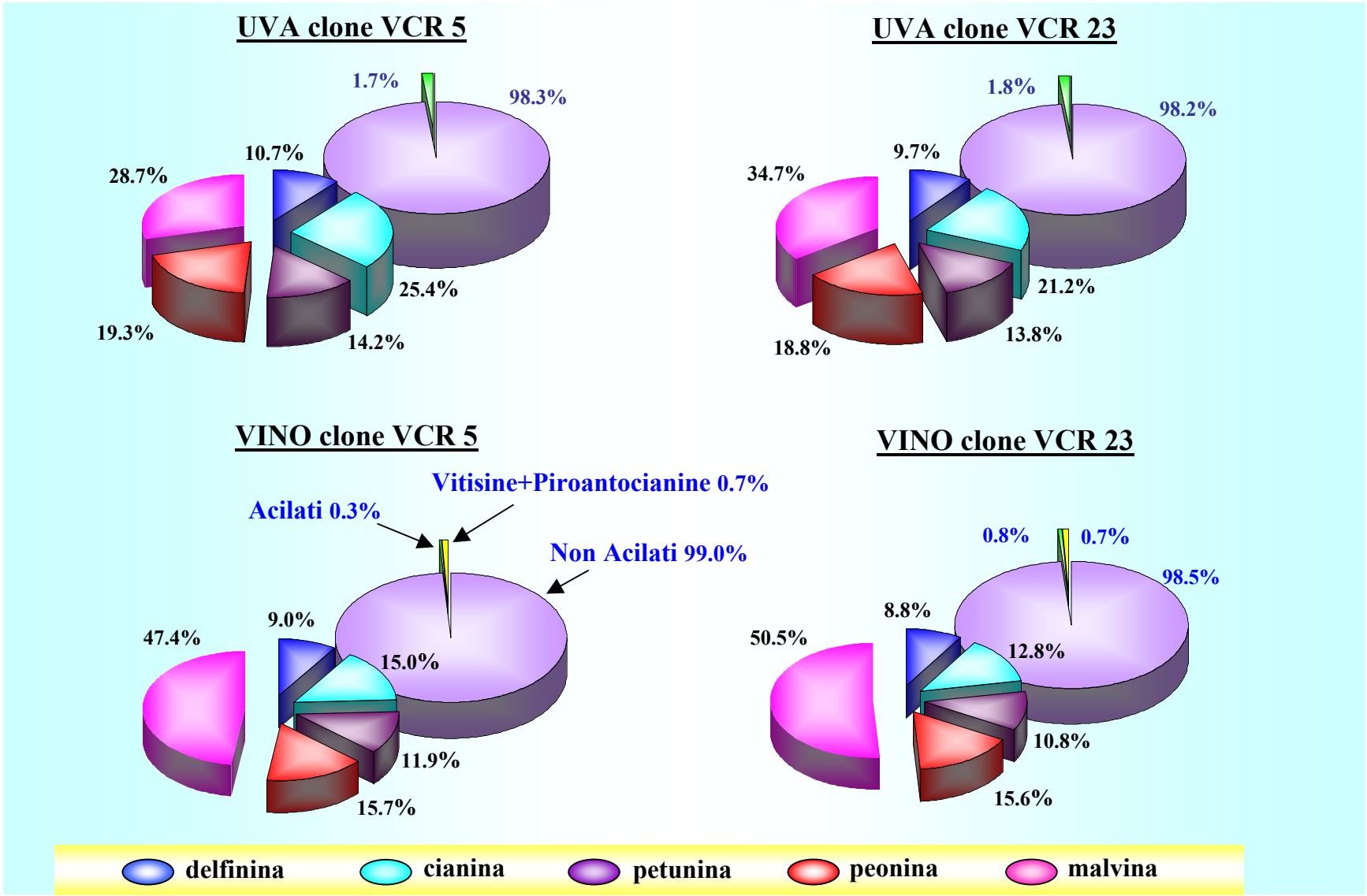

Fig. 2: Rappresentazione grafica degli antociani delle uve della cv Sangiovese (cloni VCR5 e VCR23) e dei corrispondenti vini.

BIBLIOGRAFIA

Asenstorfer R.E., Markides A.J. and Jones G.P., 2003. Formation of vitisin A during red wine fermentation and maturation. Australian J. Grape and Wine Res., 9, 40-46.

Bakker J. and Timberlake C.F., 1997. Isolation, identification and characterization of new color-stable anthocyanins occurring in some red wines. J.Agric. Food Chem., 45, 35-43.

Baldi A;Romani A;Mulinacci N;Vincieri FF., 1993. Polyphenolic compounds in Tuscan varieties of Vitis vinifera L.. J. Intern. Sciences Vigne et Vin, 27 201-215.

Bucelli P;Faviere V;Giannetti F;Gigliotti A., 1992. Sviluppo e caratterizzazione delle bacche e delle foglie di Sangiovese dall'invaiatura alla maturazione. Vignevini, 19 (1/2) 61-66.

Bucelli, P.; Storchi, P., 2007. Valutazione viticola ed enologica di alcuni vitigni autoctoni e innovativi per la Toscana. 2007 Riv.Vit.Enol., Conegliano, 60 (2) 3-21.

Calò A., Tomasi D., Cravero M.C., Di Stefano R., 1994. Contributo alla caratterizzazione e classificazione varietale attraverso la determinazione degli antociani e degli acidi idrossi cinnamiltartarici della buccia di varietà a bacca rossa. Riv.Vit. Enol.,Conegliano, 47 (3).

Di Stefano et al. 1994, Estrazione degli antociani dalle bucce dell'uva durante la fermentazione. L'Enotecnico, 30, (5), 75-83.

Fulcrand H., Benabdeljalil C., Rigaud J., Chenyer V. and Moutounet M., 1998. A new class of wine pigments generated by reaction between pyruvic acid and grape anthocyanins. Phytochemistry 47, 1401-1407.

Hayasaka Y., Birse M., Eglinton J., Herderich M., 2007. The effect of Saccharomyces cerevisiae and Saccharomyces bayanus yeast on colour properties and pigment profiles of a Cabernet Sauvignon red wine. Australian J. Grape and Wine Res., 9, 40-46.

Mangani S., Capece A., Favilli C., Buscioni G., Vincenzini M., 2008. Gli antociani dei vini rossi: indicatori varietali in pasto ai microrganismi. L'Enologo, 44 (1), 95-100.

Mattivi F., Guzzon R., Vrhovsek U., Stefanini M., Velasco R., 2006. Metabolite profiling of grape: Flavonols and anthocyanins. J.Agric. Food Chem., 54 (20) 7692-7702,

Mazza, G.; Marchi, D.; Cascio, P.; Barbieri, E., 2005. Effetto dell'impiego di tannini ed enzimi nella vinificazione di uve Nebbiolo e Sangiovese. Vignevini, Bologna, 32, 62-67.

Romero and Bakker, 2000. Effect of storage temperature and pyruvate on kinetics of anthocyanin degradation, vitisin a derivative formation, and color characteristics of model solutions. J.Agric. Food Chem, 48, 2135-2141.

Schwarz M., Picazo-Bacete J.J:, Wintheralter P., Hermosin-Gutierrez I., 2005. Effect of copigments and grape cultivar on the color of red wines fermented after addition og copigments. J.Agric. Food Chem, 53, 8372-8381.

REDUCCIÓN DE 4- ETILFENOL MEDIANTE EL EMPLEO DE LÍAS DE LEVADURA

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RESUMEN

La contaminación de *Dekkera/Brettanomyces* y la producción de cantidades significativas de 4-etilfenol suponen importantes depreciaciones en vinos tintos, generalmente, de alto valor añadido. En estudios previos se ha evidenciado cómo la liberación autolítica de fragmentos parietales varía considerablemente entre diferentes especies de levadura que muestran arquitecturas moleculares particulares en sus paredes celulares (Palomero *et al*., 2009). En este trabajo se ha estudiado el potencial para adsorber 4-etilfenol, de diferentes biomasas procedentes de cultivos puros de levaduras vínicas liofilizadas,- *Saccharomyces cerevisiae* G37, *Schizosaccharomyces pombe* 936-, y reducir así la percepción sensorial negativa de este tipo de compuestos. Mediante análisis cromatográfico SPME-GC/MS, se han obtenido elevadas correlaciones entre la dosis de lías de levadura utilizadas y la reducción de 4-etilfenol, existiendo diferencias en cuanto a la capacidad de retención de este compuesto entre las distintas cepas de levaduras estudiadas. Por otra parte, se ha investigado la repercusión de este tratamiento paliativo sobre los parámetros, cuantificándose mediante cromatografía HPLC-PDAD-ESI/MS reducciones relevantes en el contenido de antocianos cuando se emplean dosis de lías efectivas para anular el impacto sensorial producido por el 4-etilfenol.

Palabras clave: 4-etilfenol, adsorción, lías, no *Saccharomyces*, antocianos

ABSTRACT

Dekkera/Brettanomyces development and the consequent production of significant amounts of 4-ethylphenol, entail important constraints in red winemaking. In previous studies we demonstrated that the autolytic release of parietal polysaccharides varies depending on the yeast species according to their particular cell-wall architecture (Palomero *et al*., 2009). In this work the bioadsorption capacity of 4 ethylphenol of different wine yeast biomasses have been studied- *Saccharomyces cerevisiae* G37, *Schizosaccharomyces pombe* 936-, in order to diminish the negative impact on the sensorial profile of this type of compound. Through SPME-GC/MS, high correlations between the doses of yeasts added and the reduction of 4-ethylphenol initial concentrations from wine have been obtained; existing differences between adsorption kinetics between yeast were also measured. Therefore the repercussions of this palliative treatment over the anthocyanin concentration have also been studied by means of HPLC-PDAD/ESI-MS analysis. Relevant reductions in anthocyanin concentrations were detected when effective doses of yeast lees are used.

Keywords: 4-ethylphenol, adsorption, lees, non-*Saccharomyces*, anthocyanins

INTRODUCCIÓN

Las lías de levadura y sus propiedades bioadsorbentes se han señalado recientemente como posible solución para paliar los efectos sensoriales negativos de *Dekkera/Brettanomyces* (Pérez-Serradilla *et al.*, 2009). Chassagne *et al.* (2005) estudiaron la capacidad para reducir las concentraciones de 4-etilfenol en vinos sometiendo los mismos a cortos tiempos de contacto con diferentes concentraciones de biomasa de levaduras, obteniendo reducciones estadísticamente significativas. El presente trabajo tiene por objetivo el estudio de la capacidad para reducir las concentraciones de 4-etilfenol de diferentes biomasas de levaduras y, al mismo tiempo investigar los posibles efectos de este tipo de tratamientos sobre el contenido de antocianos.

MATERIALES Y MÉTODOS

Los experimentos se realizaron utilizando vino tinto de Vitis vinifera L. cv *Merlot* de la D.O. Vinos de Madrid. Dicho vino fue adicionado con una concentración conocida de 4-etilfenol (Tabla 1).

Las diferentes biomasas utilizadas se obtuvieron mediante la fermentación de medios sintéticos (Palomero *et al.*, 2009). Dichas biomasas liofilizadas se pusieron en contacto durante periodos de 90 minutos con los vinos adicionados de 4-etilfenol siguiendo la metodología de Chassagne *et al*., 2005.

El seguimiento analítico de las concentraciones de 4-etilfenol en las diferentes muestras propuestas (dosis de lías 5, 10, 15, 20, 25 30 g/l) se realizó mediante SPME-GC/MS (Benito *et al*., 2009). Se determinaron las concentraciones de antocianos mediante HPLC-PDAD-ESI/MS después de los tratamientos y previa centrifugación (4000 rpm) según Palomero *et al.*, 2009.

RESULTADOS Y DISCUSIÓN

La Tablas 1 muestra la reducción de 4-etilfenol en función de las dosis de levadura empleada. Se observa una gran correlación entre los decrementos en las concentraciones de 4-etilfenol y las dosis de levadura utilizada. Como es lógico, las mayores disminuciones se obtienen cuando se emplearon las dosis más elevadas (30 g/l). Por otra parte, *Schizosaccharomyces pombe* (936) mostró mayor capacidad en la retención de 4-etilfenol a dosis más bajas de lías que *Saccharomyces cerevisiae* (G37) (Figura 1).

Tabla 1. Concentraciones de 4-etilfenol (ppb) en un vino de *Vitis vinifera* L. cv. Merlot a diferentes dosis de lías adicionadas. Valores como ms±ds y diferencias mínimas significativas.

Yeast lees (g/l)	*Saccharomyces cerevisiae* (G37)	*Schizosaccharomyces pombe* (936)
0	434,11±23,96 [a]	434,11±23,96 [a]
5	415,01±29,29 [ab]	379,90±24,88 [b]
10	390,98±12,25 [bc]	371,21±10,48 [bc]
15	362,25±21,06 [cd]	359,86±11,17 [bcd]
20	342,67±16,14 [d]	343,40±7,43 [cde]
25	338,64±4,61 [d]	325,84±24,50 [de]
30	334,11±21,10 [d]	314,26±30,15 [e]

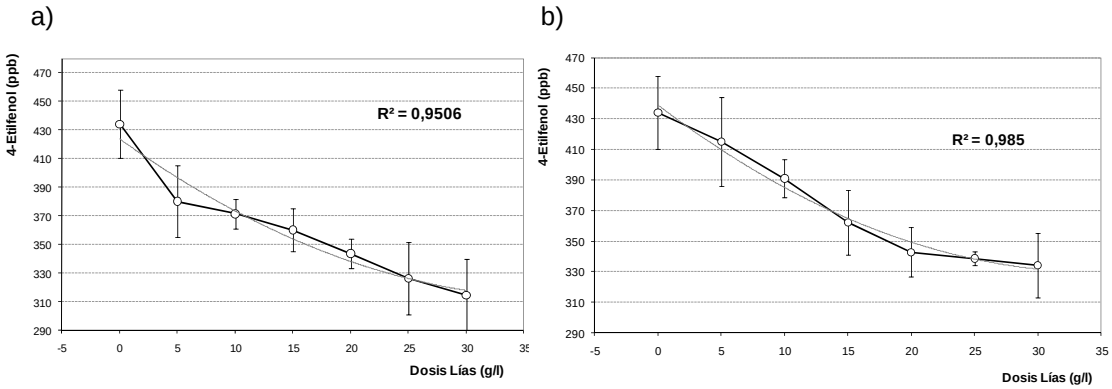

Figura 1**. a)** Reducción de las concentraciones de 4-etilfenol con el empleo de lías de *Schizosaccharomyces pombe* (936) en un vino de *Vitis vinifera* L. cv. Merlot. **b)** Reducción de las concentraciones de 4-etilfenol con el empleo de lías de *Saccharomyces cerevisiae* (G37) en un vino de *Vitis vinifera* L. cv. Merlot

Por otra parte, en las Tablas 2 y 3 se recogen las disminuciones en las concentraciones de antocianos para las levaduras *Schizosaccharomyces pombe* (936) y *Saccharomyces cerevisiae* (G37) respectivamente. En ambos casos utilizando este tratamiento paliativo se produce de forma análoga una reducción significativa de los contenidos de antocianos, siendo esta reducción, al igual que en el estudio de la concentración de 4-etilfenol, más importante en el caso de la biomasa de *Schizosaccharomyces pombe* (936).

Tabla 2. Reducción del contenido de antocianos en función de la dosis de levadura empleada de *Schizosaccharomyces pombe* (936). Valores como ms±ds y diferencias mínimas significativas.

Lías (g/l)	Antocianos monómeros	Piranoantocianos	Antocianos Acetilados	Antocianos Cumarilados
0	210,39±9,10	18,85±0,14	104,55±5,29	29,46±1,82
15	179,14± 6,61	16,56±0,37	89,76±3,60	16,77±0,86
30	157,62±7,59	14,53±0,24	7,95±4,43	10,52±64,29
% Reducción	**25,08**	**22,92**	**23,89**	**64,29**

Tabla 3. Reducción del contenido de antocianos en función de la dosis de levadura empleada de *Saccharomyces cerevisiae* (G37). Valores como ms±ds y diferencias mínimas significativas.

Lías (g/l)	Antocianos monómeros	Piranoantocianos	Antocianos Acetilados	Antocianos Cumarilados
0	237,17±1,82	18,19±0,21	124,79±0,04	37,84±1,03
15	212,27± 0,51	16,41±0,89	108,14±1,87	25,29±1,07
30	197,48±1,92	15,44±0,75	100,91±2,71	19,51±1,22
% Reducción	**16,73**	**15,11**	**19,40**	**48,44**

CONCLUSIONES

El empleo de lías de levadura para reducir concentraciones elevadas de 4-etilfenol muestra cierta eficacia con dosis de levadura muy elevadas. Pero dicha técnica provoca una reducción importante en los contenidos de antocianos lo que cuestiona su posible aplicación práctica, al menos en las condiciones experimentales estudiadas.

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BIBLIOGRAFÍA

Pérez-Serradilla, J.A., & Luque de Castro, M.D. (2008).
Role of lees in wine production: A review. Food Chemistry, 111, 447-456

Chassagne, D., Guilloux-Benatier, M., Alexandre, H., & Voilley, A. (2005)
Sorption of wine volatile phenols by yeast lees. Food Chemistry, 91, 39-44

Benito, S., Palomero, F., Morata, A., Uthurry, C., & Suárez-Lepe. (2009)
Minimization of ethylphenol precursors in red wines via the formation of pyranoanthocyanins by selected yeasts. Internatinal Journal of Food Microbiology, 132, 145-152

Palomero, F., Morata, A., Benito, S., Calderón, F., & Suárez.Lepe (2009).
New yeast genera for over-lees ageing of red wine. Food Chemistry, 112, 432-442

VALORIZZAZIONE E CARATTERIZZAZIONE DI UN VITIGNO AUTOCTONO PIEMONTESE:L'UVALINO

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RIASSUNTO

Il recupero di antiche varietà autoctone è una tappa importante per la conservazione della biodiversità presente nel territorio vitivinicolo permettendo di valorizzare varietà "dimenticate" attraverso l'ottenimento di utili informazioni genetiche, viticole ed enologiche. In questo lavoro è stata effettuata una caratterizzazione varietale del vitigno Uvalino con analisi su uve e vino. Dall'analisi dei composti glicosilati varietali delle uve, si è osservato un elevato tenore in composti benzenici, in particolare alcol benzilico e 2-feniletanolo ed un rilevante contenuto in eugenolo. Fra i composti monoterpenici prevalgono il geraniolo e i suoi derivati. L'idrolisi chimica ha fatto osservare un buon contenuto in ocimenoli, actinidoli e Riesling acetale; tali composti risultano caratterizzare anche il profilo aromatico di fermentazione insieme ad un elevato contenuto in esteri. La malvidina e la peonidina sono composti prevalenti nel profilo degli antociani monomeri delle uve. Inoltre risulta di notevole interesse l'elevato tenore in resveratrolo, noto per le sue importanti proprietà antiossidanti, che risulta presente in concentrazioni elevate anche nel vino.

ABSTRACT

The recovery of old native varieties can play an important role to preserve the biodiversity of wine area, allowing to obtain useful information about genetic, viticultural and enological knowledge. In this work, Uvalino, a Piedmont indigenous *cv*, was characterized by the analysis of grape and wine. The analysis of varietal glycosides aromatic compounds had shown a prevalence of benzenoids, particularly benzyl alcohol and 2–phenilethanol; moreover a relevant content in eugenol was observed. Within monoterpenic compounds, geraniol and its derivates were prevalent. Ocimenols, actinidols and Riesling acetal were the main molecules obtained by the chemical idrolysis reaction on the grape extract. These latter compounds, together with esters, charaterized also the wine aromatic profile. The malvidin and peonidin were prevalent monomeric anthocyanins in the Uvalino grapes. Moreover the content in resveratrol, a phenolic compound known for its remarkable antioxidant properties, was high both in grapes and wines.

INTRODUZIONE.

Nel *Catalogo Nazionale delle Varietà di Viti* si annoverano oggi più di 350 vitigni, la maggior parte dei quali autoctoni del nostro Paese. Questo elenco si arricchisce ulteriormente se si considerano tutte le varietà minori che recentemente sono in fase di recupero. Al fine di valorizzare il patrimonio di biodiversità è senz'altro fondamentale riuscire a caratterizzare il

più finemente possibile le numerose cultivar presenti sul nostro territorio. Le informazioni relative a distribuzione geografica, morfologia, fenologia e attitudini colturali delle varietà viticole, insieme alla descrizione sensoriale dei vini ottenuti da ciascuna di esse, compongono solo una parte, sebbene fondamentale, della caratterizzazione varietale dei vitigni. Grazie alle tecniche scientifiche acquisite, le caratteristiche varietali di una cultivar possono essere descritte oggi anche attraverso tecniche di biologia molecolare e cellulare volte alla ricerca di specifiche porzioni geniche, e/o alla comprensione di precise fasi biosintetiche, responsabili della formazione dei caratteristici metaboliti secondari. In quest'ottica, quindi, la descrizione delle uve e dei vini dal punto di vista del profilo polifenolico e del corredo aromatico risulta di notevole importanza per la caratterizzazione di una varietà viticola. Si è osservato infatti che questi profili mantengono una certa specificità varietale, una sorta di *fingerprinting* metabolico della cultivar esaminata (Cravero M.C., Di Stefano R., 1990). Tra le varietà autoctone piemontesi si inserisce l'Uvalino, vitigno del territorio astigiano, dalle particolari caratteristiche che ne hanno reso interessante il recupero colturale e lo studio dal punto di vista scientifico. L'Uvalino è una *cv* la cui maturazione tardiva si colloca intorno alla prima decade di ottobre (circa una settimana dopo le uve Barbera) e le cui bacche risultano fortemente resistenti all'attacco di muffe e marciumi; in particolare, la resistenza all'attacco della *Botrytis cinerea* lo rende estremamente interessante. Proprio le sue proprietà di resistenza, rendevano in passato l'Uvalino utile per la rifermentazione delle vinacce nelle vasche di fermentazione dopo l'ottenimento del vino da uve di altra varietà. Oggi l'Uvalino è stato nuovamente portato alla ribalta ed è vinificato in purezza, ottenendo un vino dal colore rosso porpora con riflessi granati, dalle note olfattive decisamente speziate e un gusto marcatamente tannico e asciutto. Ulteriore interessante aspetto di questa varietà è il caratteristico elevato tenore in resveratrolo, un polifenolo associato a importantissime proprietà benefiche, antiossidanti e antitumorali. Uno studio farmacologico indirizzato relativo ai benefici salutistici dell'Uvalino ha dimostrato i significativi effetti prodotti da questa varietà nell'inibizione della produzione dei radicali idrossili (ROS) coinvolti nelle malattie in cui lo stress ossidativo gioca un ruolo fondamentale (Bertelli A. *et al.*, 2004).

MATERIALI E METODI.

Campionamento uve. Il prelievo dei singoli acini è effettuato in modo da renderlo il più rappresentativo possibile raccogliendo gli acini da più zone del grappolo. Gli acini sono stati asportati dal grappolo con l'uso di forbicine, tagliando il pedicello all'altezza della zona centrale. Gli acini dell''intero campione sono stati sottoposti a flottazione (Fournand et al. , 2006) in vasche contenenti concentrazioni crescenti di NaCl. Quindi, si sono analizzate le due classi di flottazione più numerose corrispondenti alle vasche con aggiunta di 120 g/l e 140 g/l di NaCl, rispettivamente denominate classe 1 e classe 2. Tali classi corrispondono a un diverso grado di maturazione dell'uva in campo, in particolare evidenziata da una differente concentrazione zuccherina (19,1°Brix per la classe 1 e 21,2° Brix per la classe 2). Un'ulteriore classe, denominata massa, è stata preparata unendo frazioni di acini numericamente proporzionali al quantitativo di acini flottati nelle singole vasche. La massa così costituita è quindi rappresentativa del campione di uva presente in vigneto alla vendemmia, con grado Brix pari a 20,4. Per ogni analisi sono state effettuate due ripetizioni di 100 acini ciascuna; in laboratorio si è proceduto al congelamento dei campioni, che sono stati conservati in freezer fino al momento dell'analisi dei composti precursori d'aroma, mentre la preparazione degli estratti per la determinazione dei composti fenolici è stata effettuata al momento della raccolta.

Analisi dei composti aromatici glicosilati varietali derivanti da idrolisi enzimatica e chimica da uve. Per la determinazione del profilo dei precursori aromatici varietali sono preparati degli estratti in tampone tartarico a partire da gruppi di 100 acini secondo quanto riportato in Ummarino e Di Stefano, 1997. Dopo estrazione dei composti aromatici in fase solida su cartucce C18, si è effettuata l'analisi GC-MS secondo quanto riportato in Di Stefano, 1991.

Determinazione dei composti di fermentazione nel vino. Per la valutazione dei composti dei fermentazione si sono prelevati 30 ml di vino ottenuti da micro vinificazioni di due differenti annate (2004 e 2006), previamente filtrati e diluiti con acqua in modo tale da ottenere un tenore in etanolo non superiore al 4%, dopo addizione di standard interno, il tutto è passato su una cartuccia C18 da 1 g. Per estrazioni successive e con solventi affini, sono estratte frazioni costituite da composti idrofili e lipofili. I particolari della preparazione del campione e le condizioni cromatografiche sono riportate in Gianotti e Di Stefano, 1991.

Condizioni Cromatografiche. Per le analisi si è utilizzato un gascromatografo Agilent 6890 equipaggiato con detector MSD Agilent 5973 Network. La colonna capillare: HP-Innovax (30 m di lunghezza x 0.25 mm di diametro interno, 0.25 µm di film). Gas di trasporto: elio a 1 ml/minuto. Iniezione splitess a 40 °C, tempo di iniezione 2 minuti. T° del iniettore: 250 °C; T° dell'interfaccia: 230 °C. Programmata di temperatura: da 40° a 60 °C a 30 °C al minuto; isoterma a 60 °C per 2 minuti; da 60 °C a 190 °C a 2 °C/min; da 190 °C a 230 °C a 5 °C/min; 15 min di isoterma finale. I composti sono stati identificati per confronto con le librerie NBS75K e Wiley 275. Tutti i composti sono espressi in µg/kg di 1-eptanolo (*standard* interno) utilizzando il fattore di risposta 1.

Analisi dei composti polifenolici. Le bucce sono separate dalla polpa e dai semi e poste in tampone tartarico a pH 3,2. Dopo circa 2 ore le bucce e i semi sono omogeneizzati e centrifugati come specificato in Ummarino et al., 2001. Sugli estratti ottenuti dalle bucce o direttamente sul vino si effettuano le determinazioni spettrofotometriche degli indici di proantocianidine, di polifenoli totali (Di Stefano e Cravero, 1991), il profilo degli acidi idrossicinnamil tartarici e dei flavonoli via HPLC (Ummarino et al., 2001).

Indice di vanillina. I flavani a basso grado di polimerizzazione sono determinati mediante la reattività alla vanillina. Gli estratti delle uve o il vino sono concentrati per passaggio su cartuccia C18 Sep Pak da 500 mg ed eluiti con metanolo. 1ml di soluzione metanolica è posta in un tubo da saggio in vetro scuro e aggiunto di 2,5 ml di vanillina all'1% in metanolo e 2,5 ml di H_2SO_4 3,6 N in metanolo. Si prepara una prova in bianco sostituendo la soluzione di vanillina con ugual volume di metanolo. Le due provette sono poste in bagno termostatico per 15 min a 30°C. Si legge infine l'assorbanza a 500 nm, su 1 cm di P.O.

RISULTATI E DISCUSSIONE

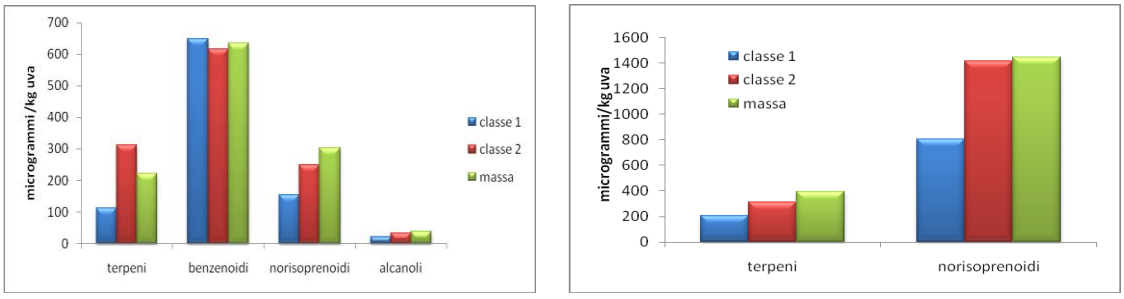
Dall'analisi dei composti glicosilati varietali delle tre classi (classe 1, classe 2 e massa) si osserva che nel vitigno Uvalino i composti benzenici risultano prevalenti senza importanti differenze associabili al diverso grado zuccherino delle uve. Al contrario, per gli altri gruppi di composti si evidenzia che un loro maggiore contenuto è sempre correlato ad un più elevato grado Brix (fig .1).

Composti volatili derivanti da idrolisi enzimatica.

Fra i composti monoterpenici prevalgono il geraniolo e i suoi derivati, in particolare l'acido geranico. Si osserva, invece, un basso tenore di linalolo per tutte le classi esaminate accompagnato da un ridotto contenuto in ossidi piranici e furanici. Fra i derivati del linalolo risultano invece sempre presenti il trans-8OH linalolo e il diolo 1. Nella classe 2 con un maggiore grado Brix (21,2° Brix) è interessante la rivelabilità, correlata ad una sua maggiore concentrazione nelle uve, del composto cis-8OH linalolo. Un basso contenuto di α-terpineolo caratterizza tutte le classi analizzate. I composti più abbonadanti tra i benzenoidi sono l'alcol benzilico e il 2-feniletanolo; sono inoltre interessanti i tenori di eugenolo e dei suoi derivati. Fra i norisoprenoidi determinati, il 3-oxo-α-ionolo e il vomifoliolo sono i due composti prevalenti.

Composti volatili derivanti da idrolisi chimica.

L'idrolisi in ambiente a cido e caldo permette di individuare i composti volatili che potenzialmente potrebbero liberarsi durante la conservazione del vino, contribuendo all'aroma del prodotto finito positivamente e/o negativamente. L'idrolisi chimica effettuata sulle uve Uvalino ha permesso di determinare quantità discrete di ocimenoli, la presenza di linalolo e relativi ossidi, non idrolizzati per via enzimatica, nonché di α-terpineolo. Gli actinidoli e il Riesling acetale, fra i norisoprenoidi, sono i composti presenti in maggiore quantità. Il TDN e i suoi derivati sono osservati esclusivamente nel campione massa; l'apporto di tali composti sembra pertanto derivare da acini con grado Brix inferiore o superiore al *range* considerato in questo lavoro.



Idrolisi enzimatica Idrolisi chimica

Fig.1 Classi dei composti aromatici glicosilati ottenuti da idrolisi enzimatica e chimica

Composti polifenolici. Tutti gli indici polifenolici risultano più elevati all'aumentare dei gradi Brix, tranne l'indice dei flavani reattivi alla vanillina che presenta un trend opposto (fig.2). La varietà risulta caratterizzata da un basso indice di antociani totali. Il profilo degli antociani monomeri ottenuto da analisi HPLC è riportato nella figura 2, dove si osserva che gli antociani monomeri prevalenti sono la malvina (35%) e la peonina (33%). Risulta di notevole interesse l'elevato contenuto di resveratrolo, polifenolo noto per le sue importanti proprietà antiossidanti. I tenori misurati si collocano intorno ai 700 mg/kg di uva. Fra gli HCTA sono stati rilevati gli acidi *trans*-caffeiltartarico e *trans* p-cumariltartarico (Tab.1). Il rapporto fra il *trans*-caffeiltartarico (CTA) ed il *trans p*-cumariltartarico (*p*-CuTA) come gli altri rapporti riportati in tabella sono caratteristici della varietà (Di Stefano et al., 1996).

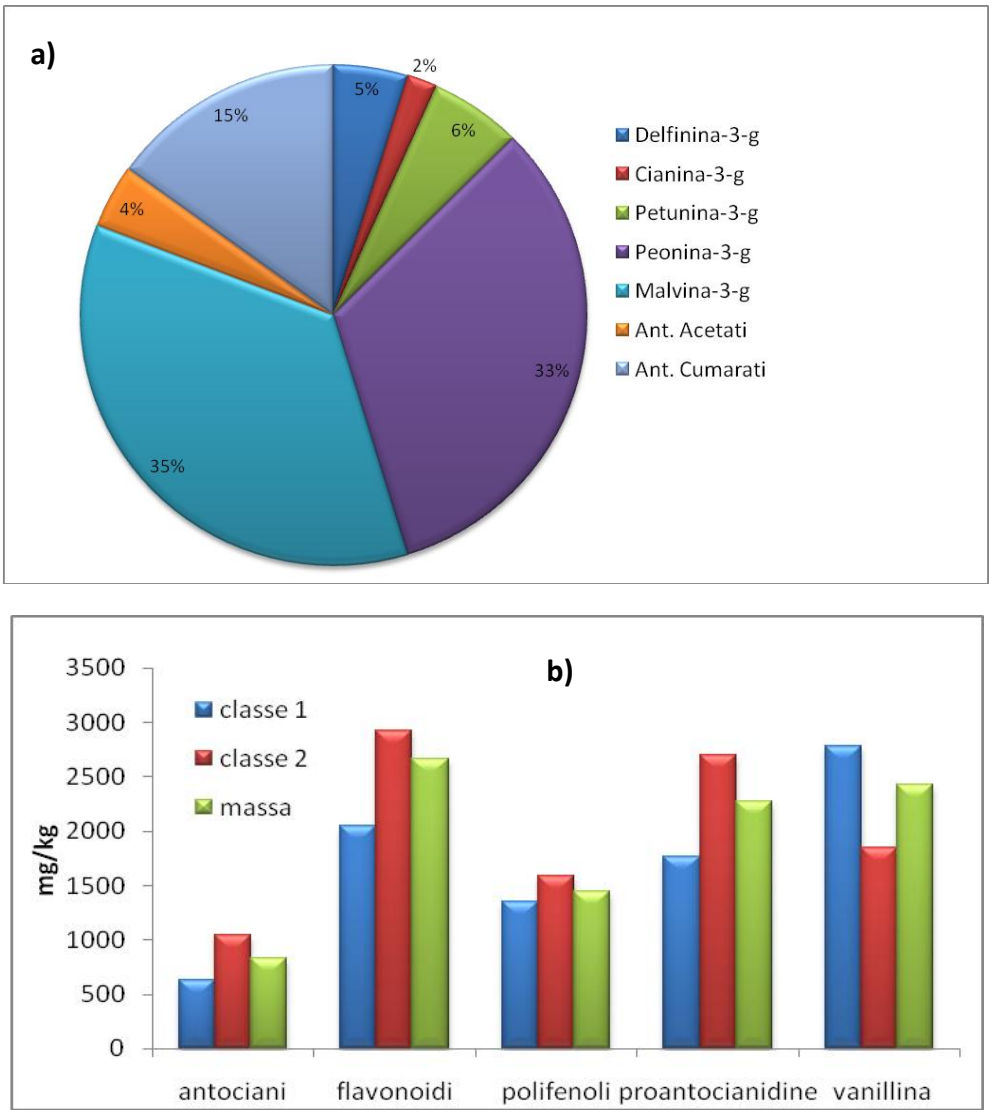


Fig.2. Profilo antocianico (a) e indici polifenolici delle uve Uvalino (b)

Tab.1 HCTA e flavonoidi delle uve Uvalino e relativi rapporti

mg/Kg	**classe 1**	**massa**	**classe 2**
trans caffeiltartarico	54,23	38,20	55,45
Trans-p-Cumariltartarico	64,23	40,10	66,11
Miricetina glucoside	12,69	16,64	22,73
Quercetina glucoronide	13,10	13,67	20,71
Quercetina glucoside	14,49	16,02	23,57
Camferolo glucoside	8,29	7,13	10,11
trans resveratrolo	711,91	755,53	757,37
Rapporti caratteristici varietali	**classe 1**	**massa**	**classe 2**
quercetina glucoronide/glucoside	<1	<1	<1
miricetina/quercetina (glucoside e glucoronide)	<1	<1	<1
CTA/pCUTA	<1	<1	<1

Aroma dei vini

Dall'analisi dei vini del 2004 e del 2006, analizzati nel 2009 (tab.2), si conferma la presenza di Riesling acetale e derivati dell'eugenolo come già osservato nelle uve Uvalino sottoposte ad idrolisi chimica. Durante la conservazione del vino si mantengono elevati i tenori dell'alcool benzilico e del 2-feniletanolo che caratterizzano, come già descritto nelle uve, il corredo varietale e possono contribuire a dare un'impronta fiorale di rosa in quanto presenti al di sopra della soglia olfattiva. Fra gli esteri di fermentazione sono presenti, anche se sotto la soglia di percezione, l'isoamil acetato e gli esteri etilici degli acidi grassi esanoico, ottanoico e decanoico, composti dall'aroma fruttato che nel tempo tendono a diminuire. La presenza di dietil succinato e di altri composti caratteristici del vino invecchiato sono prova che nei vini esaminati sono già avvenute molte delle reazioni tipiche dell'invecchiamento.

Tab.2 Composti di fermentazione nei vini ottenuti da uve della *cv* Uvalino

Composti di fermentazione	Uvalino 2004 mg /L	Uvalino 2006 mg /L
Isoamilacetato	0,27	0,21
Etilesanoato	0,13	0,11
Etile ottanoato	0,20	0,15
Etildecanoato	0,07	0,08
2 Feniletilacetato	0,11	0,12
Dietilsuccinato	0,25	1,24
Alcol Benzilico	3,47	2,34
2 Feniletanolo	135,2	161,8
Riesling acetale	0,04	0,04
Metossieugenolo	0,13	0,14

Resveratrolo nei vini.

Il contenuto di reveratrolo, elevato nelle uve, si mantiene tale anche nei vini (tab.3). E' particolarmente interessante il confronto con altre varietà a bacca rossa, per le quali il contenuto medio di questo composto si aggira su valori dei 3 mg/l. Nell'Uvalino il resveratrolo raggiunge anche tenori superiori ai 10 mg/l; comportamento intermedio è stato osservato nei vini Negroamaro e Pinot analizzati.

Tab.3 Tenore in resveratrolo di alcuni vini italiani.

Resveratrolo mg/l	
Barbera	2,19
Barbaresco	2,32
Barolo	2,37
Dolcetto	2,10
Freisa	1,82
Grignolino	2,15
Nebbiolo	1,41
Pinot	5,46
Negroamaro	6,80
Uvalino	11,86

CONCLUSIONI

L'Uvalino è un vitigno con particolari caratteristiche di resistenza a condizioni climatiche sfavorevoli e agli attacchi di *Botrytis*. Il suo corredo polifenolico è caratterizzato da un livello medio di antociani e buona concentrazione di tannini. Si tratta di una cultivar neutra in cui prevalgono i benzenoidi glicosidi, soprattutto 2-fenil etanolo e alcol benzilici insieme a derivati dell'eugenolo. Il vino presenta un'intensità del colore stabile nel tempo anche se con un modesto contenuto di antociani e il suo aroma risulta interessante. La presenza nelle uve di composti fenolici della classe degli stilbeni e degli acidi idrossicinnamil-tartarici fa si che siano favorite le reazioni che portano a fenomeni di copigmentazione e quindi alla stabilizzazione del colore nel tempo. Sarà interessante studiare i vini prodotti in purezza su scala di cantina per la valutazione della componente aromatica, considerato che si tratta di un vino che richiede un tempo di affinamento abbastanza lungo.

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BIBLIOGRAFIA

Bertelli A., Morelli R., Falchi M., 2004. Effects of uvalino, a new autochthonous wine, on the inhibition of the production of hydroxyl radicals. *Drugs Exp Clin Res.* n. 30 (4): 177-181.

Cravero M.C., Di Stefano R., 1990. I composti fenolici e l'origine varietale delle uve *Riv. Vitic. Enol.* n.1 : 33-44.

Di Stefano R., Cravero M.C.,1991. Metodi per lo studio dei polifenoli dell' uva. *Riv. Vitic. Enol.*, n. 44 (2): 37-45.

Di Stefano R., 1996. Metodi chimici nella caratterizzazione varietale. *Ann. Ist. Sper. Enol. di Asti, XXVII,* 33-53.

Fournand D., Vicens A., Sidhoum L., Souquet J.M., Moutounet M., Cheynier V., 2006. Accumulation and extractability of grape skin tannins and anthocyanins at different advanced physiological stages. *J Agric. Food Chem,* n.54: pag 7331-7338.

Ummarino I., Di Stefano R. 1997. Influenza del numero di semi per acino sulla composizione dell'uva. Nota II. *Riv. Vitic. Enol*., n. 50 (3): 9-24.

Ummarino I, Ferrandino A., Cravero M.C., Di Stefano R. 2001. Evoluzione dei polifenoli di uve di biotipi di Pinot nero durante la maturazione. *L'Enologo XXXVII,* n. 4: 71-82

EFFETTO DELLA DEALCOLIZZAZIONE MEDIANTE MEMBRANA A CONTATTORE SU VINI ITALIANI: VALUTAZIONI COMPOSITIVE E SENSORIALI.

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RIASSUNTO

Nella produzione dei vini è attualmente sempre più frequente riscontrare elevati tenori alcolici determinati, in primo luogo, dagli andamenti climatici delle ultime vendemmie ed alla ricerca di stati di maturazione avanzati dell'uva, quindi alla produzione di vini di struttura e di forte carattere territoriale, che spesso si può tradurre in una eccessiva presenza di zuccheri nei mosti e, conseguentemente, di alcol nei vini. L'eccesso di alcol, che sempre più frequentemente può superare il limite del 15 %, oltre a costituire un elemento di non equilibrio qualitativo, è per il consumatore l'elemento di maggior criticità nella valutazione della salubrità del prodotto. In seguito la recepimento della nuova OCM (reg. CE 606/2009) si sta diffondendo la dealcolazione come pratica di cantina. Nel presente lavoro vengono illustrati i risultati della dealcolazione mediante membrana a contattore su diversi vini Italiani, dealcolati con diverse intensità variabili tra un minimo di 0,5 % vol. fino a 2 % vol. Si riportano i dati relativi alle modificazioni indotte dalla tecnica sui principali parametri enochimici, sulle stabilità proteica e tartarica ed in particolare sulle caratteristiche sensoriali. I risultati hanno messo in evidenza come la dealcolazione mediante tecnica a membrana contattore possa essere una tecnica valida per la dealcolazione dei vini.

ABSTRACT

In the production of wine is now increasingly common to find high alcohol content determined, in first instance, by the climatic trends of the last harvests and by the search of advanced stages of ripening of the grape, therefore to the production of well structured wines with strong territorial characters. This can be often translated in an excessive presence of sugars in musts and, consequently, of alcohol in wines. The excess of alcohol, which is more often exceeding the limit of 15%, whilst providing an element of non-equilibrium quality, is for the consumer the most critical element in assessing the wholesomeness of the product. Following the achievement of the new OCM (Reg. EC 606/2009) the dealcoholization is becoming a diffusion cellar practice. The present paper discusses the results of dealcoholization through membrane contactor on different Italian wines, dealcoholized with different intensities ranging from a minimum of 0.5% vol. up to 2% vol. We report data on changes induced by the technique on the main oeno-chemical parameters, on protein and tartaric stability, and in particular on the sensory characteristics. The results had shown how the dealcoholization technique using membrane contactor can be a feasible technique for wines dealcoholization.

INTRODUZIONE

Negli ultimi dieci anni il contenuto alcolico dei vini è aumentato in tutte le regioni viticole. Questo fenomeno è dovuto a diversi fattori. In primo luogo l'andamento climatico delle ultime vendemmie, poi il miglioramento delle pratiche colturali e la riduzione delle rese hanno contribuito soprattutto nelle regioni peninsulari e temperate. Inoltre la ricerca di avanzati stati di maturazione delle uve, per la produzione di vini di buona struttura ed elevata identità territoriale, spesso può risultare nella eccessiva presenza di zuccheri e, conseguentemente di alcool nei vini.

L'eccesso di alcol, che spesso supera il limite di 15% vol., oltre ad essere un elemento di non equilibrio, è il fattore più critico dal punto di vista salutistico. Inoltre l'approccio proibizionistico adottato dalle autorità di molte regioni della CE, ha contribuito alla riduzione del consumo del vino, come evidenziato da diversi studi statistici. Infine la presenza di alcool nel vino è il fattore che limita il consumo di vino in gruppi dove per motivi sociali, religiosi e culturali l'alcool non può essere assunto.

La riduzione della concentrazione di alcool può essere realizzata mediante diversi approcci:

- Pratiche agronomiche per limitare la concentrazione di zucchero
    - Vendemmie precoci
    - Incremento delle rese
    - Altre pratiche (colturali, selezione clonale, miglioramento genetico, etc...)
- Diminuzione della resa in alcool durante la fermentazione alcolica, ad esempio mediante l'utilizzo di lieviti selezionati ad hoc
- Modificazione del contenuto zuccherino del mosto o della concentrazione alcolica nei vini attraverso:
    - Diluizione del mosto con acqua
    - Riduzione del contenuto zuccherino mediante Ultrafiltrazione e Nanofiltrazione
    - Concentrazione del vino mediante osmosi inversa e diluizione con acqua
    - Dealcolazione del vino
        - Spinning cone column
        - Osmosi inversa e distillazione
        - Peraporazione
        - Estrazione mediante CO_2 supercritica
        - Contattore a membrana

Il presente lavoro è stato realizzato utilizzando il contattore seguendo le indicazioni riportate nel reg. CE 606/2009 il quale ammette la dealcolizzazione dei vini mediante tecniche fisiche di separazione, fino ad un massimo di 2 % vol. ed il prodotto finale deve essere conforme al reg. CE 478/2008. Questo sistema è stato scelto in primo luogo per la semplicità realizzativa, per l'economicità e l'elevata efficienza.

MATERIALI E METODI

Contattore

Il contattore a membrana consiste in una membrana idrofobica in cui è intrappolato un gas che ha il compito di tenere separati il succo e la soluzione estraente (Fig.1). Attraverso il gas le sostanze volatili possono migrare seguendo il principio della distillazione osmotica.
In accordo con i meccanismi di diffusione molecolare, il flusso di massa transmembrana , ν è legato alla differenza di pressione di vapore del composto tra i due lati della membrana secondo la relazione:

$$v = K_m \frac{P_{w1} - P_{w2}}{P_{A_{lm}}}$$

Dove P_{Alm} è la media logaritmica della pressione dell'aria attraverso i pori e K_m è la permeabilità che dipende dallo spessore e dalla porosità della membrana.

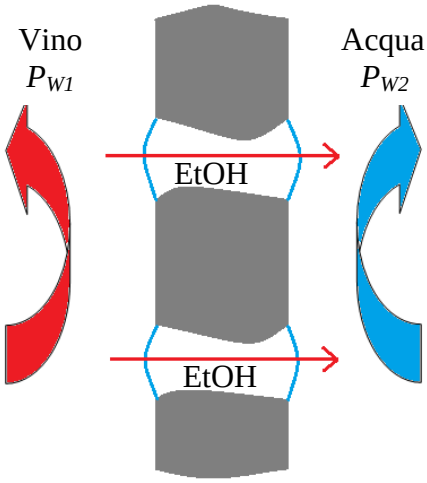

Fig. 1: Schema funzionamento contattore

Il sistema di dealcolazione impiegato per queste esperienza utilizza membrane a fibre cave (Ø est. 1 mm, spessore 200 μm) in PTFE (Teflon®), con uno sviluppo di 20 m^2; i flussi sulle superfici delle membrane, del vino in trattamento e della soluzione estraente (acqua), erano di 0,1-0,3 ms^{-1}. Tutte le esperienze sono state eseguite a temperatura ambiente. E' stata riscontrata una efficienza evaporativa di membrana compresa fra gli di 0,1 e 0,2 $l/m^2/h$ (come alcol anidro) a 20 °C.

Vini

Per la sperimentazione sono stati de alcolati vini rappresentativi della produzione italiana:

- Sfurzat 2007
- Sfurzat 2006
- Valpolicella
- Chianti
- Moscato

Mentre per i vini Sfurzat, Valpolicella e Chianti le dealcolazioni sono state condotte a fine fermentazione alcolica, per il moscato sono state realizzate quattro dealcolazioni: due durante la fermentazione alcolica e due alla fine di questa, questo per poter stabilire il momento migliore per la dealcolazione e le conseguenze di questo trattamento su un vino aromatico.

Metodiche analitiche

Le determinazioni analitiche eseguite sui mosti e vini sono state effettuate utilizzando le metodiche comunitarie [CEE, 1990] e quelle indicate dall'OIV [OIV, 2007].
Nelle prove effettuate su Moscato si è inoltre valutata la stabilità tartarica mediante analisi della T°sat determinata strumentalmente, e test di Mini Contatto determinati con apparecchiatura Criosmall della Steroglass – Pe –I.

I profili aromatici sono stati determinati dopo tecniche di arricchimento come SPE (estrazione in fase solida) utilizzando una cartuccia ENV+, e SPME (microestrazione in fase solida). Tutti i composti così arricchiti sono stati quantificati tramite HRGC-MS.
In alcuni casi si è cercato di quantificare altri composti come metanolo, acetaldeide e acetato di etile mediante iniezione diretta in GC-FID, dopo una preliminare distillazione della matrice. Questi composti potrebbero contribuire infatti ad alcuni sentori di sapore erbaceo e aceto-simili.

RISULTATI E DISCUSSIONE

Analisi Chimiche

Tab. 1: Analisi chimiche dei vini delcolati

Determinazione analitica	U.d.M.	Sforzato 2007 TQ	Sforzato 2007 De	Sforzato 2006 TQ	Sforzato 2006 De	Valpolicella TQ	Valpolicella De	Chianti TQ	Chianti De
Alcool	% vol.	16,6	14,95	15,93	14,6	14	12,3	13,9	12,89
Acidità titolabile	g/L	5,2	5,25	5,1	5,2	6,1	6,2	4,95	5,05
pH	---	3.49	3.50	3.51	3.50	3,17	3,13	3.38	3.37
Acidità Volatile	g/L	0,49	0,49	0,62	0,62	0.52	0.52	0,37	0,36
SO_2 Libera	mg/L	67	54	59	53	Nd	Nd	19	19
SO_2 Totale	mg/L	116	98	96	95	12	5	56	53

Valutazione dei composti aromatici in vini rossi tipici Italiani

Tab. 2: Analsisi SPME dei vini prima e dopo la dealcolazione

		Sfurzat		**Sfurzat**		**Valpolicella**		**Chianti**	
Classe composti	**Composti (SPME)**	**16,6 %v/v**	**14,95 %v/v**	**14,0 %v/v**	**13,9 %v/v**	**12,89 %v/v**	**12,30 %v/v**	**15,9 %v/v**	**14,60 %v/v**
Acetati	Hexyl acetate	4,3	3,8	21,4	8,8	5,1	12,6	13,6	14,5
	Isobutyl acetate	2,8	2,2	2,8	1,3	1,0	0,9	2,4	2,2
	Isoamyl acetate	79,4	71,5	339,6	107,6	83,3	204,0	161,9	147,1
	Beta-phenylethyl acetate	102,1	42,0	38,1	47,3	42,6	32,2	41,9	33,2
	Ethylphenyl acetate	7,3	5,4	2,4	4,9	4,8	2,2	4,5	4,5
	Somma Acetati	**196,0**	**124,9**	**404,3**	**169,8**	**136,7**	**251,9**	**224,4**	**201,5**
Esteri Etilici	Ethyl butyrate (C4 ethyl)	33,9	26,8	42,2	16,5	14,0	26,7	31,1	27,2
	Ethyl caproate (C6 ethyl)	327,7	294,6	493,0	240,9	222,1	342,0	350,3	320,7
	Ethyl caprylate (C8 ethyl)	1.400,1	862,2	2.163,6	1.018,3	1.345,8	1.639,5	1.070,3	945,9
	Ethyl Caprino (C10-ethyl)	783,5	343,3	742,8	731,6	946,4	513,0	537,5	431,2
	Ethyl-2-methyl-butanoate	6,6	5,9	1,7	5,6	5,7	1,2	6,1	5,2
	Ethyl-3-methyl-butanoate	10,5	9,1	2,9	6,8	6,0	2,1	8,9	7,8
	Somma Esteri etilici	**2.562,3**	**1.541,8**	**3.446,1**	**2.019,7**	**2.539,9**	**2.524,5**	**2.004,3**	**1.738,0**
Alcoli C6	Hexanol	696,4	619,3	247,7	360,6	323,4	214,5	856,0	672,7
	Trans-3-hexenol	9,7	7,6	4,3	9,0	7,1	3,4	8,8	6,7
	Cis-3-hexenol	20,0	12,7	11,0	10,7	10,3	7,5	16,9	11,8
	2-Hexen-1-ol	1,4	1,3	0,6	0,9	0,7	0,4	1,5	1,0
	Somma Alcoli C6	**727,5**	**640,9**	**263,6**	**381,2**	**341,5**	**225,8**	**883,2**	**692,2**

		Sfurzat		Sfurzat		Valpolicella		Chianti	
Classe composti	**Composti (SPME)**	**16,6 %v/v**	**14,95 %v/v**	**14,0 %v/v**	**13,9 %v/v**	**12,89 %v/v**	**12,30 %v/v**	**15,9 %v/v**	**14,60 %v/v**
Vari alcoli	Benzyl alcohol	49,8	37,6	13,7	57,4	55,6	11,5	33,1	22,8
	Beta-Phenyl Ethyl Alcohol	8.318,6	5.442,0	2.949,9	7.095,8	6.745,8	2.443,3	7.582,2	5.304,0
	3-Methyl-thio-propanol (Methionol)	36,8	28,5	28,6	64,0	69,2	23,7	42,2	25,1
	Somma alcoli	**8405,2**	**5508,1**	**2992,2**	**7.217,2**	**6870,6**	**2478,5**	**7657,5**	**5351,9**
Alcoli terpenici	Linalool	32,8	32,4	141,0	44,5	42,4	155,2	28,9	24,4
	HO-Trienolo	4,1	2,2	0,7	0,4	0,3	0,7	1,8	1,6
	Alpha-Terpineol	18,4	15,5	34,4	9,8	9,0	38,2	13,1	10,5
	Citronellol	11,9	10,3	20,2	21,7	20,9	21,4	12,3	10,7
	Nerol under	3,9	2,6	4,3	3,0	3,5	4,8	3,9	3,1
	Geraniol	3,8	2,8	7,6	6,6	5,8	8,9	3,4	2,6
	D-Limonene	9,2	3,5	20,8	3,8	3,1	14,7	7,1	3,6
	4-Terpineol	103,3	89,8	2,0	2,8	2,7	1,8	143,7	115,7
	Somma alcoli terpenici	**187,5**	**159,0**	**231,0**	**92,7**	**87,6**	**245,8**	**214,2**	**172,3**
Etilfenoli	4-Ethyl Phenol	18,6	14,1	3,8	19,8	59,8	24,3	13,3	10,8
	4 - Ethyl Guaiacol	4,7	3,6	1,0	5,9	17,5	21,1	5,7	4,6
Fenoli Varietali	Eugenol	7,2	5,0	3,6	1,5	1,4	3,8	3,3	3,0
	Guaiacol	4,8	3,5	1,3	0,9	0,8	1,2	3,3	2,3
	Ortho-Cresol	1,9	1,0	1,1	0,9	0,7	0,8	1,4	0,8
	Paracresol	2,7	1,5	1,0	0,8	1,0	0,7	1,9	1,5
	Phenol	7,6	4,7	7,9	3,4	2,9	6,6	6,8	4,7
	Somma fenoli varietali	**24,2**	**15,7**	**14,9**	**7,5**	**6,9**	**13,2**	**16,7**	**12,4**

In tabella n°2 si possono osservare le analisi aromatiche di varie esperienze di dealcolazione limitate al 2% vol, su tre vini rossi tipici Italiani.
Si può evidenziare una riduzione degli esteri etilici simile tra i vari vini, variabile tra il 60 e l'87%, con delle eccezioni nel caso del Chianti, probabilmente dovute ad una limitata fermentazione da *Brettanomyces* nel campione dealcolato, favorita dalla limitata concentrazione dell'anidride solforosa, dalla riduzione dell'effetto antisettico dell'alcol e dal possibile arieggiamento intervenuto nel corso del trattamento.
Per quanto riguarda l'intera somma degli acetati, molto importanti nel contribuire al profumo fruttato, sia il più rappresentativo, quale l'acetato di isoamile, si può evidenziare una riduzione di circa il 20%. Per il 2-feniletanolo e per l'alcol benzilico si può evidenziare una lieve riduzione, variabile dal 5 al 20%, un pò più alta per quanto riguarda il metionolo, ad eccezione del vino Valpolicella, con una possibile alterazione dovuta al metabolismo di *Brettanomyces*. Si può evidenziare una riduzione del 20% anche dei fenoli varietali, quali eugenolo, guaiacolo, p-cresolo e fenolo. Questo anche per i lattoni di quercia quando presenti.
Nel caso degli alcoli C6 si sono osservati dei cali di circa il 10%. I composti varietali subiscono una lieve diminuzione, variabile tra il 5 e il 10%, sia come concentrazione dei singoli composti, sia come somma. Questo anche per il 4-terpineolo, un terpene collegato all'intensità della disidratazione/appassimento dell'uva. Infine si può osservare il comportamento di alcuni norisoprenoidi che contribuiscono ai sentori floreali e fruttati dei vini giovani, come il β-damascenone e β-ionone; e di quelli che contribuiscono alla caratterizzazione dei vini invecchiati, come TDN (1,1,3-trimetil-1,2-diidronaftalene), vitispirani (VTP), actinidioli ed eteri etilici.
Le concentrazioni di questi ultimi sono abbastanza costanti, mentre per gli altri composti possono subire importanti diminuzioni, come nel caso del TDN (fino al 50%) e del VTP.

Moscato

Tab. 3: Analisi chimiche dei vini Moscato, T = Testimone, W -1,5 = delcolato a fine fermentazione di circa 1,5 % vol., W -2,5 = delcolato a fine fermentazione di circa 2,5 % vol., MF -1,5 = delcolato durante la fermentazione alcolica di circa 1,5 % vol., MF -2,5 = delcolato durante la fermentazione alcolica di circa 2,5 % vol. .

Determinazine analitica		**T**	**W -1,5**	**W – 2,5**	**MF – 2,5**	**MF -1,5**
Alcool	% vol.	12,40	11,00	9,70	9,90	10,70
Acidità titolabile	g/L	6,60	6,60	6,70	5,75	6,50
Acidità volatile	g/L	0,07	0,06	0,06	0,17	0,11
pH	unit	3,46	3,44	3,49	3,63	3,54
Estratto	g/L	23,5	23,9	23,2	23,3	24,6
SO2 libera	mg/L	11	7	19	3	16
SO2 totale	mg/L	69	63	77	48	61
Acido tartarico	g/L	1,82	1,79	1,90	1,39	1,29
Acetaldeide	g/L	35	34	36	61	69
Glicerolo	g/L	10,1	10,8	10,8	10,5	9,1
K	mg/L	638	664	653	632	626
Ca	mg/L	102	101	102	92	90
Mg	mg/L	65	66	68	63	62
Ceneri	g/L	1,89	1,8	1,81	1,93	2,17
TSS	°C	22,1	20,4	20,1	20,0	22,1

Sono state condotte diverse esperienze di dealcolazione fino a due gradi alcool.
Dal punto di vista chimico la diminuzione del grado alcolico corrisponde, come atteso, alla concentrazione di composti fissi quali glicerolo, potassio, magnesio, contribuendo ad un aumento degli estratti e delle ceneri. Nonostante l'aumento della concentrazione in potassio si può osservare come la diminuzione del tenore alcolico vada ad aumentare la stabilità tartarica, come dimostrato dalla diminuzione della temperatura di saturazione.

Tabella 4: Analisi SPME dei vini Moscato, T = Testimone, W -1,5 = delcolato a fine fermentazione di circa 1,5 % vol., W -2,5 = delcolato a fine fermentazione di circa 2,5 % vol., MF -1,5 = delcolato durante la fermentazione alcolica di circa 1,5 % vol., MF -2,5 = delcolato durante la fermentazione alcolica di circa 2,5 % vol. .

Classe composti	**Composti (SPME)**	**Test 12,5% vol**	**- 1,5% vol**		**- 2,5% vol**	
			MF	**W**	**MF**	**W**
Acetati	Hexyl acetate	241	134	200	101	161
	Isoamyl acetate	747	287	643	217	516
	Ethyl acetate	398	151	286	133	201
	Somma Acetati	**1386**	**572**	**1128**	**451**	**879**
Esteri Etilici	Ethyl butyrate (C4 ethyl)	56	26	45	21	36
	Ethyl caproate (C6 ethyl)	701	401	546	367	451
	Ethyl caprylate (C8 ethyl)	4762	1214	3486	1131	2816
	Ethyl Caprino (C10-ethyl)	4953	1661	3847	1297	2723
	Ethyl-2-methyl-butanoate	1	1	1	1	1
	Ethyl-3-methyl-butanoate	1	0	1	0	1
	Somma esteri etilici	**10475**	**3303**	**7925**	**2818**	**6027**
Alcoli C6	Hexanol	169	170	172	115	116
	Trans-3-hexenol	4	5	5	3	3
	Cis-3-hexenol	28	28	32	24	18
	Somma Alcoli C6	**201**	**202**	**210**	**142**	**137**
	Beta-Phenyl Ethyl Alcohol	12071	6475	11243	5577	6868

Classe composti	Composti (SPME)	Test 12,5% vol	- 1,5% vol	- 2,5% vol	Test 12,5% vol	- 1,5% vol
		2895	2786	2566	2201	1885
	HO-Trienolo	263	242	354	244	173
	Alpha-Terpineol	330	274	472	262	209
	Citronellol	26	41	23	45	16
	Nerol under	36	40	45	41	25
	Geraniol	209	76	166	73	85
	D-Limonene	155	77	173	64	57
	Somma Alcoli terpenici	**3914**	**3536**	**3800**	**2931**	**2450**
Ossidi Linalolo	Trans-linalool oxide	24	23	29	22	20
	Cis-Rose oxide	3	3	3	3	2
	Trans-Rose Oxide	1	0	1	0	0
	Somma ossidi linalolo	**28**	**27**	**33**	**25**	**22**
Esteri Terpenici	Linalyl Ethyl ether	479	294	535	237	160
	Neryl Ethyl ether	12	9	13	8	4
	Geranyl ethyl ether	259	174	276	150	15
	Alfa Terpenil Ethyl ether	12	13	14	12	10
	Somma esteri terpenici	**763**	**490**	**838**	**407**	**190**
Vinilfenoli	4-Vinyl-Guaiacol	41	27	28	29	26
	4 Vinyl Phenol	0	13	14	14	12
	Somma Vinilfenoli	**41**	**40**	**43**	**44**	**38**
	Benzaldehyde	12	5	34	4	19
Acidi Grassi	Butyric	16	8	18	8	13
	Caprylic acid (C 8)	2075	1143	2262	963	1716
	Somma acidi grassi	**2091**	**1152**	**2280**	**971**	**1729**
Norisoprenoidi	Beta Damascenone	19	28	23	27	12
	Beta-Ionone	0	0	0	0	0

In generale si può evidenziare una diminuzione dei composti aromatici di origine fermentativa, direttamente proporzionale al grado di dealcolazione del vino, che possono causare delle perdite del profumo fruttato dei vini. Il trattamento effettuato durante la fermentazione alcolica risulti più invasivo del trattamento di pari intensità effettuato a fine fermentazione. In particolare la diminuzione degli acetati vari dal 19% fino al 67%, con diminuzioni molto elevate a carico dei due composti più rappresentativi di questa classe, quali acetato di isoamile e acetato di etile. La diminuzione di concentrazione degli esteri etilici varia tra il 24% ed il 73%.

Come nelle analisi effettuate sui vini rossi, anche in questo caso si possono evidenziare delle lievi diminuzioni di composti aromatici varietali variabili tra il 5 ed il 26% per i dealcolati a fine fermentazione. I vini dealcolati in fermentazione, invece, subiscono delle diminuzioni fino al 40%.

Analisi sensoriale

L'analisi sensoriale condotta sui vini dopo la delacolazione rivela che il vino de alcolato a fine fermentazione di 1,5 % vol. risulta simile al vino testimone (dato confermato dal test triangolare). Risultano invece distinguibili il vino

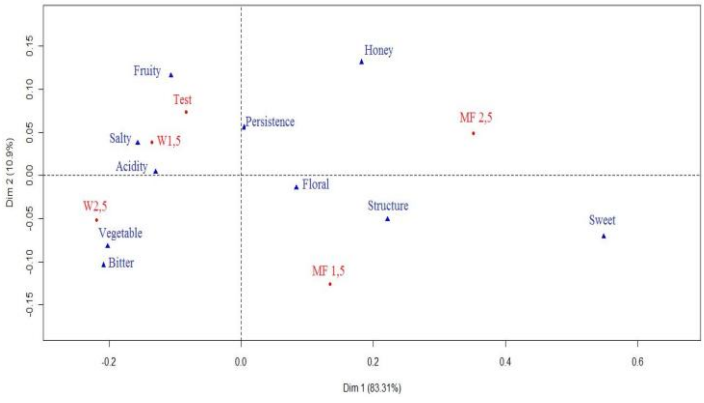

Figura 2: PCA moscato dealcolato durante e a fine fermentazione

dealcolato durante la fermentazione di 1,5 e 2,5 % vol. ed il vino de alcolato a fine fermentazione di 2,5 % vol. In particolare si può notare come il vino de alcolato durante la fermentazione di 2,5 % vol. risulta caratterizzato da una nota vegetale ed amara, mentre il vino de alcolato della stessa intensità però a fine fermentazione risulta caratterizzato da una nota più dolce.

CONCLUSIONI

Queste esperienze di delcolazione condotte indicano come il contattore risulti essere un sistema efficiente per il processo. Al momento risulta inoltre l'unico sistema che non prevede il frazionamento del vino. Le analisi condotte sui vini rivelano, come atteso, la concentrazione dei composti non volatili. Per quanto riguarda i composti aromatici il processo diretto comporta una discreta perdita della frazione aromatica, anche se questa viene difficilmente percepita a livello organolettico.

Indubbiamente sono ipotizzabili altre soluzioni impiantistiche rivolte al rispetto della frazione aromatica (Fig. 3 e Fig. 4)

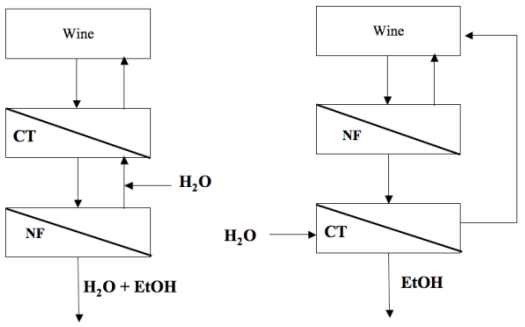

Fig. 3: Possibili schemi di dealcolazione.

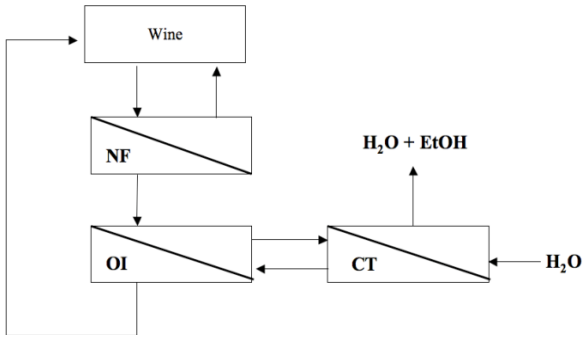

Fig. 4: Possibile schema di dealcolazione (Brevetto JUCLAS)

RINGRAZIAMENTI

Enologica Vason Srl – Verona (Italy)
JU.CLA. S. Srl – Verona (Italy)

BIBLIOGRAFIA.

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Bregoli M., Cervellati A., Ferrarini R., Leoni C., Zani A., Zardi G., Gostoli C. (2001) "Development and modelingof a plate & frame membrane contactor for juice and wine processing", oral comunication 3–6 June, Granada, Spain, pp. 259–263

Diban N., Athes S V., Bes. M., Souchon I. (2008) Ethanol and aroma compounds transfer study for partial dealcoholization of wine using membrane contactor", Journal of Membrane Sciente, 311, 136–146.

Ferrartini R., Bocca E., Gostoli C. (2008). "Membrane technique to guaranty the quality and the territorial identy", oral comunication, OIV 2008

Gomez-Plaza E., Gomez-Nicolas J.M., Lopez-Roca J.M., Martinez-Cutillaz A. (1999)"Dealcoholization of Wine. Behaviour of the Aroma Components during the Process", Lebensm.-Wiss. u.-Technol., 32, 384 } 386.

Gostoli C. (1999). "Thermal effects in osmotic distillation", J.Membrane Sci. 163/1, 75-91.

Kunz W., Benhabiles A., Ben-Aim R. (1996). "Osmotic evaporation through macroporous hydrophobic membranes: a survey of current research and applications", J. Membrane Sci., 121, 25-36.

Meillon S., Urbano C., Guillot G., Jacquet N., Schlin P. (2009). "A methodological approach to simultaneously measure the relative sensory and information impact of alcohol reduction on the overall liking of wines in real life settings", 8th Pangborn Sensory Science Symposium, Florence, 26-30th July 2009.

Evaluation de la typicité des vins liés au terroir : proposition de méthodes pour les professionnels de la filière

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Mots Clés : typicité, caractérisation sensorielle, expert, consensus

Résumé

La définition de la typicité (Casabianca et al, 2006) repose sur l'existence de propriétés d'appartenance à un type distingué et identifié par un groupe humain de références. Partant de cette définition, la mise au point de méthodes d'évaluation de la typicité doit être basée sur l'accord entre l'avis de différents experts. L'utilisation d'une question simple (adaptée de Ballester et al, 2007) « pour vous, ce vin est-il un bon exemple de l'appellation X » et l'analyse du consensus entre les juges ont été étudiées sur deux espaces produits différents : l'un composé de vins d'Anjou rouge et d'Anjou rouge Village Brissac et l'autre composé devins blancs de Savennières, Anjou Blanc et Vouvray. Les professionnels interrogés devaient donner une note à chaque vin pour évaluer s'il était un bon exemple respectivement d'Anjou Villages Brissac ou de Savennières. En fonction de l'espace étudié et du groupe de dégustateurs, le consensus observé est plus ou moins important. Dans le cas des Savennières, le consensus n'était pas satisfaisant. Cependant, dans le cas des Anjou Village Brissac, les juges étaient en accord et distinguaient assez nettement les deux types de vins. Par ailleurs, le croisement de cette analyse avec une caractérisation sensorielle a permis de mettre en évidence les caractéristiques sensorielles distinguant les deux familles de vins. Cette question est donc pertinente pour évaluer la typicité sensorielle des vins. L'espace produit étudié et le groupe d'experts, doivent cependant être correctement définis en amont de l'évaluation.

Cette méthode simple peut permettre de donner des éléments de réponse à la filière viticole en quête d'outils méthodologiques pour la définition et l'évaluation de la typicité de ces vins.

Introduction

D'après le proposition de définition (Casabianca et al., 2005), la typicité repose sur des propriétés à la fois d'appartenance et de distinction. Elle serait également une construction sociale, nécessitant un consensus au sein d'un groupe humain de référence, afin de repérer les caractéristiques constitutives de la typicité, d'orienter la production en fonction de ces choix, puis d'évaluer ces caractéristiques afin de juger de la typicité sensorielle ou non des produits. La vérification de l'existence d'un espace sensoriel propre à la catégorie, donc d'une typicité, est sans doute la condition nécessaire à la caractérisation des propriétés d'appartenance et de distinction de cette catégorie.

La méthodologie proposée par Ballester (2004) et développée dans le cadre de la typicité sensorielle des vins d'AOC au sein des travaux de l'UMT Vinitera (Perrin, 2008; Cadot, 2009) permet de vérifier l'existence d'un consensus au sein des experts locaux, preuve de l'existence d'un concept commun, donc d'une typicité. En intégrant des vins de catégories voisines, il est également possible de vérifier que les vins de la catégorie étudiée sont globalement plus représentatifs de la catégorie en question que les vins de catégories voisines. Cette étape constitue une étape préalable à la caractérisation des spécificités d'une catégorie donnée.

Derrière les groupes humains de référence se retrouvent les producteurs, les metteurs en marché et éventuellement des consommateurs avertis. Cependant, on peut s'interroger sur l'existence d'un consensus entre et au sein de ces différentes populations. L'historique, l'implication d'un individu au sein d'une appellation donnée peut le conduire à une perception différente de la typicité des vins.
Les travaux réalisés dans cette thématique ont pour objet d'étudier la perception la typicité d'appellation donnée par des différents groupes de référence et d'analyser le consensus entre les dégustateurs pour ensuite essayer de comprendre l'origine des divergences d'appréciation de la typicité.

Matériels et méthodes

Les vins

Une expérimentation s'intéressant à la perception de la typicité par différentes populations a été réalisée. Douze vins ont été sélectionnés issues de travaux de l'action 2.2 mené par Yves Cadot. Parmi ces vins, six proviennent de l'AOC Anjou Village Brissac, (AVB2, AVB3, AVB8, AVB10, AVB13, AVB14), 3 de l'AOC Anjou Rouge (AR4, AR5 et AR7), un vin de AOC Saumur (OUT7), un vin de l'AOC Chinon (OUT5) et un Vin de l'AOC Bourgueil (OUT1).

Les groupes humains de référence

Onze professionnels producteurs d'Anjou Villages Brissac ont participé à cette étude. Par ailleurs, 9 cavistes recrutés dans la région angevine et commercialisant des Anjou Villages Brissac ont pris part à cette dégustation et représentent les metteurs en marché et prescripteurs du produit. Enfin, 28 consommateurs avertis ont été également interrogés. Idéalement, nous aurions souhaité recruter des consommateurs avec un lien fort avec l'AOC Anjou Village Brissac, mais suite aux difficultés de recrutement, nous avons interrogé des membres de groupes amateurs d'œnologie de la région angevine.

La dégustation et le protocole

Les dégustations ont pour la plupart eu lieu dans une salle de dégustation permettant de meilleures conditions de dégustation. Cependant afin de toucher un maximum de professionnels, certaines dégustations ont eu lieu directement chez eux. Dans tous les cas, les dégustateurs n'avaient pas connaissance des vins qu'ils allaient déguster et les bouteilles étaient masquées. Les cavistes et les consommateurs ont par ailleurs rempli un questionnaire pour connaitre leur familiarité avec le monde du vin, la dégustation et l'AOC Anjou Village Brissac.

Les dégustateurs devaient répondre à la question simple « Considérez vous que ce vin est un bon ou un mauvais exemple d'Anjou Village Brissac » sur une échelle à 11 point de Mauvais Exemple à Bon Exemple. Les vins étaient servis dans un ordre suivant un carré latin de William.

Résultats

Les caractéristiques des vins

Un panel qualifié a caractérisé les 12 vins de l'étude et permis d'observer les différences entre les produits. Les vins AVB 2, 8, 14 et 13 se distinguent des autres par une astringence plus marquée, des notes de fruits noirs et le vin AVB13 se distinguent pour une note animale un peu plus soutenue. Le vin OUT5 révèle des notes boisées. Le vin AVB3 contient des notes de fruits rouges, il est peu astringent avec une couleur plus rosée.

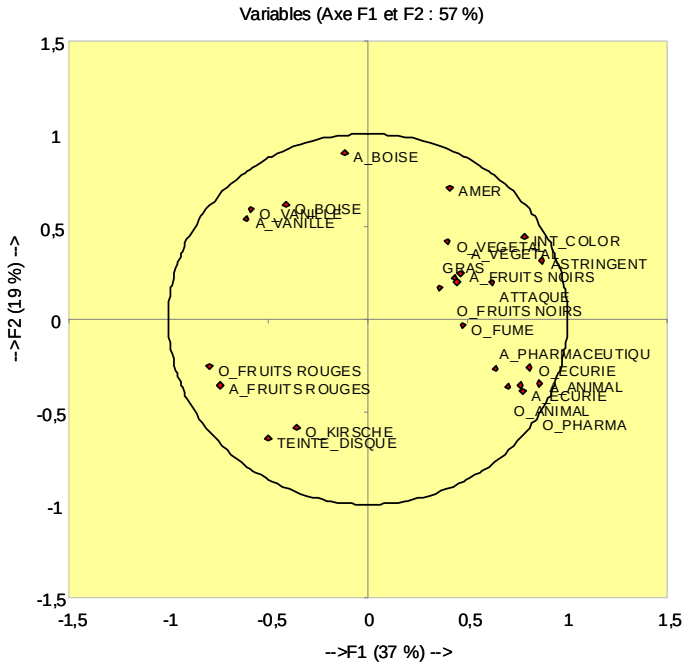
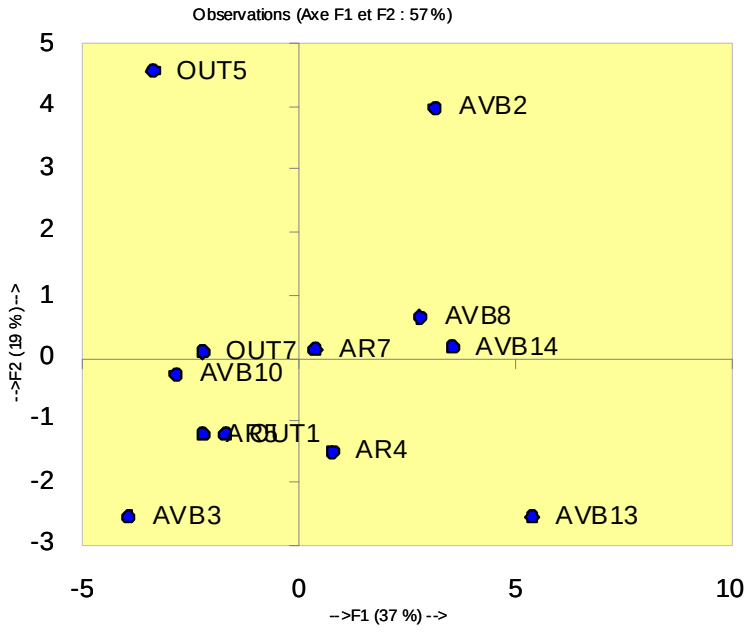


Figure 1 : ACP sur les données du panel qualifié ESA



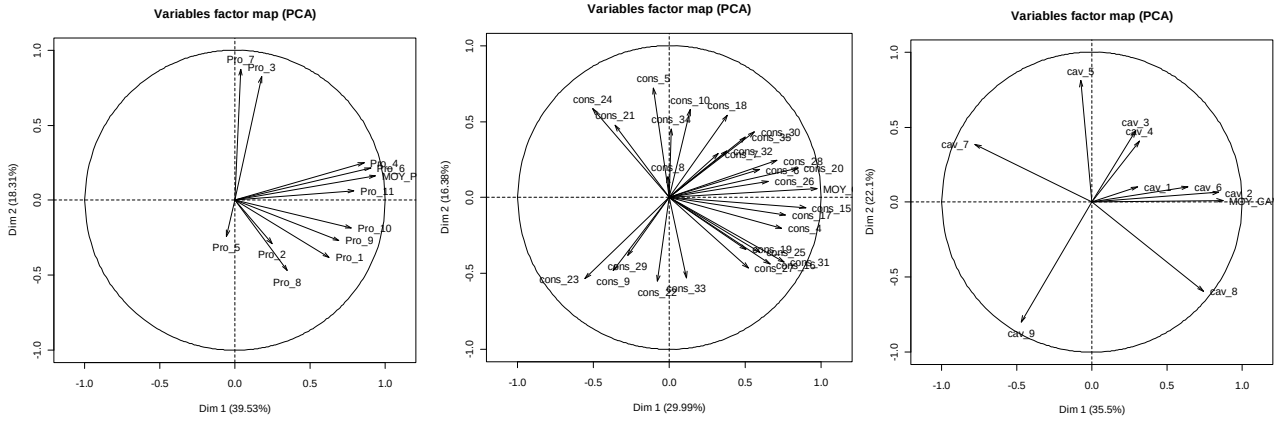
L’évaluation de la typicité par les 3 groupes de dégustateurs

Les professionnels d’AVB distinguent certains vins (p = 0.006) et notamment le AVB3 qu’ils identifient nettement comme étant un mauvais exemple de Anjou Village Brissac. Avec une moyenne d’amplitude des notes de 6,25, ils démontrent qu’ils ont fait des différences importantes entre les vins par ailleurs.

L’analyse de variance réalisée sur les 9 cavistes n’est pas significative laissant entendre qu’ils n’ont pas fait de différence entre la typicité des vins. Cependant, la moyenne des amplitudes de leurs notes est de 6,74. Il semble donc que chaque caviste a bien identifié de bons et de mauvais exemples d’Anjou Village Brissac mais qu’ils ne sont pas d’accord sur ce qui est un bon exemple ou un mauvais exemple.

Pour les consommateurs avertis, l’analyse de variance est significative (p=0,0001). L’amplitude des notes est de 6,94 donc les consommateurs ont également mis en évidence des différences de typicité entre les vins. Les AVB 8,10 et 2 ainsi que l’AR 7 sont de bons exemples d’Anjou Village Brissac. A l’inverse, l’AR 5 n’est pas un bon exemple.

Figure 2 : ACP sur les données brutes par catégorie (Viticulteur - Conso - Caviste)



Les analyses en composantes principales réalisées sur les données brutes par catégories de consommateurs permettent de visualiser l’accord entre les dégustateurs. Il apparait un assez bon consensus entre les professionnels de l’appellation avec cependant quelques divergences d’opinion. Une majorité de consommateurs semblent en accord entre eux mais avec cependant un certain nombre d’autres ayant des avis divergents. Enfin, chez les cavistes,

malgré quelques accords entres certains juges, il apparait des oppositions très marquées.

Ces différences de perception au sein de chaque catégorie amène à vérifier si il existe des groupes de dégustateurs quelque soit leur catégorie qui ont des perceptions similaires de la typicité des Anjou Village Brissac. Pour se faire, une Classification Ascendant Hiérarchique a été réalisée en regroupant les 48 dégustateurs après normalisation de leurs notes par juge. Cette CAH conduit

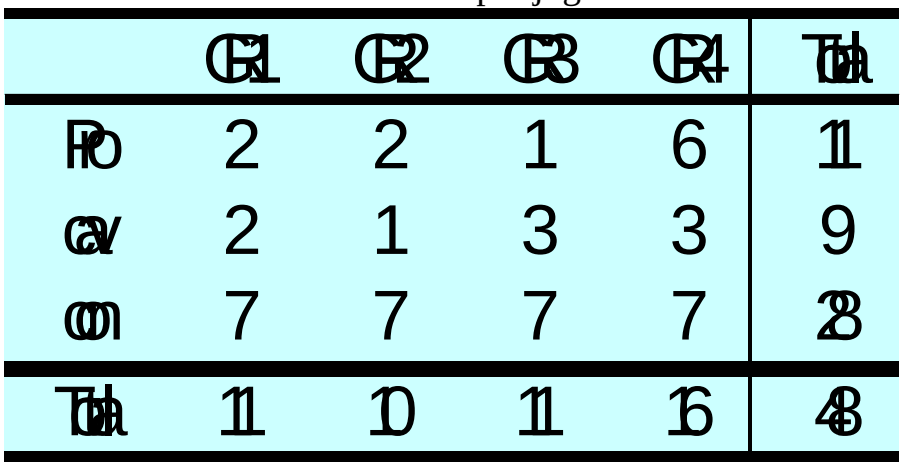


Figure 3 : Répartition des catégories par groupe

à la formation de 4 groupes avec des perceptions différentes. Ces groupes sont composés à la fois de consommateurs, de cavistes et de professionnels et l'analyse du Khi2 indique qu'aucune catégorie n'est sur ou sous représentée dans ces différents groupes.
Des différences d'appréciations entre les différents groupes apparaissent. Le Groupe 1 jugent que le produits AVB8 et OUT1 sont les meilleurs exemples et AVB14 et 2 et OUT5 sont de mauvais exemples. Pour la classe 2, AVB 13

Classe	Classe 1	Classe 2	Classe 3	Classe 4
AVB13	0,42	-1,16	-0,58	1,05
AVB8	0,74	-0,60	0,87	0,37
AR4	0,37	-0,54	0,54	-0,72
OUT1	0,85	-0,26	-0,56	-0,52
AR5	-0,05	-0,07	-1,05	-0,51
AVB14	-0,71	-0,01	0,38	0,35
AR7	-0,31	0,02	0,97	0,36
AVB3	-0,07	0,06	-0,65	-0,88
OUT7	-0,30	0,45	-0,45	-0,43
AVB10	0,24	0,52	0,22	-0,15
AVB2	-0,70	0,59	0,79	0,36
OUT5	-0,49	1,00	-0,46	0,72

Figure 4 : Appréciation moyenne des vins par groupe (sur la base des données normées)

et 8 ainsi que AR4 ne sont pas typiques des AVB et contrairement au groupe précédent, ces consommateurs jugent AVB10, AVB 2 et OUT5 plus typiques. Pour la classe 3, les meilleurs exemples sont

AVB8 et AVB2 ainsi que AR7, ils rejettent AR5 et AVB3. Enfin le groupe 4 juge AVB13 et OUT5 plus typiques et trouve AR5 et AVB3 moins typiques. Malgré ces divergences d'appréciations, quelques éléments communs apparaissent : Le vin AVB3 est perçu comme un mauvais exemple ou un exemple moyen. AVB2 est plutôt un bon exemple, même si un groupe n'est pas d'accord.

Il ressort donc une complexité dans la perception de la typicité des vins par les différentes catégories et qu'à l'intérieur de chacune d'entre elles, les différents dégustateurs ne sont pas d'accord entre eux. La mise en relation avec les données sensorielles fait apparaître que pour chaque classe ainsi constituée, les caractéristiques de l'appartenance ou de la non-appartenance aux Anjou Village Brissac diffèrent.

Du fait du nombre de consommateurs, de la présence des 4 groupes, les essais de croisement entre les caractéristiques des consommateurs et leur appartenance à l'un des groupes n'est pas significatif et ne permet pas de mettre en exergue un lien entre la perception de la typicité et le type de consommateurs.

A la fin de la dégustation, il était proposé aux cavistes et aux consommateurs de décrire en quelques mots ce qui fait la typicité des Anjou Villages Brissac. Une analyse textuelle a permis de faire ressortir que la notion d'astringence et de tanins (citée par 48,6% des dégustateurs), la couleur foncée (40.5%), les fruits rouges (29.7%), les arômes de sous-bois pour 24% et la longueur en bouche (18,9%) sont les critères les plus cités par les dégustateurs.

Conclusion

Une analyse plus approfondie devra essayer de faire le lien entre ces commentaires individuels et l'appartenance à telle ou telle classe. Les dégustateurs d'une classe ont-ils une vision similaire de ce qui constitue la typicité des vins quand il la décrive par écrit ? La limite de cette approche sera à nouveau dans le nombre de consommateurs disponibles pour avoir des conclusions robustes.

Apport des méthodes spontanées pour l'analyse sensorielle des vins

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Mots clés : Analyse sensorielle, Profil libre, Napping, Profil conventionnel, experts

Abstract

In oenology, sensory characterisation of wines is more and more used. To obtain a characterisation of the products, the sensory analyst generally uses the competences of a trained panel that carries out conventional profiling. This method requires a long period to train judges, that generally takes over several months. In the particular case of wine, professionals are not trained together and their performance is not checked. They are however used to taste wines and to describe them. Unlike conventional profiling, spontaneous methods such as Free choice profiling or Napping do not require common preliminary training of judges and give a great freedom to the taster, particularly in the choice of the descriptors. These methods thus appear to be adapted to juries of professionals. The purpose of this study was to evaluate the interest of Free profiling and Napping carried out by professionals (winemakers, winegrowers, and technicians) compared with a conventional profiling carried out by a trained panel. Case was applied to 10 white wines of the Loire Valley. Results show that free profiling and conventional profiling are very close in terms of characterisation. Data from conventional profiling is easier to interpret but free profiling is more adapted to professionals. The Napping method is also adapted to professionals but it provides a slightly different representation of the wines because of its decisional character: only the most important criteria for the judges arise.

Introduction

En œnologie, la caractérisation sensorielle des vins est de plus en plus utilisée, pour définir l'impact de différents itinéraires techniques, pour sélectionner la souche de levure la plus adaptée (Gerland et Dumont, 2000), ou pour mettre en évidence des différences sensorielles entre plusieurs terroirs (Fischer *et al.*, 1999).

En agro-alimentaire, la référence d'usage en terme de caractérisation sensorielle est le profil conventionnel (Lawless et Heymann, 1998). Cette méthode fait appel aux compétences d'un panel de juges entraînés. L'objectif de l'entraînement est de générer une expertise commune entre les juges du panel et de vérifier leur performance sensorielle. La particularité de cette approche réside dans l'absence de tout jugement qualitatif des vins, l'absence de descripteurs «subjectifs» (harmonie, équilibre, qualité…) pour lesquels l'expérience technique, l'origine du dégustateur va

conditionner la réponse et peut empêcher une évaluation strictement objective des caractéristiques des vins. La phase de formation des juges (entraînement et mise en commun du vocabulaire) s'étale généralement sur plusieurs mois.

Dans le cas particulier du vin, les professionnels ne sont pas été entraînés ensemble et leur performance n'est pas vérifiée mais ils ont l'habitude de déguster les vins et de les décrire. Chaque professionnel possède une expertise propre mais celle-ci s'accompagne généralement d'un vocabulaire qui lui est spécifique et qui correspond plus ou moins aux notions des autres dégustateurs. Certains sont très attentifs à certaines caractéristiques ou qualités du vin quand d'autres vont s'arrêter sur d'autres éléments. Les méthodes dites «spontanées» comme le Profil libre choix (Williams et Langron, 1984, Gerland et Dumont, 2000), le Profil Flash (Dairou and Sieffermann 2002, Delarue and Sieffermann 2004), ou le Napping (Pagès, 2003 et 2005) partent de cette hypothèse et utilisent la complémentarité entre les dégustateurs en leur offrant une liberté totale dans la caractérisation des vins. Elles ne nécessitent pas de mise en commun préalable et laissent une grande liberté au dégustateur, notamment dans le choix des critères. De plus, les traitements statistiques associés comme l'Analyse Factorielle Multiple (Escofier et Pagès, 1998) prennent en compte les différences interindividuelles et chaque juge a un poids équivalent dans la configuration finale.

L'objectif de ce travail est donc d'évaluer l'intérêt de descriptions professionnelles par rapport à la démarche classique utilisée en analyse sensorielle (Lawless et Heymann, 1998). Cette étude compare un Profil libre et un Napping réalisés par des professionnels (œnologues, viticulteurs, techniciens) avec un profil conventionnel réalisé par un panel entraîné, sur 10 vins blancs du Val de Loire.

2. Matériel et méthodes

2.1 Les produits

L'espace produit se compose de dix vins blancs secs et demi-secs du Val de Loire, de cépage Chenin : un Vin de Pays (VDP), trois Anjou blanc (ANJ_A, ANJ_B et ANJ_C), quatre Saumur blanc (SAU_A, SAU_B, SAU_C et SAU_D) ainsi que deux Savennières (SAV_A et SAV_B). Ces vins ont été choisis par des professionnels et représentent a priori la diversité des types de vins blancs pour ces appellations.

2.2 Recueil des données

2.2.1 Le Profil conventionnel

Le profil conventionnel est réalisé par le panel entraîné du laboratoire GRAPPE (ESA), composé de 17 juges. Les descripteurs sont générés par le panel sur l'espace produit étudié (les dix vins de l'étude). La réduction du nombre de termes se fait par consensus (Lawless et Heymann, 1998) : seuls les descripteurs pour lesquels la majorité des panélistes sont d'accord sont conservés. Une fois

la liste établie, chaque juge attribue une note pour chacun des vins et pour chaque descripteur de la liste commune. Une validation ayant été effectuée (Jourjon *et al.*, 2005), les juges travaillent sur des échelles linéaires non structurées. Les vins sont présentés de façon monadique et selon un ordre basé sur un carré latin de Williams.

2.2.2 Le Profil libre

Le profil libre est réalisé par douze professionnels de la région Val de Loire (œnologues, viticulteurs ou techniciens) au cours d'une seule séance. Les vins sont présentés de façon simultanée. On demande à chaque juge de goûter l'ensemble des produits et de générer des descripteurs lui permettant de discriminer les produits. Ainsi, chaque juge établit sa propre liste de descripteurs. On présente alors de nouveau aux juges la série de produits. Afin d'équilibrer les effets d'ordre et de report, les vins sont présentés de façon monadique et selon des ordres s'appuyant sur un carré latin de Williams. Les juges attribuent une note à chaque produit pour chacun des descripteurs générés précédemment, sur des échelles linéaires non structurées.

2.2.3 Le Napping.

Le Napping est une méthode récente, mise au point par Pagès, 2003 et 2005. Ce sont les mêmes douze professionnels que précédemment, qui réalisent le Napping au cours d'une autre séance. Les vins sont présentés de façon simultanée. Les juges doivent positionner les produits sur une nappe de papier en fonction de leur ressemblance ou dissemblance. Plus les vins sont proches entre eux et plus ils devront être placés près les uns des autres. A l'inverse, des vins jugés comme étant très différents d'un point de vue sensoriel devront être éloignés sur la nappe. Chaque dégustateur base son jugement sur ses propres critères. Une fois les vins positionnés sur la nappe et les codes des produits recopiés, on demande aux juges d'écrire, à coté des vins, des termes qui lui semblent bien caractériser le vin (ou groupe de vins).

2.3 Traitement des données

Chaque groupe de données a été analysé séparément. Les données du profil ont été traitées de manière classique par Analyse en Composantes Principales (ACP). Les données du profil libre et du Napping® ont été traitées par Analyses Factorielles Multiples (AFM) (Escofier et Pagès, 1998). Cet outil statistique permet d'équilibrer le rôle de chaque juge dans l'analyse et d'obtenir une configuration moyenne des produits.

3. Résultats et discussion

Avec chacune des méthodes testées, les résultats obtenus permettent une caractérisation sensorielle des vins de l'étude. Les outils statistiques multidimensionnels conduisent à des représentations graphiques, ou cartes sensorielles, sur lesquelles les vins et les descripteurs les caractérisant sont projetés. Ces outils permettent de synthétiser les principales différences entre les produits. Dans le cas du profil libre et du Napping, du fait du grand nombre de descripteurs (respectivement, 228 et

156 descripteurs), les cercles des corrélations pour ces deux méthodes ne sont pas présentés. Néanmoins, les valeurs des corrélations entre chacun des descripteurs et les axes ont été étudiés et permettent d'identifier les descripteurs associés aux vins extrêmes.

Les figures correspondant aux trois méthodes montrent l'opposition entre les vins SAV_B et SAU_D et les autres vins de l'étude. Ces deux vins sont sensoriellement proches, ils ont une couleur plus soutenue, ils sont boisés, lactés, empyreumatiques, ils présentent des notes d'épices, de fruits exotiques et de fruits secs et ils sont plus «alcooleux», plus ronds. Les professionnels complètent cette description en soulignant la complexité de ces vins, leur maturité. Cette complexité peut être associée à un nombre important de descripteurs olfactifs et aromatiques utilisés par le panel expert en analyse sensorielle pour les caractériser.

L'ANJ_B est également un vin qui se distingue nettement dans les différentes dégustations. Il se distingue par des notes chimiques, pharmaceutique et certains professionnels l'identifient comme étant un vin présentant un défaut.

Avec les profils sensoriels du panel entraîné et les profils libres des professionnels, les caractérisations de ANJ_C et SAU_A sont également assez proches : ce sont tous deux des vins perlants avec un peu de gaz, de CO2. Les professionnels ajoutent qu'ils sont vifs et le panel entraîné identifie des notes d'agrumes pour ces deux vins. Au niveau du Napping®, ces deux vins se distinguent très peu des autres. Le côté perlant de ces vins n'a pas été mis en évidence. Pourtant, les résultats du profil libre montrent que les professionnels sont capables d'identifier cet aspect. La différence est donc attribuable à la méthode et non à une moindre sensibilité du jury.

Les sucres résiduels présents dans VDP et ANJ_A sont perçus par tous les dégustateurs et apparaissent sur une troisième dimension sensorielle (carte non présentée ici), quelle que soit la méthode.

Avec le profil libre et la méthode du Napping®, il est difficile d'obtenir une caractérisation précise des produits. En revanche, le profil conventionnel réalisé par le panel entraîné en analyse sensorielle autorise la réalisation d'analyses statistiques dites «inférentielles» qui permettent une caractérisation plus précise. Ainsi, grâce aux analyses de la variance (ANOVA) et aux tests de comparaison de moyennes réalisés descripteur par descripteur, il est possible mettre en évidence des nuances entre les vins jugés globalement proches par les méthodes précédentes. Ainsi, SAV_B est perçu un peu plus sucré et légèrement moins perlant que SAU_D. SAU_A de distingue un peu d'ANJ_C par une acidité et des notes minérales plus marquées. ANJ_A et VDP se rapprochent par leur sucré, leurs notes de fruits blancs, leur faible acidité, leur faible amertume, leur attaque peu agressive et leur faible arôme minéral. Mais VDP se distingue par sa rondeur, ses notes de miel et lactées qu'ANJ_A ne présente pas.

4. Conclusion

Le profil conventionnel est une méthode lourde qui nécessite un temps important pour la réalisation d'une étude. Il est basé sur la caractérisation par des descripteurs objectifs, non qualitatifs. Le profil libre et le Napping® sont des méthodes très rapides et sont donc plus adaptées à la disponibilité des professionnels. Elles permettent l'utilisation du vocabulaire propre à chaque dégustateur, sans limitation. Cette spontanéité couplée à la prise en compte des avis individuels dans le traitement des données permet de ne pas oublier de dimensions sensorielles. Malgré l'intérêt évident de ces deux méthodes, leur limite se situe au niveau de la précision des caractérisations obtenues. Les méthodes spontanées sont des méthodes assez fastidieuses à analyser du fait de la diversité du vocabulaire employé par les dégustateurs. Avec le profil libre, les vins sont décrits par un total de 228 variables et les mots associés au Napping® sont au nombre de 156. Les résultats montrent que ces méthodes permettent une caractérisation globale des vins mais qu'il est difficile de zoomer et d'identifier les nuances entre les vins. A *contrario*, les données du profil conventionnel sont exploitables par des techniques statistiques inférentielles et offrent des caractérisations précises. Enfin, l'approche globale utilisée dans le Napping® permet à chaque juge de construire sa représentation en fonction des dimensions sensorielles qu'il juge importantes. Le fait que les dégustateurs n'aient pas fait ressortir le perlant avec cette méthode semble témoigner de la possibilité de hiérarchiser les critères sensoriels. Le Napping® pourrait ainsi servir à mesurer l'influence de différents éléments techniques (effet du millésime, du cépage, du producteur, etc.) les uns par rapport aux autres.

A l'issue de cette première expérimentation, il est possible de distinguer deux types d'approches nécessitant deux méthodes. Dans le cas, d'une caractérisation globale d'un nombre important de produits pour lesquels on veut identifier les principales caractéristiques sensorielles et regrouper des produits proches sensoriellement, les méthodes spontanées semblent très intéressantes et bien adaptées au milieu professionnel. Cependant, quand une caractérisation plus fine est attendue, notamment suite à un protocole expérimental ou dans une recherche de lien entre analyses physico-chimiques et sensorielles, un profil conventionnel complet serait à privilégier.

Bibliographie

Dairou, V., & Sieffermann, J.-M., 2002. A comparison of 14 jams characterized by Conventional Profile and a quick original method, the Flash Profile. *Journal of Food Science*, **67** (2), 826-834.

Delarue, J., & Sieffermann, J.-M., 2004. Sensory mapping using Flash profile. Comparison with a conventional descriptive method for the evaluation of the flavour of fruit dairy products. *Food Quality and Preference*, **15** (4), 383-392.

Escofier, B. & Pagès, J., 1998. Analyse factorielles simples et multiples. Dunod.

Fischer, U., Roth, & D., Christmann, M., 1999. The impact of geographic origin, vintage and wine estate on sensory properties of *Vitis vinifera* cv. Riesling wines. *Food Quality and Preference*, **10**, 281-288.

Gerland C. & Dumont Ann, 2000. Analyse sensorielle descriptive libre choix (F.C.P.) : utilisation pratique. *Revue Française d'œnologie*, **182**, 36-39.

Jourjon, F., R. Symoneaux, C. Thibault & M. Réveillère. 2005. Comparaison d'échelles de notation utilisées lors de l'évaluation sensorielle de vins. *J. Int. Sci. Vigne Vin*. **39**, 23-29.

Lawless, H. T. & Heymann, Hildegarde, 1998. Sensory evaluation of food - Principles and practices. Kluwer Academic/Plenum Publishers. New York. pp 362-378.

Pagès, J., 2003. Recueil direct de distances sensorielles : application à l'évaluation de dix vins blancs du Val-de-Loire. *Sciences des Aliments*, **23**, 679-688.

Pagès, J., 2005. Collection and analysis of perceived product inter-distances using multiple factor analysis: application to the study of 10 white wines from the Loire Valley. *Food Quality and Preference*, **16** (7), 642-649.

Perrin, L., Jourjon, F., Symoneaux, R., Maître, I., Asselin, C. & Pagès, J., 2006b. Comparison between a conventional profiling and a free profiling carried out by professionals, using Multiple Factorial Analysis. *In* 8th Sensometrics Meeting – Imagine the senses, 2 - 4 August 2006, Ås, Norway (Communication orale)

Perrin, L., Maître, I., Symoneaux, R., Jourjon, F., Asselin & C., Pagès, J., 2006c. Comparison of three sensory methods to explicit Napping®: Case of ten wines from Chenin grape variety. *In* A Sense of Diversity – Second European Conference on Sensory Consumer Science of Food and Beverage, 26-29 september 2006, The Hague, Netherlands (Poster).

Williams, A.A. & Langron, S.P., 1984. The use of free-choice profiling for the evaluation of commercial ports. *J. Sci. Food Agric*. **35,** 558-568.

Figures

Fig. 1 : représentation des vins issue du profil conventionnel (ACP).

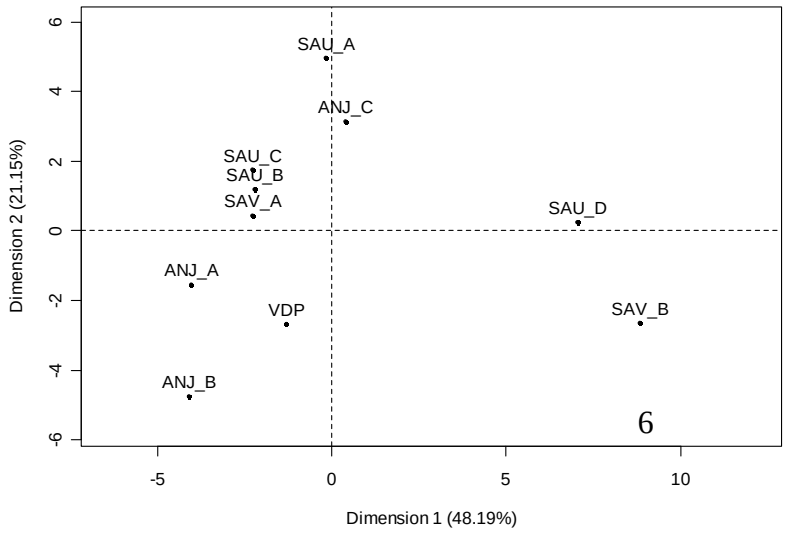


Fig. 2 : représentation des vins issue du profil libre (AFM)

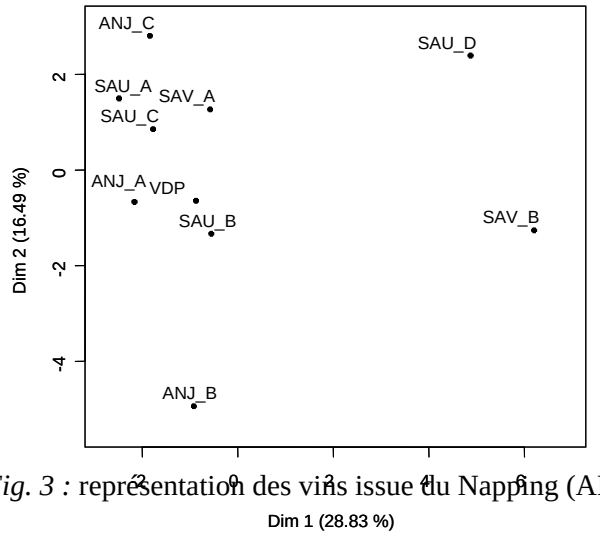
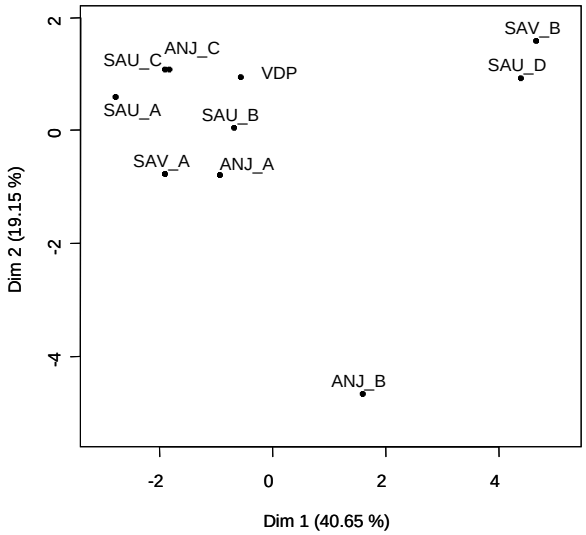


Fig. 3 : représentation des vins issue du Napping (AFM).



FRACCIONAMIENTO ISOTOPICO DEL AGUA DEL VINO DESALCOHOLIZADO MEDIANTE OSMOSIS INVERSA EN LA FASE DE SEPARACION DEL ALCOHOL A TRAVES DE UN SISTEMA DE MEMBRANA PEREXTRACTIVA

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RESUMEN

Se llevaron a cabo pruebas a nivel industrial de un equipo que se utiliza para la desalcoholización parcial de vinos. Este equipo posee una membrana perextractiva hidrofóbica para la separación del alcohol de la mezcla hidroalcohólica extraída por ósmosis inversa del vino. De un lado de la membrana se hizo circular la mezcla hidroalcohólica, y del otro lado, agua de arrastre de origen mineral. Luego de un estudio de la composición del vino tratado, por espectrometría de masas se encontró una alteración de la relación isotópica de $^{18}O/^{16}O$. Se concluyó que este tratamiento afecta la genuinidad del vino. Las mismas pruebas se repitieron a escala laboratorio, encontrándose resultados similares.

ABSTRACT

Trials were carry out on industrial-scale of a equipment that is used for the partial desalcoholization of wine. This equipment has a perstractive hydrophobic membrane for separating alcohol from the hidroalcoholic mixture extracted by reverse osmosis from wine. On one side of the membrane was circulated hidroalcoholic mixture, and on the other side, strip water from mineral source. After a study of the composition of the wine treated, by mass spectrometry, was found an alteration of isotope ratio $^{18}O/^{16}O$. The conclution was that this treatment affects the genuineness of the wine. The same trialas were repeated on a laboratory-scale, were found similar results.

INTRODUCCION

Debido al aumento de la graduación alcohólica que se ha producido en los últimos años en los vinos elaborados, en especial en el hemisferio sur, consecuencia del aumento de las temperaturas medias y escasas precipitaciones, sumado al concepto de madurez manejado hoy por lo técnicos, basado en la componente polifenólica de la uva, y considerando ~~a~~ la tendencia del consumo de vinos con baja graduación alcohólica, han surgido diferentes tecnologías que han logrado reducir el grado alcohólico de los vinos. Se estima que estos sistemas no deberían modificar la autenticidad natural de los mismos.

Tomando en consideración que La Organización Internacional de la Viña y el Vino (O.I.V.) en el Código Internacional de Prácticas Enológicas (OENO 10/04 II-3.5-17), establece que el procedimiento para eliminar parte del etanol, puede ser alcanzado mediante técnicas denominadas sustractivas, se llevó a cabo el ensayo que se presenta en este trabajo.

El proceso de desalcoholización empleado en este estudio puede verse en la Fig. 1. Del tanque contenedor de vino sale una corriente hacia el equipo de ósmosis inversa (O.I.) donde se extrae una mezcla hidroalcohólica (permeato), los componentes que no atraviesan el filtro

de O.I. retornan al tanque, mientras que el permeato es enviado hacia un equipo llamado contactor desalcoholizador, que posee en su interior una membrana perextractiva hidrofóbica que separa el alcohol por un gradiente de concentración de alcohol a través de esta membrana. Como resultado de ello, una pequeña cantidad de etanol se vaporiza en la estructura de la membrana desde la solución de alta concentración a la de baja concentración (lado del agua de arrastre). En el proceso, el permeato de la O.I. es reducido en concentración de alcohol y la corriente de agua aumenta su concentración en alcohol.

El permeato desalcoholizado también vuelve al tanque de vino.

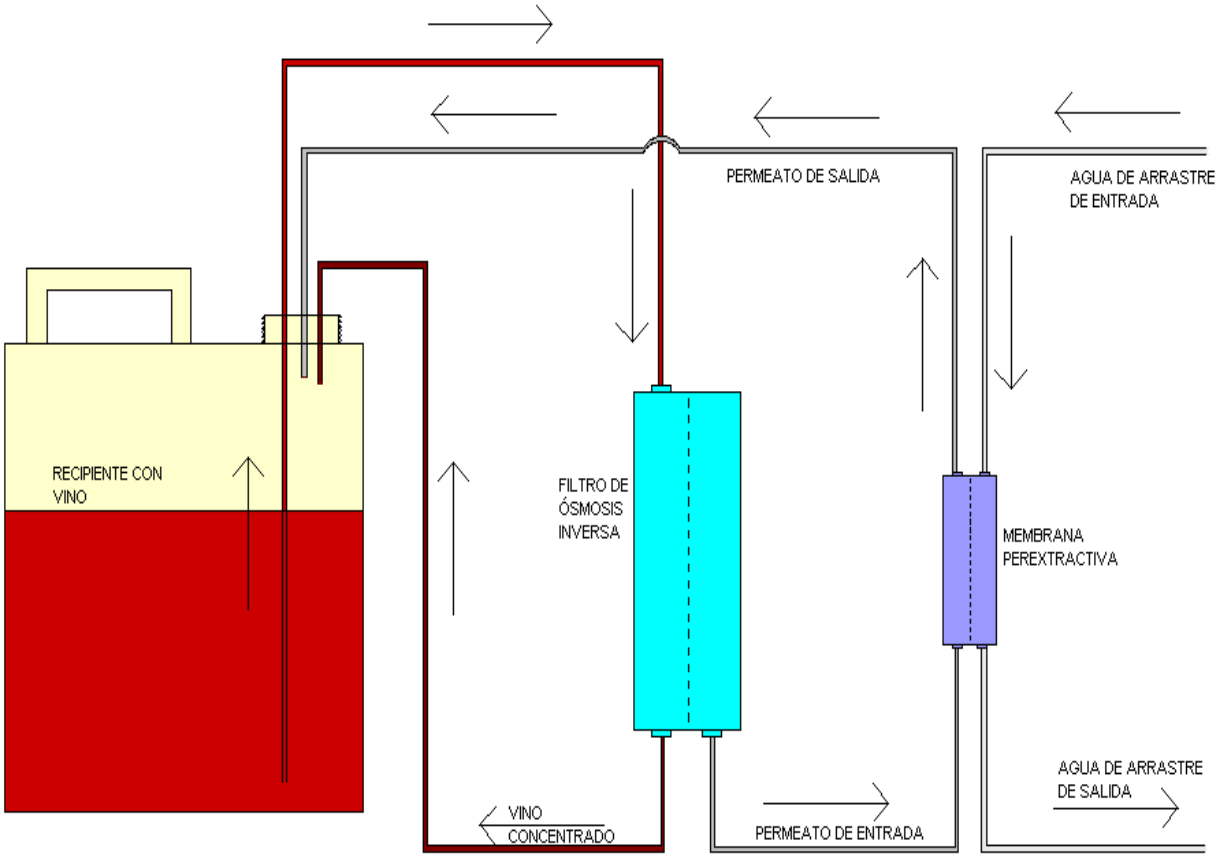

Fig. 1. Esquema del circuito

En teoría el agua de arrastre utilizada para despojar el alcohol, no pasaría al lado del permeato, por las siguientes razones:

-La concentración y presión de vapor de agua a ambos lados de la membrana son similares.

-Mediante el uso de la O.I. para pretratar el permeato, los sólidos disueltos, y por tanto, la presión osmótica del permeato es reducida relativamente a la del agua de arrastre, a fin de superar cualquier diferencia de presión osmótica que puede introducir agua hacia el permeato a través de la membrana.

-El lado de la membrana donde se encuentra el permeato se mantiene a una presión mayor que la del agua. (1 a 0.5 bar del lado del "agua vegetal y alcohol", y de 0.3 bar del lado del "agua de arrastre y alcohol").

MATERIALES Y METODOS

Se utilizó un equipo industrial en los primeros ensayos donde se trató mil litros de vino en cada uno de ellos, las variedades empleadas en los ensayos fueron cv. Chardonnay y cv. Cabernet Sauvignon. En dos de los cuatro ensayos se desalcoholizó todo el volumen de vino, mientras que en los otros dos, se trató una fracción donde se disminuyó el alcohol de tal forma que al ser cortado con el resto del vino, el vino final obtenido tuviese con una graduación alcohólica de dos grados menos que el de partida.

La capacidad de desalcoholización de este equipo, dependiendo del tipo de vino que se trate, es de 35 a 45 litros de alcohol puro por hora. El material de la membrana perextractiva utilizada fue poliolefinas y polipropileno. Ver Cuadro 1.

Posteriormente se efectuaron ensayos a escala laboratorio, sobre un equipo que funciona con el mismo principio que el industrial. Se efectuaron cinco ensayos con cuatro muestras de vino de cv. Criolla, cv. Ugni Blanc y cv. Cabernet Sauvignon. Uno de estos ensayos tuvo como objetivo un balance de masas.

Las muestras de vino fueron sometidas a análisis químicos. También se determinó la relación isotópica del agua de arrastre y el permeato antes y después de estar en contacto con la membrana perextractiva.

Para la determinación de la relación isotópica del agua se utilizó el método O.I.V. Resolución OENO 2/96. El equipo utilizado fue un Espectrómetro de masa marca FINNIGAN DELTA PLUS ADVANTAGE.

	Ensayos a escala industrial	**Ensayos a escala laboratorio**
Volumen tratado por ensayo	1000 litros de vino	4 litros de vino
Variedades empleadas	cv. Chardonnay cv. Cabernet Sauvignon.	cv. Criolla cv. Ugni Blanc cv. Cabernet Sauvignon
Cantidad	4 ensayos	5 ensayos
Reducción de grado alcohólico	2º % v/v de alcohol	2º % v/v de alcohol
Capacidad del equipo	35 a 45 l/h de alcohol	0.15 l/h de alcohol
Caudal de permeado de O.I.	150 l/hora	de 12 y 16 l/h

Cuadro 1. Datos de los ensayos

RESULTADOS Y DISCUSIÓN

En el Cuadro 2 se pueden ver los resultados de los análisis de las muestras extraídas en el tratamiento a escala industrial y en el Cuadro 3, Cuadro 4, Cuadro 5 y Cuadro 6 se muestras los resultados a escala laboratorio.

DETERMINACIÓN	Vino cv. CHARDONNAY			Vino cv. CABERNET		
	Inicial	Desalcoh. Completo	Desalcoh. Fracción	Inicial	Desalcoh. Completo	Desalcoh Fracción
Alcohol % en vol a 20°C	13.5	10.9	10.3	14.8	11.9	11.3
Extracto seco g/L	19.42	19.44	17.81	28.80	26.46	26.44
Azucares reductores g/L	2.10	2.08	2.05	2.45	2.12	2.01
Acidez Total g/L	6.45	6.30	6.00	4.98	4.50	4.42
Acidez volátil g/L	0.24	0.23	0.21	0.31	0.47	0.42
Índice de color	---	---	---	1582	1387	1439
Sodio mg/L	13	19	15	8	9	9
Calcio mg/L	96	110	84	63	63	61
Potasio mg/L	818	731	671	1300	1090	1110
Magnesio mg/L	90	95	78	129	117	112
Glicerina g/L	6.57	6.43	5.90	10.00	9.47	9.12
Relación isotópica δ 13C/12C	No se observa variación			No se observa variación		
Relación isotópica δ 18O/16O	4.4	2.7	1.8	5.7	3.2	2.5

Cuadro 2. Resultados a escala industrial

DETERMINACIÓN	Vino 1 inicial	Vino 1 tratado	Vino 2 inicial	Vino 2 tratado
Alcohol % en vol a 20°C	14,4	12,3	13,1	11,6
Extracto seco g/L	19,35	21,65	18,99	20,77
Acidez Total g/L	4.95	5,06	6,07	6,49
Acidez volátil g/L	0,4	0,43	0,42	0,44
Anh. Sulfuroso total mg/L	51	59	77	84
pH	3,7	3,5	3,2	3,2
glicerina g/l	8,92	9,55	8,23	8,27
sodio mg/l	67	73	47	49
potasio mg/l	1166	1274	829	973
cobre mg/l	0,01	0,01	0,02	0,01
hierro mg/l	1,66	1,51	1,74	1,59
plomo mg/l	0,02	0,04	0,06	0,05
estaño mg/l	< de 10	< de 10	< de 10	< de 10
cadmio ppb	1,93	1,31	1,74	1,19
Rel, isotópica δ^{18}0‰	**2,21**	**1,23**	**3,26**	**2,34**

Cuadro 3. Resultados a escala laboratorio ensayos 1 y 2.

DETERMINACIÓN	Vino 3 inicial	Vino 3 tratado	Vino 4 inicial	Vino 4 tratado
Alcohol % en vol a 20°C	13,9	12,8	15,5	13,3
Extracto seco g/L	30,52	28,08	25,12	25,56
Acidez Total g/L	3,64	3,9	5,44	5,4
Acidez volátil g/L	0,47	0,49	0,82	0,82
Anh. Sulfuroso total mg/L	38	28	56	52
pH	4	4	3,6	3,6
glicerina g/l	7,56	7,9	8,19	8,37
sodio mg/l	12	13	32	30
potasio mg/l	1980	1980	1467	1530
cobre mg/l	0,05	0,05	0,04	0,04
hierro mg/l	1,63	1,18	1,15	1,03
plomo mg/l	0,03	0,02	0,03	0,05
estaño mg/l	< de 10	< de 10	< de 10	< de 10
cadmio ppb	1,63	1,18	1,15	1,03
Rel, isotópica $\delta^{18}0‰$	**5,17**	**4,29**	**4,2**	**3,59**

Cuadro 4. Resultados a escala laboratorio ensayos 3 y 4.

$\delta^{18}0‰$	
AGUA DE ARRASTRE DE ENTRADA	AGUA DE ARRASTRE DE SALIDA
-15,83	**-14,66**

Cuadro 5. Resultados a escala laboratorio de la relación isotópica para el agua de arrastre.

$\delta^{18}0‰$	
PERMEATO DE ENTRADA	PERMEATO DE SALIDA
0,08	**-0,93**

Cuadro 6. Resultados a escala laboratorio de la relación isotópica para el permeato.

La relación isotópica de $^{18}O/^{16}O$ en la muestra con respecto a la relación de la media del Estándar de la Norma de Viena con agua de Océano (VSMOW) se obtiene de la siguiente manera:

$$\delta^{18}O_{MUESTRA} = \left[\frac{\left(^{18}O/^{16}O\right)_{MUESTRA}}{\left(^{18}O/^{16}O\right)_{VSMOW}} - 1 \right] \times 1000 \quad (1)$$

($^{18}O/^{16}O_{VSMOW}$ = 0.0020052 o 2005.2 ppm)

a- En los compuestos fijos del vino, no destilables, no se observó diferencias significativas en los distintos parámetros estudiados.

b- Se observa una disminución apreciable del alcohol en todos los ensayos.

c- No se observó fraccionamiento isotópico en relación a los isótopos 13C/12C.

d- Se observa fraccionamiento isotópico del agua entre el vino inicial y final, en todos los ensayos, incluyendo el agua del permeato y agua de arrastre, antes y después de estar en contacto con la membrana.

e- El balance de masas a escala laboratorio se realizó por pesajes del vino y del agua de arrastre. El mismo mostró que hubo una disminución de la masa de vino y un aumento en la masa de agua de arrastre (en 0,04%), por lo tanto habría un pasaje desde el vino hacia el agua. Debido a que las cantidades pesadas fueron muy pequeñas, este resultado tienen un gran margen de error y es poco confiable.

CONCLUSIONES

1- Si bien las condiciones de trabajo, de presión y concentraciones, así como el balance de masa indicarían que no sería posible la entrada de agua al sistema de desalcoholización, observando el cambio en la relación isotópica del agua de arrastre versus la de los vinos antes y después de tratar, se podría sugerir la teoría de que existe un pasaje de $H_2{}^{18}O$ desde el lado del permeato al agua de arrastre, por lo tanto se debería demostrar si el fraccionamiento isotópico ocurre por intercambio de agua de diferente composición isotópica o por el pasaje selectivo de uno de los isótopos, pero aún así, el fraccionamiento ocurre y la genuinidad del vino es alterada por el proceso.

2- La etapa posterior a la presente consistirá en efectuar nuevamente ensayos pero trabajando solo con agua de diferente composición isotópica y distintas concentraciones de compuestos marcadores, de bajo peso molecular, para establecer el mecanismo de fraccionamiento. Para complementar este estudio se debería realizar un ensayo a escala industrial efectuando un balance de masa combinando los resultados con los datos analíticos, este ensayo posibilitaría pesadas de mayor precisión.

3- Para evitar el fraccionamiento isotópico de este equipo debería reemplazase la unidad perextractiva por arrastre con agua mineral por alguna alternativa, como por ejemplo, realizar una destilación o efectuar una pervaporación, es decir no realizar el arrastre extractivo sino realizar vacío y una posible condensación o no del alcohol.

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REFERENCIAS BIBLIOGRAFICAS

Moutounet M., et al. 2007. Las tecnologías de elaboración de vinos con bajo nivel de etanol. Revista ACE de enología. Nº84

Flancy C. 2003. Enología: fundamentos científicos y tecnológicos. Segunda edición. Madrid. AMV y Mundi-Prensa.

Jackson R. Wine science, principles and applications. 2008. Tercera edición. Burlington. Elsevier.

Nath K. 2008. Membrane Separation Processes. Nueva Delhi. Prentice-Hall.

Palacio Martinez L. 1999. Caracterización estructural y superficial de membranas microporosas. Universidad de Valladolid.

Rahman S. Handbook of food preservation. 2007. Segunda Edición. Boca Raton. CRC Press.

UTILISATION DES LEVAINS MIXTES non-*Saccharomyces/ Saccharomyces cerevisiae* DANS LES FERMENTATIONS EN BLANCS SECS

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RESUME

Dans le but de diversifier la palette aromatique des vins blancs secs, des essais d'ensemencements mixtes séquentiels ont été effectués à l'aide de levures *non-Saccharomyces* appartenant aux genres *Candida*, *Hanseniaspora* et *Willopsis* , une LSA étant incorporée à J+5 . Les fermentations réalisées avec ces levains sont généralement plus lentes, phénomène vraisemblablement lié à la concurrence en alimentation azotée. Les vins ne présentent pas de modifications analytiques déterminantes, à l'exception de certains composés d'arômes fruités plus abondants dans le cas des flores mixtes, et les premiers examens sensoriels sur les produits confirment une très grande complexité aromatique et gustative, notamment avec deux biomasses de *Candida* (*C. pyralidae* et *C. xestobii*).

INTRODUCTION

Traditionnellement, les fermentations alcooliques ont toujours été assurées de façon spontanée par des populations de levures indigènes où le genre *Saccharomyces* devenait rapidement très prédominant. Une amélioration notable, visant à mieux maîtriser les fermentations et à éviter les incidents organoleptiques, a consisté à préconiser le levurage par inoculation d'une souche pure de levure du genre *Saccharomyces* sous forme de Levure Sèche Active (LSA). Cet objectif a été atteint dans divers secteurs dont l'œnologie, toutefois, dans les cas où l'utilisation de LSA a été généralisée, bien qu'une amélioration de la qualité soit effectivement observée, elle est généralement accompagnée parfois d'une standardisation et d'une perte de typicité des produits finis. Aujourd'hui, les études scientifiques, montrent qu'un petit nombre de souches de levures « non-*Saccharomyces* », dites « exotiques », sont responsables du gain organoleptique supplémentaire observé lors des fermentations spontanées. Ces souches contribuent ainsi à la complexité aromatique des produits, tandis que la souche *Saccharomyces* permet le maintien des exigences technologiques propres au bon déroulement des fermentations.

MATERIEL ET METHODES

Compte tenu de ces données, la piste de cultures mixtes de levures représente donc un outil intéressant pour la valorisation des vins issus de cépages considérés comme peu aromatiques. Le travail mené au cours de la campagne vise à vérifier l'efficacité de flores mixtes associant une souche de *Saccharomyces cerevisiae* à des souches de levures *non-Saccharomyces* sur moût de cépage Melon, ceci afin de vérifier leur incidence sur les cinétiques fermentaires

ainsi que sur les modifications organoleptiques positives supposées être apportées par ces flores. Le **Tab. 1** recense la liste des espèces de non- *Saccharomyces* retenues dans cet essai.

Symboles	Genre	Variété
HOC	*Hanseniaspora*	*occidentalis*
05T2Till	*Torulosporula*	*delbruekii*
RnM05	*Candida*	*pyralidae*
94	*Williopsis*	*californica*
900	*Candida*	*xestobii*
914	*Candida*	*intermédia*
915	*Candida*	*intermédia*
930	*Candida*	*Intermédia*
984	*Candida*	*xestobii*

Tableau 1: Les différentes souches non-*Saccharomyces* utilisées

Pour cette étude, 3 lots de raisins de cépage Melon issus de terroirs différents ont été vendangés à différentes étapes de maturité :

. Le lot 1 à la Haye Fouassière (HAI) le 18 septembre,
. Le lot 2 à Vertou (VER 1) le 23 septembre,
. Le lot 3, également à Vertou (VER 2) le 28 septembre.

Dans les trois situations la récolte est effectuée à l'aide d'une machine à vendanger. Le raisin est amené à la cave dans les 2 heures qui suivent la récolte sans protection particulière ; la vendange présente dans tous les cas un excellent état sanitaire (<5% de raisins botrytisés). A la cave, les traitements mécaniques du raisin, sont les suivants :

-égrappage,
-pressurage soigné à l'aide d'un pressoir pneumatique.

Le jus de raisin extrait est transféré dans une cuve de débourbage où il subit un sulfitage (3 g/hl) couplé à une addition d'enzymes pectolytiques aux fins de clarification (1g/hl).

-le débourbage du moût est réalisé après une stabulation en cuve de 3 à 4 jours à 5°C.

Au soutirage, le moût homogénéisé entre 80 et 125 NTU est transvasé pour chacune des 3 séries dans 10 fûts de 50 L. Un échantillon de moût frais est alors envoyé au laboratoire aux fins d'analyses.

Les résultats de ces contrôles notamment ceux portant sur la richesse en sucres et la teneur en azote assimilable permettent de faire des ajustements, ou des corrections du moût afin d'obtenir des conditions de fermentation favorables. Ainsi lorsque la teneur en azote assimilable dans le moût est en dessous du seuil de carence (160 mg/L), il est alors supplémenté en azote pour l'amener à ce niveau par l'addition de sulfate d'ammonium.

De la même manière on procède à la correction des teneurs en sucre, en chaptalisant afin d'amener le titre alcoolique final à 12% ; cette opération est accompagnée d' une micro-oxygénation au 4ème jour de la phase tumultueuse. La mise en œuvre des levains mixtes dans les trois séries suit un protocole identique :

. Les 9 souches *non-Saccharomyces* sont préparées sur des milieux de jus de raisin 8 jours avant leur introduction dans les moûts des 3 séries de manière à ce qu'elles aient le

temps de se développer pour former de la biomasse, au moment de leur introduction , les populations présentes dans le levain sont proches de 10^9 UFC/ml. Ces souches sont comparées à un témoin ensemencé uniquement avec une souche de levure *Saccharomyces cerevisiae* Fermicru 4F9.

. A J_0 les souches non-*Saccharomyces* sont introduites dans les moûts débourbés avec une proportion de 2% en volume ; les populations de de levures viables sont proches de 10^8 UFC /ml. Après 5 jours de stabulation dans le milieu, la LSA *Saccharomyces cerevisiae* Fermicru 4F9 est ensuite incorporée dans les moûts pour achever les fermentations alcooliques, ces dernières étant conduites dans un local thermorégulé à 18°C au cours de l'ensemble du processus.

. La réussite de l'implantation des souches de levures mises en œuvre est vérifiée au $10^{ème}$ jour de la fermentation alcoolique par amplification PCR.

RESULTATS

Composition analytique des jus

Le **Tableau 2** récapitule la composition des jus de raisins utilisés dans les 3 bancs d'essais. L'examen des résultats montre une hétérogénéité entre les provenances de vendanges que l'on peut classer en 3 groupes :

. HAI et VER 1 qui pour l'essentiel des paramètres analysés présentent des valeurs assez comparables (acides malique et tartrique, acidité totale, pH et azote assimilable carencé),

. VER 2 dont l'acidité totale est plus faible et qui présente des teneurs plus confortables en azote assimilable mais encore légèrement carencées.

	HAI	**VER 1**	**VER 2**
Acide L malique (g/l)	3,7	3,9	4,1
Acide tartrique (g/l)	4,5	4,0	3,6
Acidité totale (g/l H_2SO_4)	4,14	4,10	3,71
pH	3,36	3,37	3,32
Azote assimilable (mg/l)	74	73	139

Tableau 2 : Composition analytique des 3 lots de jus de raisin mis en œuvre

Cinétiques fermentaires

Les paramètres concernant le temps de latence ainsi que la durée des cinétiques fermentaires des flores mixtes engagées dans les 3 lots de moûts de raisin sont répertoriés dans le **Tab. 3**. La vitesse de fermentation des 9 souches de *non-Saccharomyces* de J_0 à J_{+7} montre une activité fermentaire assez faible jusqu'à l'ajout de *Saccharomyces cerevisiae 4F9.*

Le témoin également fermenté avec cette préparation (4F9) présente une cinétique franche et très rapide (8 à12 jours). L'ajout de levures non-*Saccharomyces* conduit d'une manière générale à un allongement des durées de fermentations comprises entre 13 et 26 jours ; ce ralentissement n'a jamais conduit à des arrêts de fermentation. On peut constater également que si l'effet de la chaptalisation se manifeste clairement sur le témoin par une pseudo-stagnation de la densité, cet effet n'apparaît aucunement dans le cas des fermentations mixtes à ce stade.

	HAI		**VER 1**		**VER 2**	
	Temps de latence (jours)	Durée de la fermentation alcoolique	Temps de latence (jours)	Durée de la fermentation alcoolique	Temps de latence (jours)	Durée de la fermentation alcoolique
900	**2**	**15**	**1**	**24**	**1**	**17**
930	**2**	**15**	**1**	**24**	**1**	**19**
984	**1**	**18**	**1**	**24**	**1**	**19**
94	**1**	**18**	**1**	**26**	**1**	**9**
HOC	**1**	**17**	**1**	**26**	**1**	**22**
RnM05	**1**	**15**	**1**	**26**	**1**	**19**
05T2Till	**1**	**17**	**1**	**26**	**1**	**13**
914	**1**	**19**	**1**	**26**	**1**	**22**
915	**1**	**19**	**1**	**26**	**1**	**17**
4F9 (T)	**1**	**10**	**2**	**12**	**1**	**8**

Tableau 3 : Caractéristiques fermentaires des différents lots ensemencés

Composition des vins après fermentation alcoolique

Le **Tab. 4** relate la composition analytique des vins du lot VER 1, représentatif des deux autres lots vins, sitôt la fermentation alcoolique achevée. A l'exception de la souche *H'spora occidentalis* connue pour son activité démalicante, la fermentation un peu plus lente des lots ensemencés avec les non-*Saccharomyces* n'a pas eu de répercussions ni sur la dégradation de l'acide malique, (13 % d'acide malique seulement décomposé par les levures), ni sur l'augmentation du niveau moyen d'acidité volatile (< 0,20 g/l) ; les quantités de glucose + fructose relevées indiquent une fermentation incomplète pour la quasi-totalité des souches à l'exception du témoin, avec un rapport de transformation sucres/alcool toujours plus élevé. Ce dernier lot (4F9) qui présente la plus haute teneur en éthanol (TAV) montre également une valeur de pH la plus faible liée à une concentration plus importante en acide tartrique.

Les souches de levures à caractère oxydatif dominant étant généralement connues pour leur propriété de former des composés susceptibles de combiner le SO_2, les teneurs en anhydride sulfureux total ont été évaluées ; elles ne montrent pas de différences significatives avec le témoin ensemencé avec *Sacch. cerevisiae* seul. Les teneurs en glycérol trouvées dans les vins issus de levains mixtes sont légèrement inférieures à celles de la référence (**Tab. 5**).

	900	930	984	94	HOC	Rn MO5	05 TD2T	914	915	4F9
Acide malique (g/L)	3,2	3,2	3,2	3,2	2,6	3,2	3,2	3,2	3,3	3,3
Acide tartrique (g/L)	3,4	3,4	3,4	3,4	3,4	3,5	3,4	3,4	3,3	3,8
Ethanol (%vol)	12,23	12 ,26	12,06	12,15	12,21	12,18	12,21	12,09	12,22	12,41
Glucose+Fructose (g/L)	3,1	3,2	5,5	4,2	4,6	4,4	4,3	5,4	3,5	<0,4
Acidité totale (g/L H_2SO_4)	4,27	4,33	4,28	4,33	3,80	4,37	4,28	4,28	4,24	4,73
Acidité volatile (g/L H_2SO_4)	0,16	0,15	0,14	0,13	0,13	0,17	0,13	0,14	0,13	0,12
pH	3,25	3,24	3,25	3,23	3,22	3,22	3,22	3,24	3,23	3,2

Tableau 4 : Caractéristiques fermentaires des différents lots ensemencés (VER 1)

	900	930	984	94	HOC	Rn MO5	05 TD2T	914	915	4F9
Glycérol (g/l)	5,6	5,6	5,7	5,6	5,5	5,3	5,6	5,3	5,5	6
SO_2 total (mg/l)	84	84	89	87	88	87	84	86	85	86

Tableau 5 : Production de glycérol et teneurs en SO_2 total des différentes modalités (VER 1)

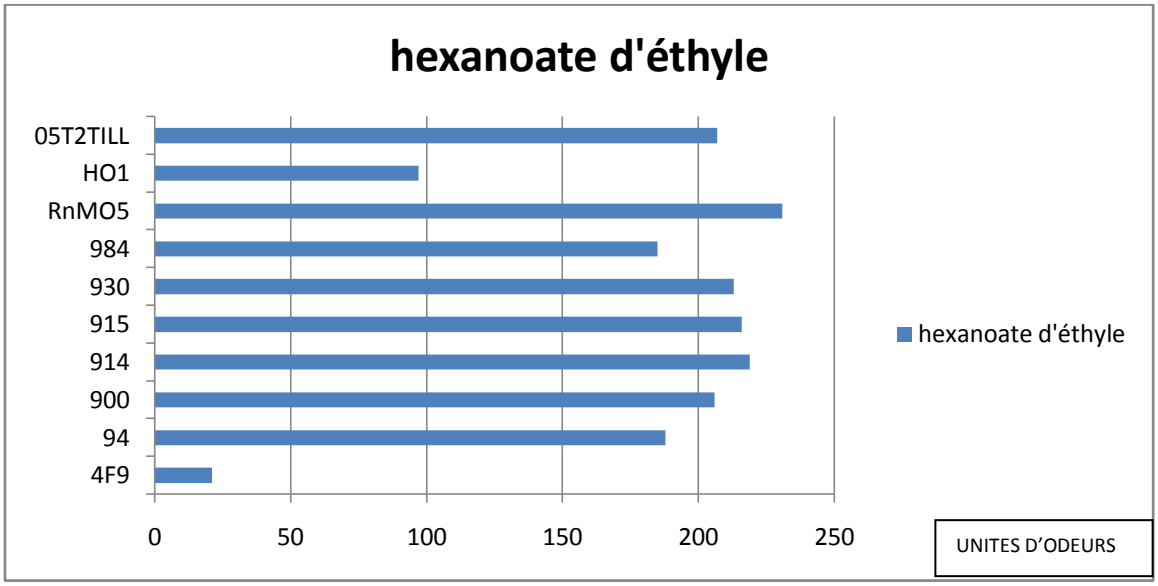

Fig. 1 Production d'hexanoate d'éthyle par les associations de flores (VER 1)

L'influence des flores mixtes sur la production d'arômes a également fait l'objet d'investigations. D'une manière générale, les vins qui en résultent possèdent une complexité aromatique beaucoup plus marquée que celui résultant de la simple intervention de

Saccharomyces cerevisiae. Ainsi, les esters éthyliques sont retrouvés dans ces vins dans des proportions significativement plus importantes que dans le témoin, c'est le cas de l'hexanoate d'éthyle (**fig.1**) où les souches de *Candida sp.* et *Torulaspora delbrueckii* sont très performantes, mais également pour une grande partie des esters qui contribuent à apporter des notes fruitées aux vins (**fig.2**). Notons que le vin élaboré avec *Hanseniaspora occidentalis* présente un profil aromatique moins complexe de la série.

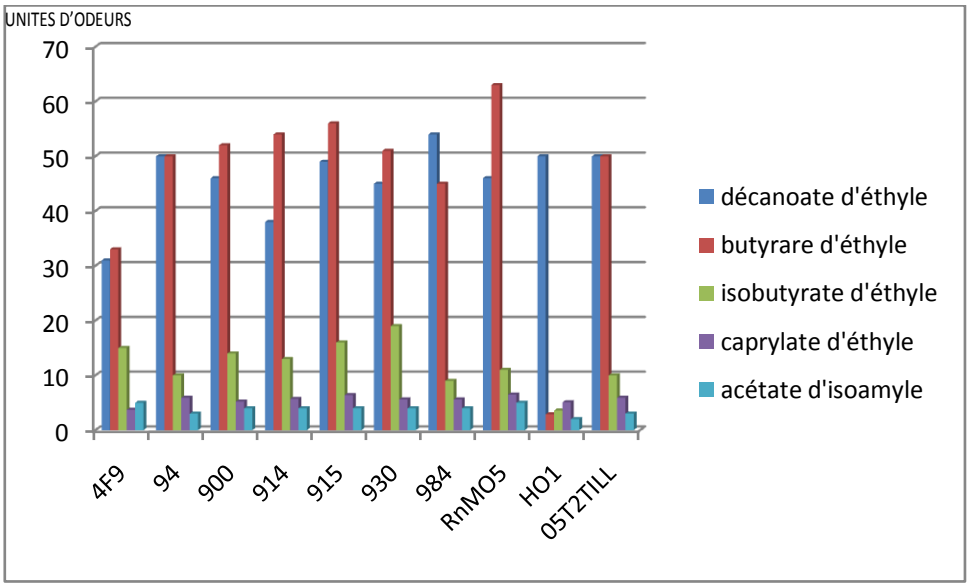

Fig. 2 Production d'esters éthyliques par les associations de flores (VER 1)

Evaluation sensorielle

L'évaluation sensorielle des vins a été réalisée sur vins jeunes à trois reprises de novembre 2009 à mars 2010 par un jury composé des professionnels de la filière.

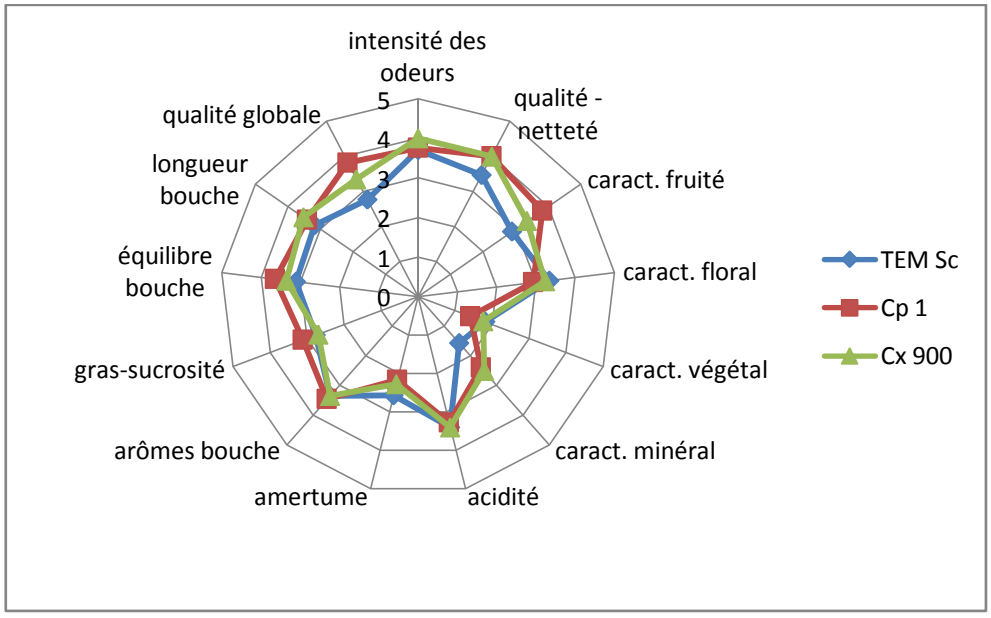

Fig. 3 Caractéristiques sensorielles des vins élaborés en flores mixtes avec *C. xestobii* 900, *C. pyralidae* 1 et *Saccharomyces cerevisiae* seul (HAI)

De manière générale, et pour les trois séries, on observe une assez grande atomisation des notes sur les descripteurs, les vins issus de levains mixtes présentant très souvent des caractéristiques olfactives marquées par des notes fruitées/florales très souvent supérieures au témoin ainsi qu'une bonne complexité en bouche. Le témoin est pénalisé par quelques notes de réduction accompagnant le caractère thiolé des vins. Sur l'ensemble des 3 essais les dégustations opérées en mars 2010, deux biomasses (*C pyralidae* et *C. xestobii)* se détachent significativement sur les descripteurs olfactifs caractères fruités/floraux ainsi que sur la qualité globale des produits (**Fig.3)**.

CONCLUSION

Ce travail a permis de tester l'incidence d'ensemencements séquentiels réalisés à partir de plusieurs espèces de levures *non-Saccharomyces* sur le déroulement des fermentations alcooliques ainsi que sur la qualité des vins qui en découlent. Ces souches présentent pour la plupart des besoins élevés pour leur développement, entraînant une carence précoce du milieu lors de l'apport de *Saccharomyces cerevisiae*, contribuant à des cinétiques fermentaires généralement plus allongées.Ce point nécessitera d'être approfondi pour les souches candidates à une éventuelle industrialisation.

Les paramètres analytiques classiques ne sont guère affectés par l'utilisation des souches à l'exception des teneurs en sucres résiduels qui restent un peu plus élevées, conséquence d'une fermentation incomplète. *H'spora occidentalis* confirme son excellent potentiel de démalication sur l'ensemble des trois séries. La production d'acidité volatile par les *non-Saccharomyces* reste très faible .L'utilisation de souches non-*Saccharomyces*, notamment celles appartenant au genre *Candida*, n'entraîne pas une combinaison plus importante du SO_2 ; l'analyse des composés aromatiques révèle la présence en quantités significatives d'esters éthyliques contribuant à exhausser le caractère fleuri/fruité des arômes des vins.

Les premières analyses sensorielles effectuées en primeur confirment l'intérêt de deux souches de *Candida* introduites en levain mixte (*C pyralidae* et *C. xestobii) ;* les vins élaborés sont caractérisés par une expression aromatique très originale qui se démarque significativement du témoin ; en outre, ils présentent une qualité globale jugée significativement meilleure vis-à-vis de la référence. Des travaux complémentaires réalisés sur des volumes industriels doivent confirmer ces observations avant d'envisager un développement commercial de ces souches.

REFERENCES BIBLIOGRAPHIQUES

Bejan V. 2006. Contribution à la mise en oeuvre de certaines espèces de levures apiculées de type *Kloeckera sp*. dans la désacidification des moûts de vins blancs. Master francophone Management viti-vinicole , Université Technique de Moldavie, Chisinau.

Ciani M., Ferraro. L**.** 1998. Combined use of immobilized *Candida stellata* cells and *Saccharomyces cerevisiae* to improve the quality of wines. J. Appl. Microbiol. 85: 247-254.

Joly N.P., Pretorius I.S. ; 2000. The distribution of non-*Saccharomyces cerevisiae* yeast strains in four wine production regions of the western cape, South Africa. Tenth Internatioanl Symposium on Yeasts. Deft University Press.

Languet P. et al. 2005. Valorisation aromatique des moûts par l'utilisation séquentielle de levure d'espèces non-*Saccharomyces* et *Saccharomyces*. Revue des Œnologues. 117 : 31-33.
Poulard A. et al. 2009. Utilisation d'*Hanseniaspora occidentalis* pour la démalication des vins blancs secs. XXXIIè Congrès mondial de la vigne et du vin. Zagreb.
Romano P. et al. 2003. Function of yeast species andstrains in wine flavour. Int. J. Food Microbiol. 86:169-180.
Soden A. et al. 2000. Effects of co-fermentation with *Candida stellata* and *Saccharomyces cerevisiae* on the aroma and composition of chardonnay wine. Austr. J. Grape Wine Res., 6 **:** 21-30.
Zott K. et al. 2009. Les levures non-*Saccharomyces.* Dynamique et caractérisation durant les étapes pré-fermentaires et la fermentation alcoolique. Revue des Œnologues, 133 : 11-12.

IL PIEMONTE DELLA VITE E DEL VINO: UN PATRIMONIO DI STORIA, TRADIZIONI E TERRITORIO. RICERCA STORICA PER LA CANDIDATURA UNESCO DEL PIEMONTE COME PATRIMONIO DELL'UMANITÀ

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RIASSUNTO

Il Piemonte, possiede un ricchissimo patrimonio di vitigni che sono unici e tipici della regione: Nebbiolo, Moscato bianco, Barbera, Freisa, Grignolino, Dolcetto, Arneis, Brachetto e molti altri ancora. Questi vitigni hanno radici storiche locali molto profonde e sono documentati da secoli nel panorama vitivinicolo piemontese. Alcuni di essi sono citati fin dal Medio Evo e hanno scritto pagine di storia importanti e ben documentate nei secoli successivi, fino ad oggi. La loro diffusione e i loro successi hanno determinato gli orientamenti dell'economia e dell'occupazione, oltre che la fisionomia dell'ambiente naturale e paesaggistico. Le provincie di Asti, Cuneo e Alessandria sono quelle più ampiamente caratterizzate per l'ampia superficie vitata e per la lunga tradizione vitivinicola. Dal Piemonte come punto d'incontro fra le antiche culture di Celti, Greci, Etruschi, Romani, si passa alla trattazione delle vicende vitivinicole medievali, passando poi alla storia del vino nel 1600 e 1700, fino alla grande svolta del 1800 che ha visto affermarsi i grandi vini e le zone di eccellenza della viticoltura regionale piemontese.

Piedmont is one of the 20 regions of Italy. It has a rich heritage of vines that are unique and typical of the region: Nebbiolo, Moscato Bianco, Barbera, Freisa, Grignolino, Dolcetto, Arneis, Brachetto, and many others.

These local varieties have very deep historical roots and are documented for centuries in the Piedmont wine landscape.

Some of them are mentioned since the Middle Ages, have written important pages of history and are well documented in the following centuries, until today.

Their spread and their successes have led to the guidelines of the economy and employment, as well as the physiognomy of the natural environment and landscape.

The provinces of Asti, Cuneo and Alessandria are the most extensively characterized for the wide area of vines and the wine tradition.

Our work studied Piedmont as meeting point between the ancient cultures of the Celts, Greeks, Etruscans, Romans, it follows the wine events of the Middle Ages, it analizes the history of wine in 1600 and 1700, until the great turning point of 1800 with the emergence of the wines of excellence and the great areas of vine growing.

INTRODUZIONE

L'UNESCO (United Nations Educational, Scientific and Cultural Organization), fondata a Londra il 16 novembre 1945, è un'Agenzia specializzata delle Nazioni Unite che opera sotto il coordinamento del Consiglio Economico e Sociale (ECOSOC), che è direttamente collegato

all'Assemblea Generale dell'ONU e si occupa di tematiche economiche, culturali, sociali, educative e sanitarie.

Una delle missioni dell'UNESCO è quella di definire una lista di "Patrimoni dell'umanità". La Convenzione sul Patrimonio dell'Umanità, adottata dalla Conferenza generale dell'UNESCO il 16 novembre 1972, ha lo scopo di identificare e mantenere una lista di siti che rappresentano delle particolarità di eccezionale importanza da un punto di vista culturale o naturale.

Quella di Sito Patrimonio dell'Umanità è la denominazione ufficiale delle aree registrate nella Lista del Patrimonio dell'Umanità, o nella sua accezione inglese World Heritage List, della Convenzione sul Patrimonio dell'Umanità.

Il Comitato della Convenzione, chiamato Comitato per il Patrimonio dell'Umanità, ha sviluppato dei criteri precisi [1]) per l'inclusione dei siti nella lista.

Secondo l'aggiornamento effettuato nella riunione del Comitato per il Patrimonio dell'Umanità a Siviglia, il 30 giugno 2009, [2], la lista è composta da un totale di 890 siti[3] (di cui 689 beni culturali, 176 naturali e 25 misti) presenti in 148 Nazioni del mondo[3].

Attualmente l'Italia è la nazione a detenere il maggior numero di siti inclusi nella lista dei patrimoni dell'umanità (44 siti), seguita dalla Spagna (41 siti) e dalla Cina (38 siti).

Nel febbraio 2008 è stato siglato un Protocollo di Intesa per attuare il progetto di candidatura dei "Paesaggi Vitivinicoli Tipici del Piemonte" tra il Ministero dei Beni e delle Attività Culturali, la Regione Piemonte e le Province di Alessandria, Asti e Cuneo.

Il bene proposto per la candidatura riguarda aree geografiche fortemente caratterizzate sotto l'aspetto ambientale e culturale, connotate da una antichissima modellizzazione del territorio collinare finalizzata alla coltivazione della vite e alla produzione di vino. Per definire la perimetrazione sono stati effettuati studi multidisciplinari che hanno esaminato le caratteristiche naturali, geomorfologiche e topografiche ed hanno espresso valutazioni relative alla presenza e significatività delle testimonianze storiche connesse alla cultura vitivinicola.

La lunga tradizione vitivinicola piemontese, che ha avuto origine più di duemila anni fa, ha prodotto un paesaggio culturale di grande bellezza, che riflette le trasformazioni e le evoluzioni sociali, tecnologiche ed economiche legate alla viticoltura e ad una straordinaria "cultura del vino", profondamente radicata nella comunità.

I "Paesaggi Vitivinicoli Tipici del Piemonte" rappresentano otto aree a più alta vocazione tra quelle vitate della regione, dove è presente un'estensione vitivinicola quantitativamente e qualitativamente unica nel panorama mondiale, in relazione alla eccezionale varietà ed originalità di vitigni locali e di produzioni enologiche d'eccellenza.

MATERIALI E METODI

Per la realizzazione della ricerca storica che è l'oggetto di questo lavoro, sono state impiegate principalmente fonti dirette e indirette tratte da archivi pubblici e privati, biblioteche universitarie, biblioteche nazionali e locali, istituzioni pubbliche, organizzazioni professionali vitivinicole, Camere di Commercio, aziende, musei. Si è inoltre verificato lo stato attuale delle conoscenze sul tema attraverso la consultazione di opere importanti ed anche di opere meno note che permettessero di ricavare informazioni sulle specificità storiche della coltivazione della vite e della produzione del vino in Piemonte.

Alla ricerca delle fonti è seguita una selezione del materiale significativo. Questo è stato vagliato, contestualizzato e commentato per dare origine ad un ampio testo del quale si riportano qui i principali risultati.

RISULTATI

L'analisi critica delle fonti ha permesso di produrre un testo che presenta un esame delle specificità storiche della coltivazione della vite e della produzione del vino in Piemonte, evidenziando ai primordi di questa storia il ruolo della regione come punto di incontro delle influenze culturali degli Etruschi, dei Greci, dei Celti.

Ogni area vitivinicola selezionata per la candidatura dalla Commissione di Esperti è stata presentata nella sua evoluzione storica, con particolari riflessioni sulla viticoltura e l'enologia locali e sulla storicità e sul ruolo economico-culturale-sociale dei più celebri ed importanti vitigni specifici del Piemonte. Si è visto come l'attuale configurazione del "paesaggio culturale" vitivinicolo sia il risultato di un complesso insieme di valori tramandato nei secoli e legato all'intreccio dell'opera dell'uomo con il particolare contesto naturale della regione.

Per le zone selezionate della provincia astigiana, alessandrina e cuneese sono state così prese in esame le storiche aree di produzione del Nebbiolo, del Moscato, del Barbera, del Dolcetto, del Freisa, del Grignolino.

Si è constatato come questi vitigni abbiano fortemente caratterizzato fino ad oggi la fisionomia, l'economia, la vita dei rispettivi territori.

Zona di eccellenza A: l'area del Freisa.

È ormai accertato che le vicende del Freisa sulle colline del Piemonte si dipanano lungo un periodo di tempo di almeno 500 anni. Delle *"carrate"* e delle *"somate fresearum",* cioè *"di fresie*", sono già citate in una tariffa di pedaggio di Pancalieri (in provincia di Torino, sulla sponda sinistra del Po) nel 1517 e secondo la studiosa Anna Maria Nada Patrone si tratta di una trascrizione da una tariffa ancora più antica.

Ma oltre all'antichità della citazione la cosa importante da leggere in questo documento è che le carrate e le somate delle *"frese"* sono poste tra i vini pregiati e sono stimate il doppio del vino comune. Prosegue poi nel 1600 l'antica storia delle *"freise",* nome al plurale, perché spesso, specialmente nei tempi più antichi, in riferimento a questo vitigno si trova usato il plurale *"fresie"* o *"freise"*.

Il conte Nuvolone nel suo libro intitolato "Sulla coltivazione delle viti", pubblicato a Torino nel 1798, inserisce la Freisa fra le uve nere di prima qualità. Alla fine del 1800 la zona del Freisa si delinea chiaramente tra i circondari di Asti e di Torino. Freisa d'Asti e Freisa di Chieri sono le due DOC legate alla regione storica di coltivazione del Freisa e ne sono indubbiamente i portabandiera.

Zona di eccellenza B: l'area del Grignolino.

Alla fine del 1800 la zona del Grignolino era delineata chiaramente tra i circondari di Asti e di Casale, ma la sua coltivazione è attestata da moltissimo tempo. *Berbexino* è infatti sinonimo riconosciuto da tutti gli ampelografi dell'attuale Grignolino. Il suo nome si trova a partire dal XIII secolo. A Casale Monferrato in un atto del 7 novembre 1249 si notifica l'affitto di terre, da parte della chiesa di S. Evasio e si stabilisce che vi si debbano piantare di *"bonis vitibus berbexinis"*.

Nel 1614 il vino Grignolino è citato fra i vini della cantina della celebre fortezza di Casale, una delle più importanti piazzeforti d'Europa. I documenti ottocenteschi continuano ad identificarlo come vitigno caratterizzante l'Astigiano e il Monferrato Casalese, che sono anche le zone attuali d'eccellenza per la sua coltivazione. "Grignolino d'Asti" e "Grignolino del Monferrato Casalese", sono le denominazioni ottenute rispettivamente nel 1973 e nel 1974.

Zona di eccellenza C: l'area del Barbera

La fama e la diffusione di questo vitigno fanno pensare ad una coltivazione molto antica nella regione. In effetti vi sono testimonianze che citano il barbera già nel 1500. Si pensa tuttavia di poter andare ancora più indietro nel tempo, individuando questo vitigno probabilmente diffuso sotto un altro nome. Secondo A. M. Nada Patrone l'uva Barbera potrebbe identificarsi con la Grisa descritta nel 1304 dal magistrato bolognese Pier de' Crescenzi nel suo celebre trattato agronomico *"Liber ruralium commodorum.* È soprattutto con la fine del 1600 che si nomina con maggior frequenza questo vitigno. La sua importanza cresce ancora sulla fine di quel secolo e anche nel successivo. Nel 1798, la Barbera viene menzionata ufficialmente fra le uve nere piemontesi di prima qualità, nell'*"Istruzione sulla coltivazione delle viti e sul modo migliore di fare e conservare i vini"* del conte Giuseppe Nuvolone Pergamo, vicedirettore della Società Agraria di Torino. Nei primi decenni del 1800 è indicata dallo studoso ligure Giorgio Gallesio come *"Uva Montisferratensis"* a sottolineare la zona d'eccellenza della sua coltivazione. Negli stessi anni Gian Secondo De Canis, nella sua *"Corografia Astigiana"*, delinea una precisa area specificamente vocata alla coltivazione delle "Barbere". Quell'antica zona dell'Astigiano è tuttora simbolo di grandi Barbera.

Queste grandi premesse ottocentesche furono disattese da condizioni sociali e politiche collegate alle due guerre mondiali e dalla crisi vissuta dal mondo agricolo negli anni '20 e '60. Il Barbera diventò oggetto di falsificazioni e speculazioni di mercato che ne fecero scendere l'immagine, presto identificata con quella di un vino di grande massa, senza pretese, senza eccellenza. La rinascita del vero Barbera partì lentamente dagli anni '60, per concentrarsi poi negli anni '80, grazie all'azione e agli investimenti di produttori, i cui nomi sono diventati sinonimo del totale riscatto e della grande valorizzazione di questo vino sullo scenario piemontese, italiano e mondiale. Coltivato su 16.600 ettari, il Barbera è il vitigno più diffuso in Piemonte ed è un classico vitigno del Monferrato. La coltivazione del Barbera, presente sulle colline che emersero dal mare nell'Era Terziaria, è tutta su colli di altitudine dai 150 ai 300 metri, con qualche punta più elevata nell'Alto Monferrato. I vigneti del Barbera sono concentrati particolarmente nel sud Astigiano, tra il fiume Tanaro e il torrente Belbo, con paesi fortemente e storicamente vocati alla sua coltivazione come Nizza, Vinchio, Agliano, Costigliole. A nord del Tanaro, nel Nord Astigiano e nel Monferrato Casalese, la coltivazione diventa meno intensa.

Zona di eccellenza D: l'area del Moscato

Il Moscato bianco è uno dei vitigni più importanti e caratterizzanti della storia enologica del Piemonte. Già a partire dal XIV secolo si afferma come produzione piemontese il Moscato bianco, uva dolce e profumata con la quale si producono il Moscato d'Asti e l'Asti spumante.

La parola "Moscato" si riferisce alla elegante nota aromatica che caratterizza questo vitigno.

Il termine "Moscato" compare infatti nella seconda metà del 1200, proprio con il significato di "profumato".

Il Moscato bianco diviene un vitigno fra i più importanti nella storia del vino piemontese e di questa regione ha condiviso e continua a condividere la vita sociale, politica ed economica.

I documenti più antichi che parlano della coltivazione del Moscato in Piemonte sono del 1300. Da quegli anni lo troviamo sempre citato e tenuto in grande considerazione. Nel 1500 il suo vino era oggetto di omaggi diplomatici, nel 1600 era considerato fra le uve bianche *"più eccellenti"* del Piemonte, nel 1700 era indicato come uno dei vitigni piemontesi in grado di dare i vini più pregiati. Nel 1800, un secolo importante per l'evoluzione delle conoscenze viticole ed enologiche, questo antico vitigno dalla lunga storia e dai dolci profumi, diventa

brillante protagonista dell'enologia piemontese che si sta aprendo nuove strade ed ottiene importanti successi. Dalla metà del 1800, il Piemonte si andò caratterizzando nettamente rispetto alle altre regioni italiane per l'estesa coltivazione di Moscato bianco.

La coltivazione del Moscato Bianco nel corso del secolo diventò sempre più significativa. Gli ampelografi di fine '800 Pier Paolo Demaria e Carlo Leardi, riferendosi alla provincia di Alessandria e al circondario di Asti, identificarono per il Moscato una zona d'elezione lungo la linea delle colline che da Ricaldone, Strevi ed Acqui va verso Canelli, Calosso, Costigliole d'Asti. Questa venne segnalata dai due Autori come *"la vera zona dei moscati bianchi"*, dove il vitigno spesso rappresentava anche la metà della coltivazione complessiva e dove raggiungeva *"la massima squisitezza e perfezione"*.

Verso la fine del 1800, in Piemonte, la produzione di uva Moscato si aggirava intorno ai 148.000 quintali. Canelli costituiva la principale area di coltivazione, con una produzione di 72.000 quintali. Era seguita da Santo Stefano Belbo, Calosso, Costigliole, Strevi, Castiglione Tinella, Acqui e Ricaldone.

La tradizionale vocazione al Moscato è rimasta costante in questi paesi.

Fino al 1875 la maggior parte del Moscato piemontese era rivolta alla produzione di Vermouth. Questo vino aromatizzato divenuto celebre come "Vermouth di Torino" era diventato l'aperitivo per eccellenza ed il suo consumo era divenuto un vero fenomeno di costume. Nel volgere di vent'anni tuttavia la situazione si modificò in modo sostanziale.

Alla fine del 1800 iniziò infatti una significativa produzione di vino spumante rifermentato in bottiglia, ottenuto partendo dal Moscato.

Nel 1895 il Moscato era ormai rivolto soprattutto alla produzione dello spumante e già si parlava della considerevole richiesta che arrivava anche dall'estero per quel vino bianco, dolce, profumato e spumeggiante, noto in commercio sotto i nomi di Moscato d'Asti o Moscato di Canelli. Le storiche cantine canellesi scavate sotto le colline diventarono "cattedrali sotterranee" dove si elaboravano dolci e profumate "bollicine" pronte a partire verso i quattro angoli del mondo Con l'affermazione di questo tipo di vinificazione, il Piemonte si caratterizzava in modo assolutamente diverso rispetto alle altre regioni di diffusione del Moscato. Le tecniche di elaborazione dello spumante ebbero diretta influenza sulla svolta radicale che prese l'impostazione produttiva ed ebbero influenza sullo sviluppo in ambito territoriale de attività collegate a questa importante produzione..

Oggi il Piemonte è per eccellenza la terra del Moscato bianco. Dei 13.500 ettari dedicati oggi in Italia a questo vitigno, ben 9900 ettari si trovano infatti sulle colline piemontesi.Quella che oggi consideriamo la zona eletta del Moscato costituita da 52 paesi delle provincie di Asti, Alessandria e Cuneo, si trovava già delineata nelle opere dei più celebri e dotti geografi piemontesi del 1600: Giovanni Botero nel 1607, nella "Relazione di Piamonte" e Francesco Agostino Della Chiesa, cosmografo e consigliere del duca Carlo Emanuele I, nella sua *"Relatione dello stato presente di Piemonte",* nel 1635.

Zona di eccellenza E ed F: l'area del Nebbiolo

Il Nebbiolo è uno dei primi vitigni piemontesi ad essere citati in testimonianze scritte.

La sua importanza è continuamente cresciuta nel tempo e si è costruita insieme alle vicende politiche, economiche, culturali della regione.

Fino dal 1200 troviamo documenti che attestano la presenza di *"Nibiol"* o di *"Nubiolio"* nell'area pedemontana. Già allora era considerato un vitigno di pregio e la sua coltivazione era specificamente protetta dalle norme degli statuti comunali.

L'alta stima di cui godeva il Nebbiolo fra 1200 e 1500, si mantenne e aumentò ancora all'inizio del 1600. A quell'epoca erano molte le varietà di vitigni conosciute in Piemonte, ma proprio a quest'uva venne riservato il titolo di regina.

Nell'opera del gioielliere ed enologo torinese Giovanni Battista Croce *"Della eccellenza et diversità dei vini che si fanno nella Montagna di Torino"*, l'uva *"Nebiol"* venne definita la regina delle uve nere per la sua eccezionale attitudine a dare vini di qualità.

Nel corso del 1700 continua ad essere indicato fra i più importanti vitigni piemontesi.

Nel 1800 la storia del Nebbiolo procede con quella di celebri famiglie nobiliari piemontesi, grandi proprietarie di vigneti che vedevano un segno di distinzione anche nella produzione di vino di qualità. Sarebbero molti i nomi da citare. Il conte Camillo Benso di Cavour, che nella tenuta di Grinzane aveva soprattutto Nebbioli e Dolcetti. Poi ancora i marchesi Falletti di Barolo, lo stesso re Carlo Alberto, che costituì l'Agenzia di Pollenzo alla quale facevano capo diverse tenute con vigne coltivate a nebbiolo. In quest'epoca e in questi luoghi si compiono passi molto importanti nella storia del successo del nebbiolo e dell'enologia del Piemonte.

Grande protagonista della tradizione e dell'evoluzione enologica piemontese dell'epoca ottocentesca, il nebbiolo è diventato un simbolo delle colline di Langa sulle quali trova un habitat ideale dando origine a vini famosi in tutto il mondo come Barolo e Barbaresco.

Il Nebbiolo è vitigno d'eccellenza anche in Roero, area a sinistra del fiume Tanaro, fra Asti e Alba, con i paesi di Canale, Vezza, Monteu, Montaldo, Santo Stefano Roero come riferimenti principali. Qui la vite ha gradualmente sottratto terreno ai boschi fino a diventare fra il 1600 e il 1700 la coltura principale. Oltre al Nebbiolo, il vitigno caratterizzante il Roero è l'Arneis, una varietà a bacca bianca. I primi riferimenti scritti che riguardano l'Arneis, vengono dal nome di un'altura collinare dietro Canale, citata nel 1478 come "*Renexium"*. Nel 1500 si parla poi di vigne *"renexi"*.

Questo vitigno che era stato quasi abbandonato, è stato oggetto di recupero e valorizzazione alla fine degli anni 1970, operazione che ha conosciuto un grande successo.

Zona di eccellenza G ed H: l'area del Dolcetto

Il Dolcetto è un vitigno che tradizionalmente ha fatto parte della storia enologica del Piemonte e continua oggi a segnarla. *"Duset e duzet, dossetti e dozzetti"* sono i nomi popolari con cui i Piemontesi l'hanno definito fin dal 1500. Che il Dolcetto fosse un vitigno fortemente legato alla tradizione delle colline piemontesi è ampiamente e chiaramente testimoniato da quando gli studi di ampelografia hanno cominciato ad analizzare il patrimonio viticolo del Piemonte. Nel 1798, il Conte Nuvolone, nella sua *"Istruzione sulla coltivazione della vite e sul modo migliore di fare e conservare i vini",* scrisse "*Dolcetto: vitigno della tradizione piemontese"*. In provincia di Cuneo, dove questa varietà è da secoli presente, i due paesi di riferimento per la concentrazione della sua coltivazione sono Dogliani e Diano.

Nell'Alessandrino la zona storica del Dolcetto è quella di Ovada.

Il Dolcetto è attualmente il terzo vitigno del Piemonte, dopo Barbera e Moscato, per l'estensione della sua coltivazione.

Nel 1824, De Chabrol de Volvic, un funzionario francese del Dipartimento, scriveva di Dogliani e delle sue produzioni di grano, di mais, di legumi, di castagne, di frutta e *"soprattutto vino"*. Rilevava che sul territorio di Dogliani predominava la cultura della vite e che la sua produzione eccedeva la metà del reddito di tutte le altre. Il vino prodotto è definito *"abbondante e di buona qualità"*. Il vitigno indicato come il più diffuso e più produttivo è il dolcetto, che costituiva i cinque sesti della totalità dei vigneti.

A Dogliani la lunga tradizione venne raccolta dal primo presidente della Repubblica Italiana, Luigi Einaudi, che nella sua storica proprietà vitivinicola doglianese dagli inizi del 1900 fece piantare migliaia di barbatelle di Dolcetto.

Il Dolcetto è attualmente il terzo vitigno del Piemonte, dopo Barbera e Moscato, per l'estensione della sua coltivazione.

CONCLUSIONI

L'analisi storica ha evidenziato il 1800 come epoca che rappresenta la base fondamentale su cui poggia la moderna e prestigiosa realtà della vite e del vino piemontesi. La svolta radicale avvenuta in quel secolo vede concorrere, rafforzandosi e integrandosi l'un l'altro, molti eventi di tipo economico, sociale, agronomico, tecnico e scientifico. Fino alla metà del 1800 era infatti proseguita senza rilevanti modifiche una lunga tradizione di viticoltura empirica che risaliva al periodo medievale. Successivamente si produssero grandi mutamenti in rapporto a quanto comportò nei vigneti la diffusione di oidio, fillossera e peronospora, provenienti da oltreoceano e prima sconosciuti in Europa.

Del modo in cui agirono queste ampelopatie e sulle ragioni per le quali ebbero tanta influenza sul nuovo volto della viticoltura, si può portare qualche esempio essenziale: l'oidio determinò la necessità dei primi interventi chimici in vigneto, la fillossera indusse ad adottare la prima misura di difesa biotecnica, ovvero l'innesto su portinnesti resistenti, la peronospora incitò agli studi dei prodotti e delle modalità da impiegare per combattere l'infezione. Queste avversità ebbero anche una fortissima influenza sui mutamenti della piattaforma ampelografica, portando un cambiamento delle varietà di uve coltivate e nella diffusione della loro coltivazione.

In questa opera di ricostituzione, la biodiversità genetica si ridusse drasticamente, molti vitigni andarono perduti; si privilegiarono varietà più resistenti e produttive e si introdussero varietà estere.

Negli Annali di Viticoltura ed Enologia di G. E. Cerletti e negli Annali dell'Accademia Agricoltura di Torino pubblicati in quel periodo, si possono ritrovare le discussioni fondamentali affrontate dagli esperti in quel periodo di grandi cambiamenti.

I miglioramenti qualitativi venivano preconizzati con degli importanti aggiustamenti di politica agraria, che erano rivolti essenzialmente su quattro fronti:

- la razionalizzazione della scelta dei vitigni maggiormente idonei alla vinificazione (studiando il collegamento esistente tra il tipo di vitigno e la qualità del prodotto finito; confrontando la qualità relativa delle diverse varietà, e soprattutto spingendo verso la vinificazione in purezza)
- la definizione della piattaforma ampelografica disponibile (che appariva caotica, confusa da sinonimie e da nomi di varietà locali)
- lo sviluppo delle conoscenze tecniche dei vinificatori (attraverso l'organizzazione di Conferenze enologiche, l'istituzione di pubbliche Sale di lettura e di Biblioteche tecniche circolanti)
- la sperimentazione di nuove tecniche enologiche (anche attraverso la creazione delle stazioni enologiche sperimentali)

Gli ultimi anni del 1800 videro una effettiva concretizzazione di tali obbiettivi.

Questo grande e impegnativo lavoro portò a delineare aree d'eccellenza vitivinicola che si sono mantenute fino ad oggi.

BIBLIOGRAFIA

Berta P., Mainardi G., 1997. Storia regionale della vite e del vino in Italia: Piemonte. Milano: Ed. Unione Italiana Vini.

Berta P. 2004, L'apparecchio Martinotti e la storia dello spumante piemontese. In: Il vino piemontese nell'800. Alessandria: Dell'Orso

Comba R., 1990. Vigne e Vini nel Piemonte Medievale. Cuneo: L'arciere.

Comba R. 1994. Vigne e Vini nel Piemonte Antico. Cuneo: L'arciere.

Di Rovasenda G., 1877. Saggio di una Ampelografia Universale. Roma-Torino-Firenze: Ermanno Loescher,

Eynard I. ed al., 1981. Il moscato bianco di Canelli fra i vitigni con uva a sapore moscato. In Atti del convegno sul Moscato di Canelli. Canelli, 16 maggio 1981 pagg 19-85

Fantini L., 1879. Monografia sulla viticoltura ed enologia nella provincia di Cuneo. Torino, 1973: Cavalieri del Tartufo e dei vini d'Alba.

Gallesio G., 1839. Pomona Italiana, ossia Trattato degli Alberi da Frutto. Pisa, 1817 – 1839. Pisa, 1817 – 1839: N. Capurro.

Gay-Eynard G., Arzone A., Gobetto M., 2004. Le trasformazioni della viticoltura piemontese causate da parassiti e insetti giunti dall'America: dopo l'oidio, la fillossera. In: Il vino piemontese nell'800. Alessandria: Dell'Orso.

Lisa L., Eynard I., 1980. La viticoltura collinare nei suoi aspetti colturali e di meccanizzazione. In "Atti convegno nazionale sulla meccanizzazione della viticoltura collinare" Torino pagg. 15-53

Lozato-Giotart J. P., 1988. Il vigneto di Asti. Asti: CCIAA.

Mainardi G., 2003. Storia dell'enologia. In Enotria: Corriere Vinicolo

Mainardi G., 2004. Il podere reale di Pollenzo, centro di sperimentazione enologica nel Piemonte dell'Ottocento. L'attività di Paolo Francesco Staglieno, enologo di Carlo Alberto. In: Pollenzo. Una città romana per una real villeggiatura romantica" a cura di G. Carità. Savigliano: L'Artistica.

Mainardi G., Berta P., 2000. Scelte socioculturali e tecniche nella storia del Moscato di Canelli. In: Quaderni della scuola di spec. In scienze viticole e enologiche 1999. Università di Torino. pp 5-14

Mainardi G., Berta P., 2005. Le strutture delle cantine: aspetti storici ed architettonici. OICCE Times N. 22. Pagg. 32-38

Mainardi, G., Berta P., 1991. Il vino nella storia e nella letteratura. Bologna: Edagricole.

Mondini S., 1900. La viticoltura e l'enologia in Italia. Roma: Tip. dell'Unione Coop. Edit.

Rapetti V., 1984. Uomini, collina e vigneto in Piemonte, da metà Ottocento agli anni trenta. Alessandria: Dell'Orso.

Les informations fournies sur le terroir ont-elles une influence sur le choix d'un vin ? Une approche séquentielle de l'analyse conjointe

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Mots-Clés : terroir, analyse conjointe, communication, consommateurs, sensoriel

Abstract
The "terroir" concept in wine includes 4 dimensions: soil, territory, know-how and identity. This is a very complex notion to be understood by consumers and we know that it needs to be clarified for most of them. It's difficult to clearly identify what is the relative importance of the different dimensions on which consumers make their choices between different wines: sensory preference, "Appellation d'Origine Contrôlée" (label quite close: "protected designation of origin") and "Terroir". One of the consumer methodologies to look into that issue is the conjoint analysis, including products tasting. References on repeated experiments are rarer and very often linked to the study of the impact of consumer information. Our objectives were double: - to test if a communication has a positive impact on consumer appreciation and – to check if conjoint analysis is a good way to measure the consumer interest for "terroir" concept.
Our experiment aimed to measure if the "terroir" dimension is important in consumer preference for wine after tasting, and to check if information on "terroir" concept was efficient. First field took place in June, 130 consumers were asked to taste 11 different wines, combinations of 3 variables: wine (fruity, astringent, woody), appellation d'origine contrôlée (Loire valley, Saumur, Saumur Champigny), communication on label and back label (on the bottle back: sensory aspects, soil, or terroir). We used a conjoint analysis with an incomplete experimental design. Consumers scored wines on a continuous preference scale. Second field took place in November, including the same consumers, half of them having been exposed to a personal communication to clarify terroir role in wine quality. Results show consumers perceive wines in a different way depending on appellation contrôlée and communication on back label. 85 people made strong differences between wines, depending on what is written on the bottle labels.
The results show that conjoint analysis is an interesting way to explore interactions between wine sensory properties and what is written on the bottle as communication or appellation. Depending on consumers, label and appellation can have a strong influence on wine appreciation. The impact on one single set of information on terroir is not clearly demonstrated. Therefore, further research should be done, as we know that the way of giving information and the frequency has a large influence.

Introduction

La connaissance du ressenti des consommateurs est un enjeu important pour la filière vin. L'écoute, la compréhension des clients sont des gages de réussite pour la commercialisation des vins. La typicité des vins, son image, sa connaissance font partie probablement des facteurs déterminants de l'acte d'achat. Si les marques ont bâti leur image sur leur savoir faire individuel, les produits d'Appellation d'Origine ont acquis une notoriété sur les notions de typicité et de terroir. Celles-ci interviennent probablement de façon significative dans la construction de l'acte d'achat. Ces dernières peuvent également jouer un rôle sur la perception sensorielle ressentie par le consommateur.

Comment ces différentes spécificités d'un type de vin : sensorielles, historique, géographique, analytiques, techniques influencent-elles l'appréciation du consommateur final et son acte d'achat ? Quel est l'impact d'un discours sur le terroir auprès des consommateurs ? Comment rendre efficace la communication sur le terroir ?
L'analyse conjointe permet de comprendre la "structure de préférence" du consommateur. Dans le cas du vin, différents éléments de la qualité influencent l'appréciation globale : la qualité perçue (toutes les informations données sur le vin contribuent à cette qualité perçue) et sa qualité ressentie lors de la dégustation. Selon les individus, plus ou moins d'importance sera donnée à tel ou tel élément de la qualité, le consommateur hiérarchise. L'analyse conjointe mesure non seulement l'importance de chaque facteur dans la décision générale, mais aussi l'influence des différents niveaux de chaque facteur dans la formation de la préférence générale. Cette

méthode est utilisée en marketing sur les différents attributs du mix marketing mais moins souvent en intégrant une variable sensorielle, notamment une dégustation.

Les objectifs de ce projet sont donc à la fois de tester l'analyse conjointe ou trade off qui est une méthodologie devant permettre de faire réagir simultanément les consommateurs à plusieurs variables, ici à trois facteurs dont une variable sensorielle. Par ailleurs, l'intérêt d'une communication sur la bouteille faisant référence aux attributs du terroir a été évalué et comparé à l'impact de l'appellation notée sur la bouteille et les caractéristiques intrinsèques du vin. Enfin, nous avons étudié la performance d'un message commercial expliquant aux consommateurs ce qu'est le terroir.
Pour mener à bien ce projet, un rapprochement avec la société Alliance Loire a été réalisé pour bénéficier de leur expertise marketing, de leur vin et d'étiquettes.

Le plan expérimental

Dans le cas de cette expérimentation, nous avons décidé de travailler avec 3 variables à trois niveaux pour répondre à nos objectifs :

La Variable Vin :

Trois vins différents ont été proposés aux consommateurs pour étudier combien leurs caractéristiques sensorielles influencent lors de la consommation du produit. Des vins aux caractéristiques très marquées ont ainsi été sélectionnés

Un vin Fruité : Saumur Champigny Réserves 2006
Un vin Puissant : Saumur Champigny Les Poyeux 2005
Un Vin Boisé : Saumur Champigny Emotions 2005

Le Panel Expert du laboratoire GRAPPE a dégusté les vins et confirmé les caractéristiques de ceux–ci.

La Variable Information Etiquette :

Dans cette variable, nous avons fait varier les informations données aux consommateurs en intégrant soit une information liée aux terroirs (le sol ou la parcelle) soit une information liée au cépage et aux caractéristiques sensorielles. Pour se faire, l'étiquette comportait un nom faisant référence aux messages présentés sur la contre étiquette.

Les Pouches (parcelle) : Cette cuvée est issue exclusivement de la parcelle dénommée Lieu-dit Les Pouches. Niché au cœur de la région, c'est un minuscule vignoble de 6 hectares. Ses qualités uniques en font une parcelle rare et étonnante donnant naissance à des vins remarquables.

Les Tuffeau (sol) : C'est son sol de calcaire tendre si particulier qui donne son caractère à cette cuvée. Egalement appelée pierre de tuffe ou tuffeau, cette roche rassemble des conditions idéales et apporte richesse et structure au vin.

Cabernet franc (sensoriel) : Ce vin présente une belle robe rouge rubis Au nez, les parfums intenses de fruits rouges frais (cerise, framboise) se mêlent aux notes douces d'épices. En bouche, il révèle son ampleur et sa richesse et associe ses tanins soyeux à des arômes fruités. Servi légèrement frais, il accompagnera délicieusement les charcuteries et les fromages de la région.

La Variable Appellation

L'appellation d'origine contrôlée est par définition un élément important dans la définition du terroir. Aussi, nous avons souhaité faire varier celle-ci sur les étiquettes pour mesurer son importance dans l'appréciation du produit. Trois niveaux ont ainsi été définis :

Vins du Val de Loire
Saumur
Saumur Champigny

En théorie, le plan d'expérience idéal découlant de ces variables à trois niveaux est un plan à 27 produits. Mais étant donné que le projet comporte une dégustation des vins avec des consommateurs, un bloc incomplet équilibré à été mis en place pour réduire le nombre de produits tout en permettant de mesurer l'impact des différents facteurs et niveaux sur la perception. Les consommateurs ont donc été confrontés à 11 produits différents composés de couples vin et bouteilles différents comme suivant :

Profil	VIN	COM	APP
P01	Puissant	Parcelle	VDL
P02	Puissant	Senso	SAU
P03	Boisé	Parcelle	SAU
P04	Puissant	Sol	VDL
P05	Puissant	Parcelle	S_CH
P06	Boisé	Senso	S_CH
P07	Fruité	Senso	SAU
P08	Boisé	Senso	VDL
P09	Boisé	Sol	SAU
P10	Fruité	Parcelle	VDL
P11	Fruité	Sol	S_CH

Figure 1 : Liste des trios présentés aux consommateurs

Afin de mesurer l'impact d'un discours commercial « vantant » ou présentant le terroir, une communication sur le terroir a été envoyée à la moitié des consommateurs après la première dégustation. Cette communication s'intitulait : « le terroir : une richesse issue de la diversité des sols et des savoir-faire des vignerons » et présentait les différents attributs du terroir. Elle a également été donnée à lire à cette moitié de dégustateurs en début de deuxième session.

Les consommateurs et les conditions de dégustation

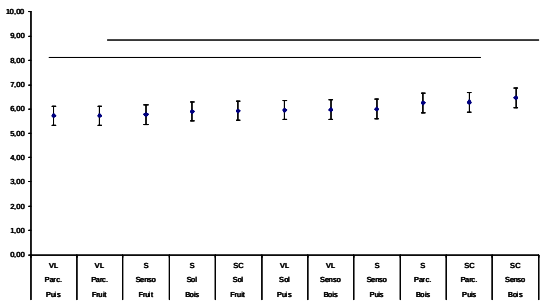
Cent trente quatre consommateurs de vins angevins âgés de 20 à 70 ans dont 52 femmes et 82 hommes ont participé à cette expérimentation. Il leur était demandé : « Nous allons vous fournir de manière successive différents vins rouges, accompagnés de leur bouteille, afin que vous puissiez connaître le vin que vous dégustez. Nous vous demanderons de bien vouloir goûter chaque vin et de prendre connaissance de l'étiquette et de la contre-étiquette qui l'accompagnent, afin d'évaluer ensuite l'ensemble du produit selon votre appréciation globale. » Ainsi, ils devaient évaluer simultanément la bouteille et le verre de vin l'accompagnant sur une échelle hédonique à 11 points.

Les dégustations ont eu lieu dans la salle d'analyse sensorielle de l'ESA en lumière blanche. Les vins étaient servis en verres INAO.

Comme indiqué précédemment, les consommateurs ont participé deux fois à l'expérimentation : une fois en juin 2008 sans information préalable et une deuxième fois en décembre 2008 avec une information sur le terroir envoyée et présentée à la moitié des consommateurs.

L'impact des différentes variables sans communication (Session 1)

De manière classique, lors d'un test consommateurs, la première analyse se fait avec tous les consommateurs confondus en observant la moyenne d'appréciation pour chaque produit. La figure 1 montre que tous consommateurs confondus, il n'y a pas beaucoup de différence entre les trios « vin-étiquette- appellation ». Le vin boisé, présenté avec une



bouteille faisant référence au cépage et avec Saumur Champigny inscrit sur la bouteille a été un peu plus apprécié mais sans que ce soit significatif

Figure 2 : Moyenne par vin tous consommateurs confondus (n=134)

La méthode de l'analyse conjointe permet cependant d'aller zoomer sur les différentes variables et les différents niveaux et de détailler l'impact de chacun sur l'appréciation des consommateurs. Cette analyse montre que ces différentes variables n'ont pas la même importance dans la construction de la perception. Le facteur Appellation pèse 46 % dans l'appréciation, le facteur Vin 41% et le message Terroir ou Senso indiqué sur la bouteille seulement 12% quand on s'intéresse à tous les consommateurs simultanément. Il apparait ainsi que l'impact de l'information présente sur l'étiquette semble peu important tous consommateurs confondus et que l'appellation influence fortement les consommateurs ainsi que les qualités intrinsèques du vin.

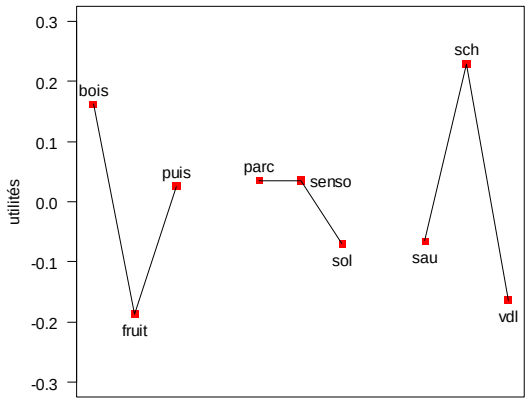


Figure 3 : Utilités calculées pour chaque niveau avec tous les consommateurs

Cet effet se manifeste en observant les différences d'appréciation entre les différents niveaux. Le Saumur Champigny est plus performant que le Saumur et que le Vin du Val de Loire. Le vin boisé est plus apprécié que le puissant et que le vin fruité. On observe peu d'influence de ce qui est écrit sur l'étiquette.

Ces résultats étant obtenus à partir de l'analyse de tous les consommateurs et partant du fait que les consommateurs peuvent avoir des appréciations différentes, nous avons utilisé une Classification Ascendante Hiérarchique pour regrouper les consommateurs ayant des appréciations similaires. Cette analyse à mis en évidence quatre groupe de 23 à 38 consommateurs.

L'analyse conjointe permet à nouveau de détailler le comportement de chacun des groupes et d'observer que les consommateurs ne sont finalement pas sensibles tous aux mêmes critères. Le niveau d'influence moyen de chaque variable au sein des groupes est beaucoup plus important que lors du traitement global, on peut en effet constater jusqu'à deux points d'écart moyen entre deux niveaux d'un facteur au sein d'un groupe (exemple du groupe 2, variable sensorielle) :

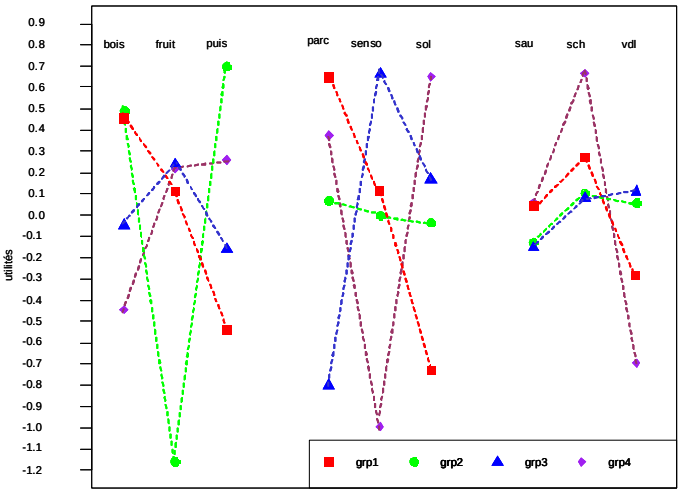


Figure 4 : Utilités par niveau et par groupe

Le groupe 1 (n=38) préfère les vins avec une étiquette sur la parcelle et le goût bois. Ces consommateurs sont très sensibles à l'information sur l'étiquette et aux caractéristiques du vin avant l'appellation. Le groupe 2 (n=36) préfère les vins puissants ou boisés et sont insensibles deux autres types d'informations. Ce sont des consommateurs qui se sont visiblement concentrés sur les qualités intrinsèques des vins. Le groupe 3 (n=37) préfère les vins avec une étiquette sur l'aspect sensoriel du produit, ces consommateurs ont porté moins d'importance aux caractéristiques des vins et à l'appellation notée sur la bouteille. Le groupe 4 (n=23) préfère les vins avec une étiquette sur la parcelle ou le sol et l'appellation Saumur Champigny. Ces derniers consommateurs ont rejeté le vin boisé et semblent plus sensibles aux notions de terroirs.

L'impact de la communication présentant ce qu'est le terroir (Session 1 vs Session 2)

Comme indiqué précédemment, la moitié des consommateurs a reçu une information sur le terroir avant la deuxième dégustation. Il a été demandé à ces consommateurs de relire cette note avant la dégustation.

A l'issue de cette deuxième dégustation, une analyse de la variance a permis de valider si l'information avait eu un impact significatif sur le comportement des consommateurs. Il apparait que les consommateurs qui n'ont pas reçu d'information n'ont pas changé leur comportement ($P_{Interaction\ Communication\ x\ Session}$). En revanche, les autres consommateurs ont légèrement modifié leur notation lors de la deuxième dégustation ($P_{Interaction\ Communication\ x\ Session}$ = 0,09). Il apparait que ces consommateurs ont baissé leurs notes pour les produits avec une « information sensorielle » sur l'étiquette.

Malheureusement, étant donné les faibles effectifs dans les clusters réalisés après la première dégustation, il n'a pas été possible de zoomer sur les clusters de consommateurs de la partie 1.

Conclusions

L'analyse conjointe apparaît être une bonne méthode pour étudier l'impact de différents facteurs sur la perception des consommateurs. Sa principale limite réside dans le nombre important de produits à tester pour valider les hypothèses ce qui n'est pas toujours possible avec une dégustation mais l'utilisation de bloc incomplet équilibré contourne en partie ce problème. En revanche pour valider l'impact du message sur l'étiquette sans dégustation, cette méthode doit être vraiment pertinente.

L'impact des informations présentes sur la bouteille est très divers selon les consommateurs. Dans certains cas, l'étiquette et plus encore l'appellation ont une forte influence sur la perception du vin. Ces résultats seront à confirmer sur un nombre de consommateurs plus important pour permettre de mieux étudier les sous groupes obtenus.

Dans les conditions du test : la communication présentant « le terroir » a eu un impact limité. Cela mérite d'être exploré à nouveau soit en testant un autre type de communication car celui-ci n'était peut être pas assez « commercial » ou en envisageant une exposition répétée pour renforcer cet impact.

Trans-resveratrol and ε-viniferin in red Georgian wines

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ABSTRACT

At present, out of the rich spectrum of the phenol substances of Georgian red wines, stilbens are not thoroughly studied. In 1991-1994, we isolated and identified from *Rkatsiteli* vine species trans-resveratrol, ε-viniferin and two Tetramers of resveratrol. The dependence of trans-resveratrol concentration in the red Georgian wines on the vine species and wine type has been identified. Of naturally semi-sweet, semi-sweet, dry and naturally cleared fortified wines, trans-resveratrol was found in the greatest concentrations in the fortified wines. 0,78-3,20 mg/l (in *Saperavi*); 0,69-2,62 mg/l (Otskhanuri Sapere); 0,65-2,87mg/l (Cabernet Sauvignon); 0,47-1,92 mg/l (Tavkveri). ε-viniferin in self-cleared dry bulk wine of *Saperavi* and *Cabernet Sauvignon* amounts to 0,98 mg/l and 0,40 mg/l, respectively.

Key words: Red wine, Trans-resveratrol, ε-viniferin.

RESUME

A nos jours parmi le spectre riche de produits phénoliques des vins rouges géorgiens les stilbènes ne sont pas encore étudiés complètement. En 1991-1994 il a été distingué et identifié du cépage Rkatsitéli le trans-resvératrol, la ε-viniférine et deux tetramètres de resvératrol. Il est découvert que dans les vins rouges géorgiens le taux de concentration de trans-resvératrol dépend des cépages de vignes et des types de vins. Le taux de concentration est élevé dans les vins naturellement demi-sucré, demi-sucré, sec et les vins fortifiés filtrés naturellement. Sapéravi 0,78 – 3,20 mg/L ; Otskhanouri Sapéré 0,69 – 2,62 mg/L ; Le taux de ε-viniférine dans les vins fins de Sapéravi et Cabernet Sauvignon filtrés naturellement, secs est : 0,98 mg/L et 0,40 mg/L.

Mots clés : Vin rouge, Trans-resvératrol, ε-viniférine.

INTRODUCTION. Georgia is the homeland of vine and wine. The gene pool of Georgian vines including approximately 525 unique species of white and versicolored vine has been tried by the Georgians for centuries. The diversified technologies to produce different types of wine developed as a result of the rich experience, have been shifted to one generation to another and as time went, they were perfected and new technologies were developed. In this respect, the merit of Georgian scientists as the foundation of Georgian viticulture and winemaking is worth mentioning. The curative properties of grape and wine always attracted a great deal of attention of different scientific branches. This is particularly true with red Georgian wines known for their curative properties from the ancient times and are a panacea in Georgian folk medicine, particularly, the bread soaked in red wine. The scientific progress allowed thoroughly explaining the close connection between the useful properties of red wine and its rich chemical content.

The search for the fundamentals of the phenomenon of the "French paradox" was followed by the intense study of the content of red wines on an international scale. Numerous experiments have demonstrated the leading role of biologically active phenol substances in the formation of the curative and nutritive value of red wines. Identification of stilbens and particularly resveratrol, which is their monomer representative, and their determination in red and pink wines made of different vine species in a range of countries is associated with these studies. **(Waterhouse,1993; Roggero, Archier, 1994; .Lamuela-Raventos et al.,1995; Romero-Perez et al.,1996; Lamuela-Rawentos,** Waterhouse, 1993; **Lamikarna, et al.,1996).**The further studies, besides resveratrol, identified its derivatives: piceids, dimmers: ε-viniferin and δ-viniferin; trimer α-viniferin, etc. as well as other stilbens. (Ribeiro de Lima et al.,1999; Guebailia et al., 2006). Out of resveratrol isomers, it is red wines with the higher content of trans-resveratrol if compared to cis-resveratrol. At the same time, trans-resveratrol is biologically more active than cis-resveratrol. The established diversified biological activities of stilbens greatly make for the positive effect of red wines against cardiovascular, cancerous, thrombotic and other diseases(**Blond et al., 1995; Klatsky et al.,1997; Yang et al., 1997; Szmitko, Verma, 2005**; **Balestrieri et al., 2008).**

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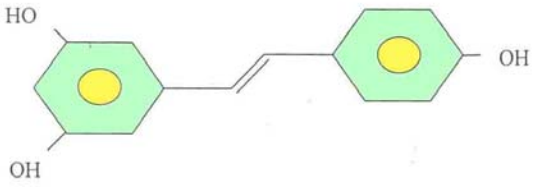
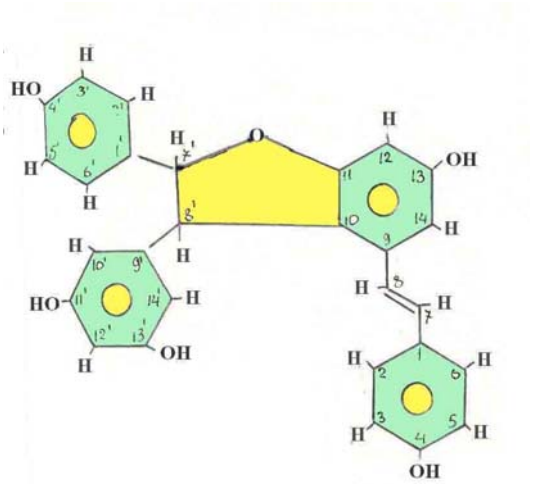
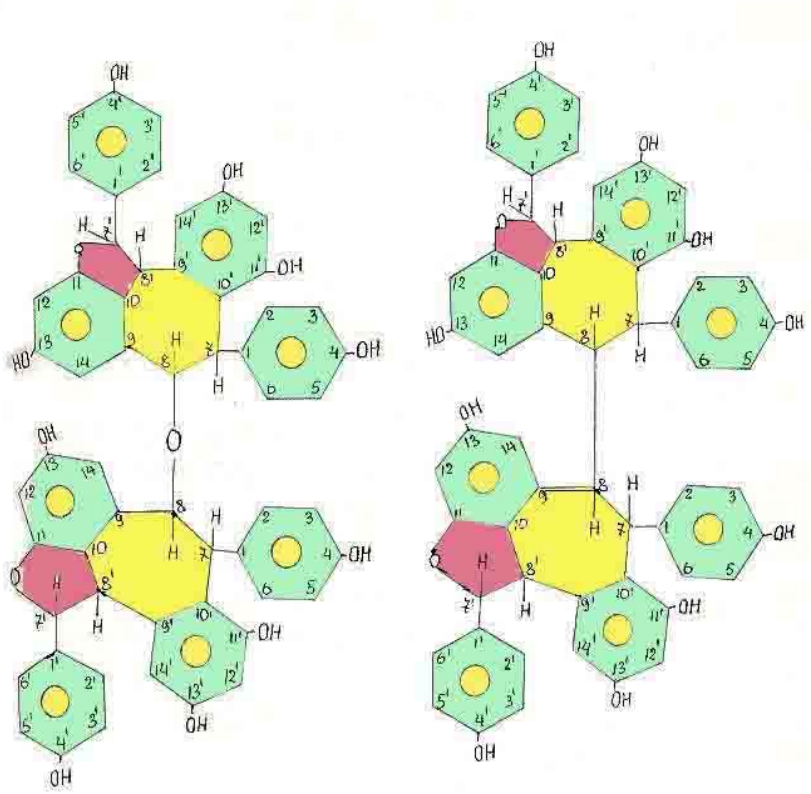
We started to study stilbens in 1991-1994, and individually isolated and identified trans-resveratrol, ε-viniferin and two tetramers of resveratrol from 1-year-old *Rkatsiteli* (*Vitis vinifera* L.) vine shoot (Bezhuashvili et al., 1991; Bezhuashvili et al., 1997; Bezhuashvili, 1994).



Trans-resveratrol



ε-viniferin



Tetramer-I **Tetramer-II**

Then, we continued studying them in red Georgian wines. It should be noted that a Georgian scientist Durmishidze S. and his colleagues have made a great contribution to the study of the diversified spectrum of phenol compounds of the vine species spread in Georgia (Durmishidze, Khachidze, 1979; 1985). Out of phenol compounds, proantocyanidines (oligomeric and polymeric), phenolic acids, anthocyanins, catechines, flavonols, etc. have been identified. *Saperavi* species growing in different regions of Kakheti have been studied by us, and we identified the differences between the chemical compositions of bulk wines. The property of an early (9-month-long) stabilization of *Saperavi* growing in Khashmi has been identified in Kakheti Region. This property is demonstrated by the wine changing for the coloured complex of anthocyanins by preserving its intense ruby color, with the colouring intensity coefficient T<1 (Kvlividze, Bezhuashvili; 2005-a, b, c) like in young wines. Besides, we have studied the anthocyanins and proantocyanidines of the grape skin of red-grape vine species – *Saperavi*, *Saperavi Budeshurisebri*, *Otskhanuri Sapere*, *Cabernet Sauvignon*, *Tavkveri* and *Shavkapito* (*Vitis vinifera* L.) and dry table wines, as the properties of generic purity (Bezhuashvili et al. 2009). We have taken the ratio between the oligomeric proantocyanidines and polymeric proantocyanidines as the conditional coefficient K=OPC/PPC, with K<1 in the experimental wines made of technical red-grape species and commercial wines (Bezhuashvili et al. 2008).

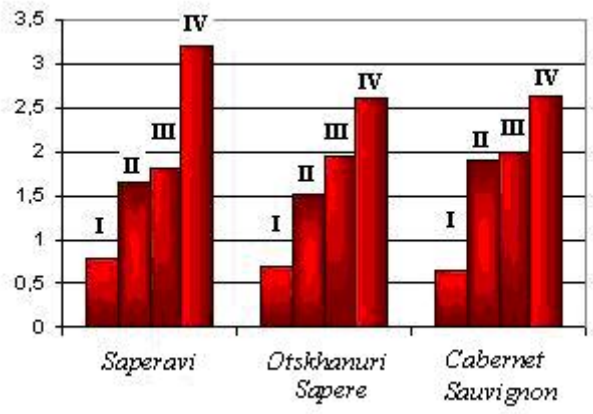
Phenol compounds have intense antioxidant activities and naturally, their high content in red Georgian wines make for their high curative and nutritive value. All these properties are the principal characteristics of the red Georgian wines as of the functional products.

By considering the fact that the stilbens in red Georgian wines are less studied, we continued their study and studied trans-resveratrol at the first stage. At present, we are

engaged in an intense study in the direction of "Wine and health" to identify specifically the stilben spectrum in red Georgian wines. At present, we have identified and determined resveratrol dimmer ε-viniferin.

MATERIALS AND METHODS. We used the different types of naturally cleared red wines made with *Saperavi*, *Otskhanuri Sapere*, *Cabernet Sauvignon* and *Tavkveri* species as the objects of the study, in particular, dry table, naturally semi-sweet, semi-sweet and fortified wines. For qualitative and quantitative analyses of trans-resveratrol, we extracted the wines in advance with ethyl acetate and used the gained fraction for analyses. We used thin-layer chromatography with silufol plates (20 cm x 20 cm), system: chloroform : methanol (80:20) and denitrated sulfanilic acid as a developer. We defined the quantity of trans-resveratrol by high-efficiency liquid chromatography in terms of gradient. The column of Nucleosil C_{18}, eluent A: water + H_3PO_4; eluent B: acetonitrile+H_3PO_4, pH=3,5-4,0. Out of naturally cleared dry table bulk wines made with the 2008 harvest of Saperavi and Cabernet Sauvignon, we isolated the total stilbens with further treatment of their ethyl acetate fractions (Ribeiro de Lima et al., 1999). We analyzed the gained fraction by high-efficiency liquid chromatography in terms of gradient (the chromatograph made by "Varian"); UV detector/visible spectrum, column – Microsorb 100 C18, 250X4,6 LxId (mm); 5μm – Particle Size. Eluent A: TF (trifluoroacetic acid) 0,025% water solution; eluent B: ACN/AA, 80/20 (v/v); (Guebailia, et al., 2006).

RESULTS AND DISCUSSION. The gained results demonstrate that the concentration of trans-resveratrol in bulk wines depends on different factors, one of which is the generic factor. In this respect, *Saperavi* and *Otskhanuri Sapere* are the most obvious examples. The concentration of trans-resveratrol in bulk wines also changes according to the sugar content of the must. In particular, within the range of 19,1-26,0%, the content of trans-resveratrol in the red wines made of *Saperavi* with different sugar contents changes from 1,69 mg/l to 2,87 mg/l; for *Otskhanuri Sapere* (with the sugar content of 19,2%-22,4%), it is 1,25 mg/l 1,95 mg/l and for *Cabernet Sauvignon* (with the sugar content of 19,3%-22,7%), it is 1,15 mg/l to 2,25 mg/l. At this point, in addition to the generic factor, the factor of alcohol-content of the pomace fermented is obviously seen. As for the wine type, it is seen as the factor making the wines made with the same grape species significantly different from one another with their concentration of trans-resveratrol. This is proved by the diagram in "Fig. 1".

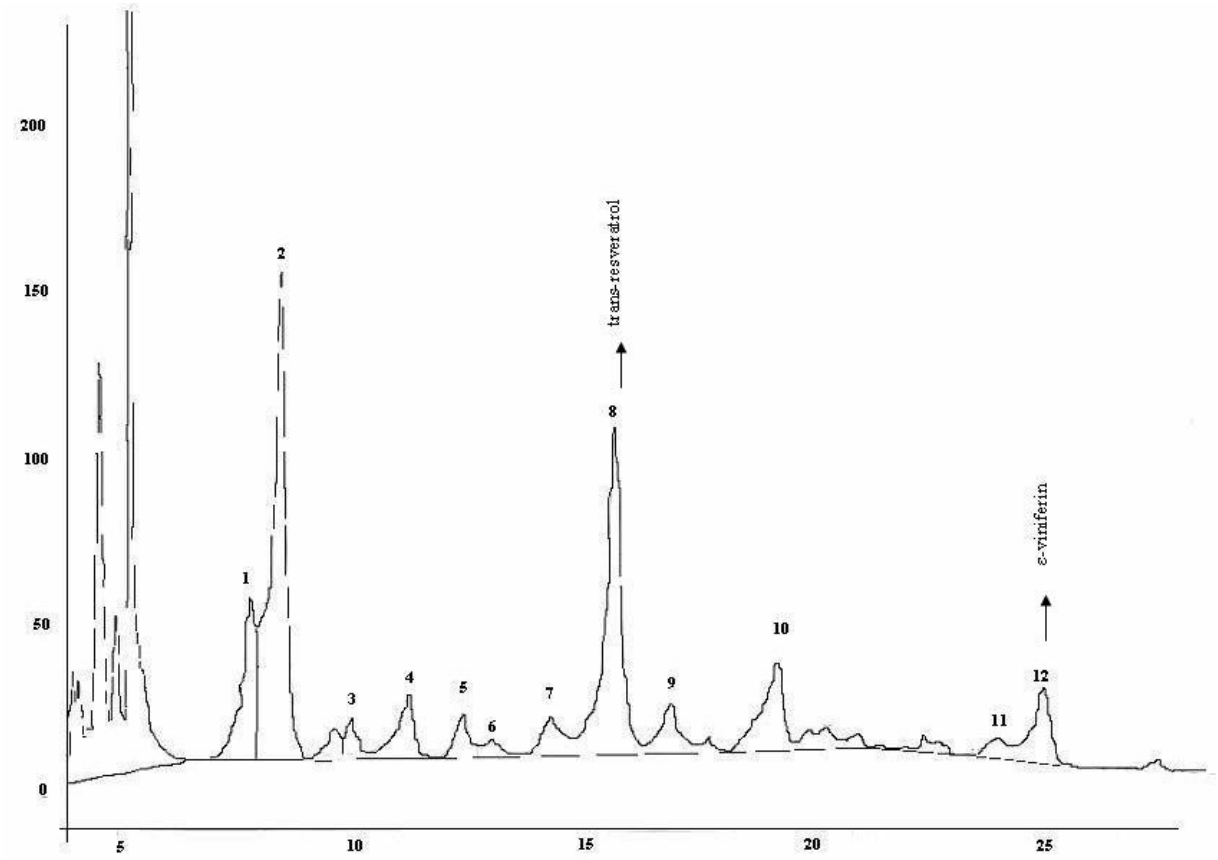


"Fig. 1". Change of the concentration of trans-resveratrol according to the types of wine. I – naturally semi-sweet, II – semi-sweet, III – dry, IV – fortified.

Among the naturally semi-sweet, semi-sweet, dry and fortified wines, the concentration of trans-resveratrol is the highest in the fortified wine and is the least in the naturally semi-sweet wine. Besides the above-mentioned factors, temperature and squeezing and mixing of

fermented pomace were also identified as the factors affecting the quantity of trans-resveratrol in bulk wines (Kokhtashvili, Bezhuashvili; 1998, Kokhtashvili et al. 2002; Kokhtashvili, 2006).

The data of thin-layer and liquid chromatographs of the total stilben fraction gained from dry table bulk wines made of *Saperavi* and Cabernet Sauvignon prove their variety. The stilben spectrum, besides trans-resveratrol and ε-viniferin, is presented by some other stilbens ("Fig. 2"). The concentration of ε-viniferin in *Saperavi* bulk wines is 0.98 mg/l and is 0.40 mg/l in *Cabernet Sauvignon*. At present, an intense research is carried out to study the stilben spectrum in red Georgian wines.



"Fig. 2". Liquid chromatogram of stilben fractions gained from dry table bulk wines of *Saperavi*.

CONCLUSIONS. The content of biologically active high-oxidant trans-resveratrol and ε-viniferin in red Georgian wines rich in phenol substances is a certain proof of the useful properties of the given wines. Thoroughly studied stilben spectrum in red Georgian wines as that of the product of a functional designation, is an important bio-chemical marker.

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Bibliography

1. Balestrieri M., Schiano C., Felice F., Casamassimi A., Balestrieri A., Milone L., Sarvillo L., Napoli Cl. 2008. Effect of low doses of red Wine and Pure Resveratrol on Circulating Endothelial Progenitor Cells. Journal of Biochemistry, 143 (2): pp. 179-186.
2. Bezhuashvili M., Mujiri L., Kurkin V., Zapesochnaya G. 1991. Resveratrol of vine. Timber chemistry, №6: pp. 75-76.
3. Bezhuashvili M. 1994. Development of theoretical basics for vine and oak timber lignin and identification of the ways to use the gained products. Doctoral thesis, Tbilisi, 260 p.
4. Bezhuashvili M., Mujiri L., Shashkov A., Chzhov O., Stomakhin A. 1997. Stlben tetramers of one-year vine shoots. Bioorganic chemistry, vol. 23, №12: pp. 979-987.
5. Bezhuashvili M., Deisadze I., Kobaidze T. 2008. Correlation of proanthocyanins and selected sorts of red bulk wines made of technical sorts and hybrids – immediate producers. Winemaking and Viticulture, №3.: pp. 20-22.
6. Bezhuashvili M., Deisadze I., Shubladze L., Sikharulidze T. 2009. Chromatographic profile of anthocyanins of the grape of red technical sorts and wines made of them. Magarach Winemaking and Viticulture, №3: pp.27-29.
7. Bezhuashvili M., Vepkhishvili N., Kobaidze T. 2010. Identification and determination of biologically active ε-viniferin in grapes and bulk wines of Saperavi and Cabernet Savignon sorts. Collection of Scientific Works of the National Institute of Grape and Wine "Magarach", Yalta,pp.71-75.
8. Bezhuashvili M., Vepkhishvili N., Kobaidze T. 2010. Identification of resveratrol dimmer ε-viniferin in versicolored vine species. Georgian National Academy of Sciences, Republican Scientific Conference "Biologically active natural and synthetic substances". Tbilisi, March 30. The Theses. pp. 25-26.
9. Blond Y., Denis M. Bezard I. 1995. Antioxidant action of resveratrol in Lipid peroxidation. Sciences Aliments, 15,: pp. 347-358.
10. Durmishidze S., Khachidze O. 1979. Chemical composition of grape. Metsniereba. Tbilisi: Editor Lashkhi A.
11. Durmishidze S., Khachidze O. 1985. Biochemistry of vine. Metsniereba. Tbilisi: . Editor Kezeli T.
12. Durmishidze S. 1955, Tannin and anthocyanins of the vine and wine. Publishing house of the Academy of Sciences of the USSR, Moscow: Editor Sisakian H.
13. Guebailia H., Chilra K., Richard T., Mabrouk T., Furiga A., Vitrac X., Monthi Jean-Pierre, delaunay Yean-Claude, Merillon Yean-Michael. 2006. Hopeaphenol: The first resveratrol Tetramer in Wines from North Africa. J. Agric. Food Chem., 54: pp. 9559-9564.
14. Klatsky A., Armstrong M., Friedman G. 1997. Red wine, white wine, liquor, beer and risk for coronary artery disease hospitalization. American Journal of Cardiology., 80: pp. 416-420.
15. Kokhtashvili M., Bezhuashvili M. Identification of trans-resveratrol in some red grape species. Georgian Engineering News. 1998, №4: pp. 104-106.
16. Kokhtashvili M., Bezhuashvili M., Pataraia M. Study of trans-resveratrol in dry table red wines. Collection of Works of Georgian Agrarian University, Tbilisi, 2002, vol. 19: pp. 79-86
17. Kvlividze D., Bezhuashvili M. 2005-a. Phenol compounds in the wine of Saperavi sort and its table bulk wines for wines controlled by origination. Winemaking and Viticulture, №2: pp. 21-22.

18. Kvlividze D., Bezhuashvili M. 2005-b.Study of anthocyanins of the Saperavi sort grape and making dry table bulk wines of it according to the place of origination. Magarach Winemaking and Viticulture, №1: pp. 25-27.
19. Kvlividze D., Bezhuashvili M. 2005-c. Economic and technological properties of Saperavi in Khashmi microzone. The Bulletin of the Academy of Agricultural Sciences of Georgia. №14: pp. 47-55.
20. Lamikarma O., Grimm C., Rodin B., Lnyang I. 1996. Hydroxylated stilbenes in Selected American Wines. j.Agric.food Chem., 44: pp. 1111-1115.
21. Lamuela-Raventos, R., romero-Perez, A., Waterhouse, A., Torre-Bornat, M. 1995.Direct HPLC Analysis of cis- and trans-resveratrol and piceid isomers in Spanish red vitis vinifera wines. J. Agric. Food Chem., 43: pp.281-283.
22. Lamuela-Rawentos R., Waterhouse A. 1993. Occurrence of Resveratrol and California wines by a new HPLQ method. J. Agric. Food Chem. 41: pp. 521-524.
23. Ribeiro de Lima M., Waffo Heguo, P., Tessedre, P., Pujolas, A., Vercauteren, I., Cabanis, I. C., Merillon, I. 1999. determination of stilbenes (trans-astrigin, cis- and trans-piceid, and cis- and trans-resveratrol) in Portuguese wines. J. Agric. Food Chem., 47: pp.2665-2670.
24. Roggero, I., Archier, P. 1994. Quantitave determination of resveratrol and one of its glycosides in wines. Sci. Alimests, 14: pp. 99-107.
25. Romero-Perez A., Lamuela – Raventos R., Waterhouse A., de la Torre-Boronat M. 1996. Levels of cis- and trans- rezveratrol and their Glucosides in white and Rose Vitis vinifera Wines from Spain. J. Agric. Food Chem., 44: pp. 2124-2128.
26. Szmitko P., Verma S. 2005. Antiatherogenic potential of red wine: clinican update. Am Journal Physiol Heart Circ. Physiol.,288.:pp. 2023-2030.
27. Yang M., Gai L., Udeani G., Slowing K., Thomas C., Beecher C., Fong H., Fansworth N., Kinghorn A., Metha R., Moon R., Perruto Y. 1997. Cancer chemopreventive Activity of Resveratrol, a natural Product Derived from Grapes. Science, 275: pp. 218-220.

CAMBIAMENTI DELL'ATTIVITÀ ANTIOSSIDANTE DURANTE IL PERIODO DI MATURAZIONE DELLA BACCA IN DUE DIVERSE VARIETÀ DI UVA E LORO RELAZIONE CON IL CONTENUTO POLIFENOLICO

CHANGES OF ANTIOXIDANT ACTIVITY DURING THE MATURATION COURSE OF TWO DIFFERENT VARIETIES OF GRAPE AND THEIR RELATION WITH POLYPHENOL CONTENT

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RIASSUNTO

I composti polifenolici nelle uve e nei vini manifestano una forte attività antiossidante. L'attività antiossidante dipende dalla cultivar, dalla regione viticola, dalla tecnica colturale, dalle condizioni climatiche e dal grado di maturazione dell'uva. In questo lavoro sono stati valutati sia il contenuto dei polifenoli totali sia l'attività antiradicalica (AA) nelle bucce e nei semi degli acini di due varietà di uva: una a bacca bianca (Verdicchio) ed una a bacca rossa (Nero d'Avola). Le cultivar sono state allevate nelle stesse condizioni pedoclimatiche e adottando il Cordone Speronato quale forma di allevamento. Le frazioni polifenoliche sono state estratte, dalle bucce e dai semi, con 125 ml di tampone tartarico a pH 3.2. Il contenuto polifenolico dell'estratto è stato determinato con il metodo Folin-Ciocalteau. L'attività antiossidante degli estratti è stata misurata con il metodo del DPPH. Maggiore attività antiossidante è stata registrata sia nelle bucce sia nei semi di entrambe le cultivar (Verdicchio e Nero d'Avola) prima della fine del periodo di maturazione dell'uva. Il contenuto polifenolico nelle stesse parti della bacca è risultato positivamente correlato con l'attività antiradicalica. I risultati ottenuti supportano l'ipotesi che il cambiamento delle quantità e dei rapporti tra i diversi composti polifenolici durante la maturazione sia correlato con le proprietà antiradicaliche.

ABSTRACT

Phenolic compounds in grapes and wines, posses strong antioxidant activity. The antioxidant activity depend on cultivars, viticulture regions, cultivar technique, climate conditions and maturation degree of the grape. The occurrence and content of total polyphenols and the AA in skin and seeds of white (Verdicchio) and red (Nero d'Avola) Vitis Vinifera L. cultivars were valued. The cultivars were grown in the same pedoclimatic conditions and with the same training system (cordon spur).The skins and seeds fractions were extracted with 125 ml of pH 3.2 tartaric buffer. The polyphenols content of the extracts was determined by Folin-Ciocalteau method. The antioxidant activity of the extracts was analysed by DPPH method. Higher antioxidant activity both the skins and the seeds of two cultivars, Nero d'Avola and Verdicchio, was found before of the end grape maturation. While the increase of polyphenols content both skin and seed of two grape cultivars was positively correlated with antiradical activities. The obtained results supported the assumption that the qualitative and quantitative variation of polyphenols composition during maturation is related with antiradical properties.

INTRODUZIONE

Lo sviluppo di una pianta è un processo influenzato da fattori endogeni, come l'espressione genica ed esogeni come le condizioni climatiche, il terroir e l'esposizione alla luce. Si chiama oxidative burst(radiazione ossidativa) la prima risposta delle cellule della pianta ai diversi stress biotici e abiotici, e nel normale corso della senescenza.(Bhattacharjee, 2005). Nelle cellule vegetali è presente un efficiente sistema di difesa antiossidante per la detossificazione dei ROS (Specie Reattive all'Ossigeno) che comprende anche costituenti non enzimatici come i composti fenolici (Basu *et al.* 2010). Vi è quindi un'attivazione del segnale ed una risposta sistemica che comprende cambiamenti metabolici ed induce fenomeni come l'espressine di determinati geni, con produzione di metaboliti secondari.(Leon *et al.* 2001)In tal senso l'attività antiossidante fornisce un sistema di tolleranza ai fenomeni di stress che normalmente accompagnano la maturazione. Le cultivar che tollerano maggiormente lo stress accumulano piccole quantità di radicali liberi. Negli ultimi anni sono stati utilizzati diversi metodi per la determinazione dell'attività antiradicalica, utilizzando diverse parti della bacca o della pianta come substrato (Balik *et al.* 2009), ed in riferimento alle proprietà nutraceutiche e salutistiche dei frutti a bacca rossa ed in particolare dell'uva. E' noto che, gran parte dell'attività antiossidante della bacca d'uva, proviene dalle bucce e semi (Garofolo et al., 2009.), i quali sono particolarmente ricchi di polifenoli, in particolare procianidine, e subiscono significative trasformazioni nel corso della maturazione delle uve (Geny *et al.* 2003).

Oggetto del presente lavoro è stato lo studio dell'evoluzione dei parametri fisici, chimici e dell'attività antiossidante di uve delle cv autoctone Nero d'Avola a bacca rossa e Verdicchio a bacca bianca (*vitis vinifera* L.) durante il periodo luglio-ottobre 2009, compreso tra la pre-invaiatura e la post-maturazione delle uve.

MATERIALI E METODI

Le prove sono state eseguite utilizzando due cultivar di *Vitis vinifera* L, Nero d'Avola e Verdicchio, rispettivamente a bacca nera e bianca.Le uve utilizzate provenivano dalla collezione del CRA - Unità per le Produzione Enologiche dell'Italia Centrale, vigneto di Velletri (Roma). Per l'estrazione e l'analisi dei polifenoli (metodo Folin-Ciocalteu) si fa riferimento a quanto riportato da Garofolo *et al.* 2009.

Per la misura dell'attività antiradicalica (ARP = 1 / EC50) è stato utilizzato e in parte adattato il metodo che impiega il radicale libero di sintesi 2,2-diphenyl-1-picryl-hydrazyl (DPPH), L'ARP è stata confrontata con quella di soluzioni standard di Trolox (EC50 = 0.3220 ± 0.005 g/L) ed espressa in μmoli di Trolox/grammo di bucce e/o semi.

RISULTATI E DISCUSSIONE

In ambedue le varietà Nero d'Avola e Verdicchio, a partire dal terzo prelievo (fine luglio), il peso dell'acino (Fig. 1, Fig. 2) cresce in proporzione diretta con l'accumulo degli zuccheri fino alla maturazione e decresce in post-maturazione (fine ottobre).

L'incidenza % delle bucce (tab. 1) sul peso dell'acino nel periodo luglio-ottobre varia in modo sensibilmente diverso per le due varietà. Nel Nero d'Avola il valore del rapporto, stabile da fine luglio ad agosto, risale in modo sensibile quando gli zuccheri raggiungono il 20%. Nel Verdicchio si registra un picco (23.75%) nel mese di settembre in coincidenza con l'inizio della maturazione (zuccheri 18.51%). Ciò è evidentemente dovuto ad un incremento forte del rapporto tra superficie e/o spessore della buccia e volume dell'acino, nonché all'aumento contemporaneo del peso specifico dell'acino. Il calo successivo in fase di post-

maturazione (zuccheri 24-27%) è apparentemente contraddittorio e non trova al momento spiegazioni certe.

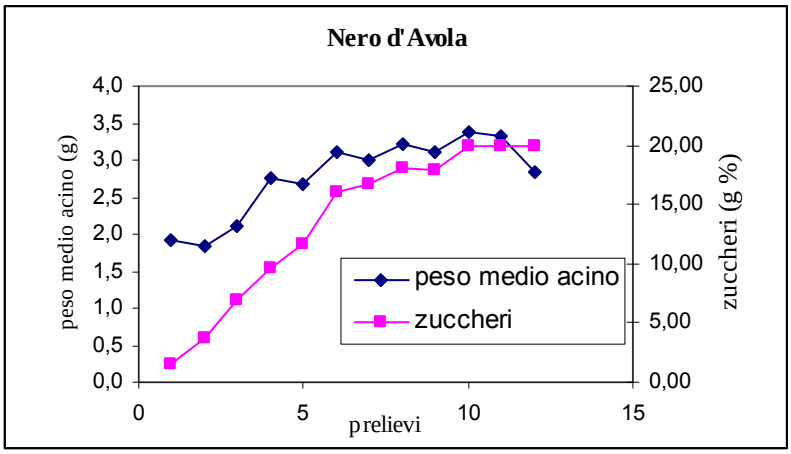

Fig.1- Evoluzione degli zuccheri(g%) e del peso dell'acino (g) durante il corso della maturazione nella cultivar Nero d'Avola

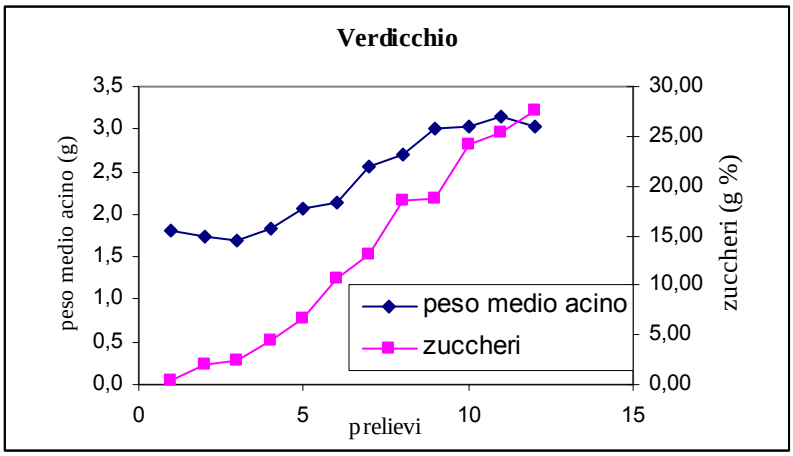

Fig. 2 – Evoluzione degli zuccheri(g%) e del peso dell'acino (g) durante il corso della maturazione nella cultivar Verdicchio

Nei semi il comportamento delle due cultivar è simile e porta ad una contrazione del peso in 100 grammi di acini rispettivamente del 45.9±1.8% e 41.8±0.7%.
L'attività antiradicalica (ARP: μmoli di Trolox equivalente per grammo) nelle bucce del Nero d'Avola (Fig. 3) presenta un massimo quando gli zuccheri accumulati nella bacca sono tra il 10–15 % per poi decrescere progressivamente. Da notare che in fase di post maturazione (punto di prelievo 12) il calo diventa sensibile ed il valore finale è inferiore a quello manifestato dalle bacche in pre-invaiatura.

Tab. 1 Evoluzione degli zuccheri e del peso di bucce e semi

v. 2009		Nero d'Avola			Verdicchio		
	date prelievo	zuccheri g%	g bucce % g di acini	g semi % g di acini	zuccheri g%	g bucce % g di acini	g semi % g di acini
1	17-lug	1,50	15,78	5,14	0,50	15,24	6,96
2	24-lug	3,73	13,29	4,65	1,95	15,11	6,83
3	31-lug	6,87	13,48	3,47	2,36	16,40	6,37
4	7-ago	9,61	14,13	3,18	4,41	16,87	6,24
5	12-ago	11,65	13,11	2,95	6,73	15,33	5,88
6	19-ago	16,04	14,50	3,04	10,68	18,42	5,51
7	28-ago	16,70*	13,78	2,94	13,16	17,51	5,49
8	2-set	18,05	15,30	2,65	18,51	23,75	4,09
9	9-set	17,83	14,34	2,55	18,63	21,38	3,91
10	23-set	20,00	18,61	2,79	24,16	20,68	3,94
11	07-ott	20,00	15,69	2,92	25,40	17,64	4,48
12	26-ott	20,01	16,46	2,78	27,58	16,32	4,05

Medie di tre repliche; *invaiatura completata

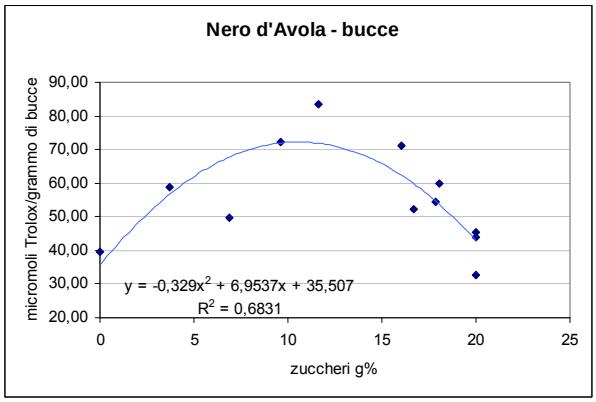

Fig. 3- Attività antiradicalica delle bucce da pre-invaiatura a maturazione dell'uva

L'evoluzione tendenziale dell'ARP è fortemente correlata con quella dei polifenoli totali (PT) che mostra anch'essa (Fig. 4) un valore di picco intorno al 12% ed una diminuizione lineare fino al 20% di contenuto zuccherino (ARP versus PT: coefficiente di correlazione lineare di Pearson r = +0.87; $p \leq 0.01$).

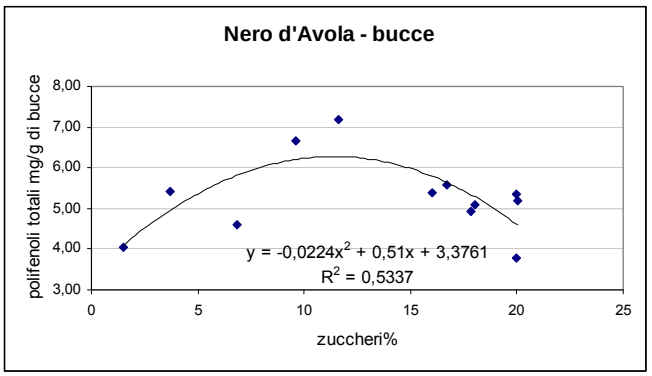

Fig. 4 -Evoluzione dei polifenoli totali (mg /g) delle bucce

Nei semi del Nero d'Avola, la crescita dell'attività antiradicalica (Fig. 5) è tendenzialmente lineare fino a maturazione tecnologica raggiunta (zuccheri 20%) rimanendo stabile nel corso dei 4 prelievi consecutivi. Nella post maturazione, in corrispondenza della evidente lignificazione del seme, il valore dell'ARP subisce un calo di circa 80 μmoli di Trolox equivalente per grammo. L'evoluzione dei polifenoli estratti da un grammo di semi (Fig. 6) è crescente fino al 15-16% di zuccheri con tendenziale diminuizione successiva. In questo caso la regressione ARP - PT presenta un livello di significatività scarso ($r = + 0.58$; $p \leq 0.05$).

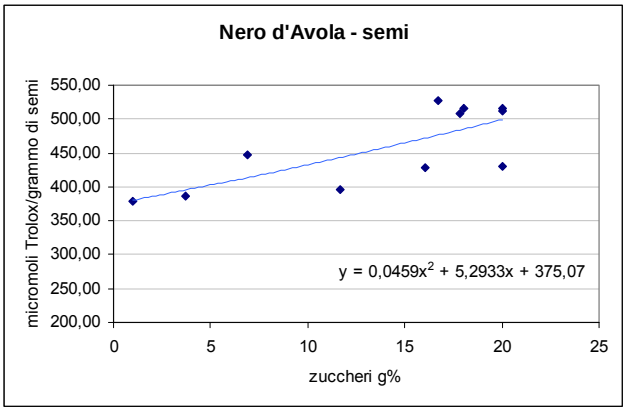

Fig. 5 -Attività antiradicalica dei semi da pre-invaiatura a maturazione dell'uva

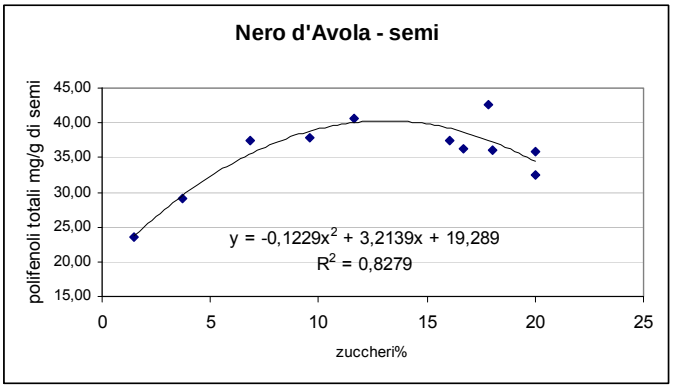

Fig. 6- Evoluzione dei polifenoli totali (mg/g) dei semi

L'attività antiossidante (ARP) nelle bucce del Verdicchio (Fig. 7) presenta un massimo estremamente precoce, quando gli zuccheri accumulati nella bacca sono tra il 4 – 8 % per poi decrescere in modo nettissimo. L'andamento dei polifenoli (Fig. 8) segue in modo soddisfacente quello dell'ARP ($r = + 0.85$; $p \leq 0.01$).
Nei semi l'attività antiradicalica finale (zuccheri 27,58%) anche se in calo rispetto al massimo tendenziale (550 μmoli di Trolox/grammo di semi) permane doppia rispetto al valore di pre-invaiatura della bacca (Fig. 9) in pieno accordo con l'evoluzione polifenolica (Fig.10). La regressione lineare ARP-PT è pertanto significativa ($r = + 0.80$; $p \leq 0.01$).

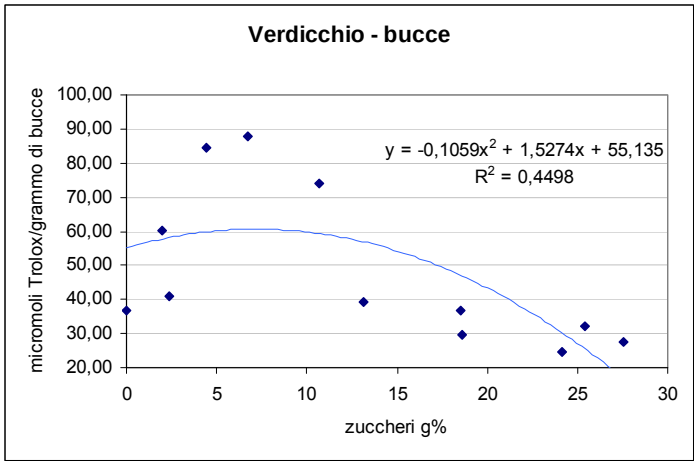

Fig. 7- Attività antiradicalica delle bucce da preinvaiatura a maturazione dell'uva

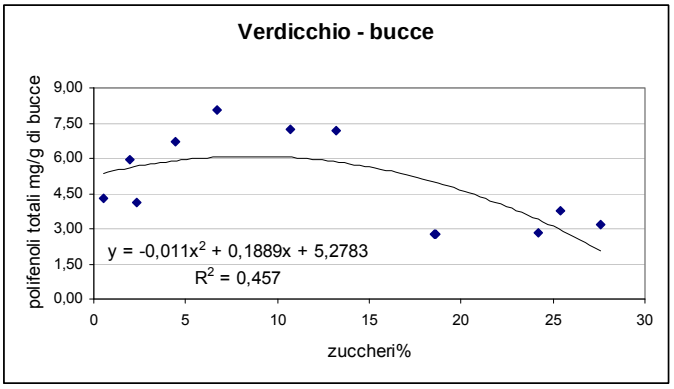

Fig. 8- Evoluzione dei polifenoli totali delle bucce

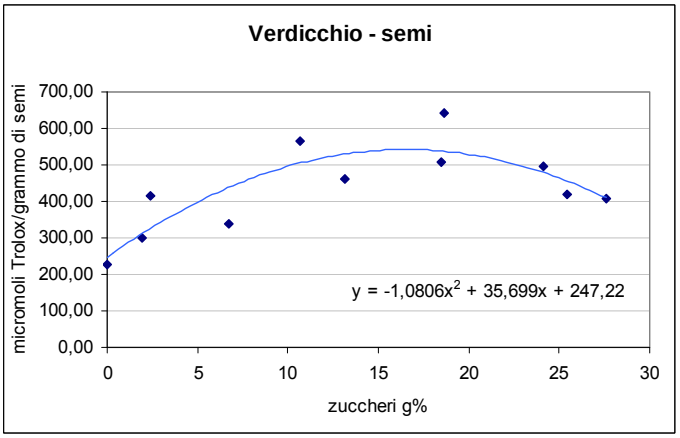

Fig. 9 -Attività antiradicalica dei semi da pre-invaiatura a maturazione dell'uva

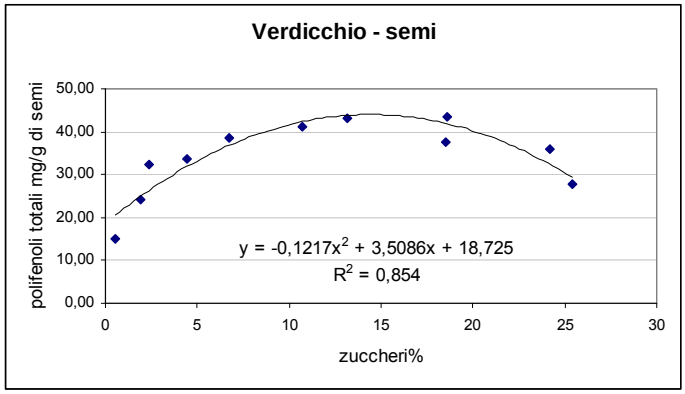

Fig. 10- Evoluzione dei polifenoli totali dei semi

L'ARP % complessiva (bucce + semi) delle uve Verdicchio (Fig.11) è stata in media quasi sempre superiore a quella del Nero d'Avola e risultava in crescita tra fine luglio e i primi di settembre 2009 (zuccheri 2.4%-18.5%) con un picco a metà agosto quando gli zuccheri non superavano ancora il 10%.

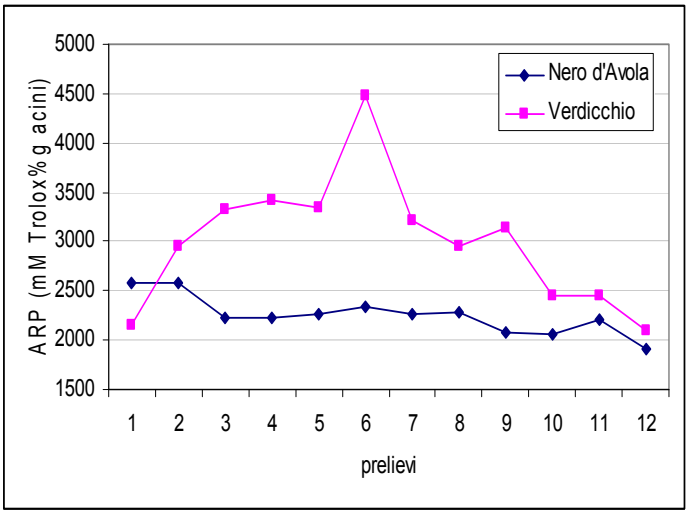

Fig. 11- Evoluzione dell'ARP complessiva di bucce e semi nelle uve

I risultati evidenziano che i criteri di valutazione dello stadio evolutivo della bacca che consente di ottenere il potenziale antiradicalico ottimale sono molto diversi da quelli enologici basati sulla maturazione tecnologica (equilibrio tra zuccheri e acidità) o fenolica (valutata sulle uve a bacca rossa in base al rapporto antocianine-polifenoli totali). Ne consegue che l'ARP migliore ottenibile dalle bucce corrisponde per ambedue i vitigni ad uno stadio di sostanziale immaturità (zuccheri < 11-15%) delle bacche (specie per il Verdicchio). Il risultato è sorprendente per il Nero d'Avola perché, nel punto di massima attività antiossidante, anche il contenuto polifenolico è massimo, nonostante l'invaiatura e quindi la biosintesi antocianica non sia completata (bacche colorate 56%). Ciò autorizza ad ipotizzare modificazioni significative dei rapporti tra differenti polifenoli;quindi, ad es. che il picco di maggior concentrazione dei polifenoli (zuccheri < 12%) derivi da un più alto contributo delle procianidine rispetto alle antocianine. Mentre l'ARP ottenibile da un grammo di semi, di

entrambe le cultivar ha manifestato valori crescenti fino a concentrazioni di zuccheri rispettivamente di circa 18-20% (Nero d'Avola) e 15-18%, (Verdicchio) non oltre, quindi, la maturità tecnologica. Questi risultati vanno, tuttavia, interpretati anche rispetto al bilancio complessivo della bacca, tenendo conto che la quantità di semi ricavabile da un kg di uva subisce, a causa dei processi di lignificazione, un sostanziale dimezzamento rispetto allo stadio di pre-invaiatura dell'acino.
Pertanto se calcoliamo il potenziale antiossidante derivante dalla somma dei contributi di bucce e semi in 100 g di acini, il Nero d'Avola ha sviluppato, un valore di ARP più elevato all'inizio, quando ancora non si manifestava presenza di antocianine (acino verde); mentre la diminuzione successiva è stata lenta e contenuta (range 2000-2500 μmoli di Trolox % g di acini). Nel Verdicchio i valori di pre-invaiatura e di post-maturazione sono risultati i più bassi, mentre a circa metà maturazione tecnologica dell'uva si è riscontrato il valore massimo (4500 μmoli di Trolox % g di acini). In conclusione l'utilizzo dei soli semi o dell'intera bacca per ricavare il massimo potenziale antiradicalico comporta la scelta di stadi di evoluzione dell'acino sensibilmente diversi.

CONCLUSIONI

E' stata effettuata l'analisi comparata dell'evoluzione dei parametri chimico-fisici e del potenziale antiradicalico di due cultivar, Nero d'Avola e Verdicchio, rispettivamente a bacca nera e bianca. I risultati ottenuti evidenziano che i criteri di valutazione dello stadio evolutivo della bacca, rispetto al potenziale antiradicalico ottimale, sono molto diversi da quelli enologici basati sulla maturazione tecnologica (equilibrio tra zuccheri e acidità) o fenolica (rapporto antocianine-polifenoli totali).
Ne consegue che la migliore attività antiradicalica (ARP) ottenibile dalle bucce corrisponde per ambedue i vitigni ad uno stadio di sostanziale immaturità delle bacche; per i semi,invece, entrambe le cultivar hanno evidenziato valori crescenti di attività antiossidante fino a maturità tecnologica.
Il potenziale antiossidante derivante dalla somma dei contributi di bucce e semi in 100 g di acini, ha evidenziato nel Nero d'Avola un valore di ARP maggiore all'inizio dell'invaiatura, mentre nel Verdicchio, l'ARP migliore si riscontra circa a metà della maturazione tecnologica dell'uva .

BIBLIOGRAFIA

Balik J., Kyselakova M., Vrchotova N., Triska J., Kumsta M.,Veverka J., Hic P., Totusek J., Lefnerova D., 2009. Relation between polyphenols content and antioxidant activity in vine, grapes and leaves. *J.Food,* 26: 25-32.

Basu S., Roychoudhury A., Saha P.P., Sengupta D.N.(2010) Differential antioxidative responses of indica rice cultivarsplant Growth Regul.60:51-59

Bhattacharjee S., 2005. Reactive oxygen species and oxidative burst: Roles in stress, senescence and signal transduction in plants. Current Science, 89:7

Garofolo A., Giannini B., Favale S., 2009. Free radical scavenging activity (DPPH) of red grapes and wines of Vitis vinifera (L.) cv Cesanese d'Affile clone. *Riv. Vitic. Enol*., 2-3: 11-27.

Geny L., Saucier C., Bracco S., Daviaud F., Glories Y., 2003. Composition and cellular localization of tannins in grape seeds during maturation, *J. Agric Food Chem.*, 51: 8051-8054.

Leon J.; Rojo E.; Sanchez-Serrano J.J (2001).*Would signalling in plants* J Eperimental botany,52(354),1-9

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CLAIM

Theme : New products, new technologies, new challenges
Sub-theme : Safety & Health - Health impact of vitivinicultural products

Type of presentation : Poster
Language : English
Title : Phenolcarbon Acids, Catechins and Antioxidant Activity of Grape Juice Concentrated with Polyphenols

Abstract

We have elaborated and offered the processing technology of juice from the Saperavi grape variety concentrated with polyphenols. The objects of research were: juice sample (test) prepared by the technology developed by us; juice and dry red wine samples (control) from the same grape variety prepared by the existing technology. The determination of phenolcarbon acids and catechins was conducted using the method of HPLC. The antioxidant activity was studied using the stable radical (DPPH). It has been established that the total amount of phenolcarbonic acids is 5.9 times higher in juice test samples compared with control, and in comparison with wine sample by 91%. In juice test samples the total of catechins increases: compared with the control in 7.6 times, and with red wine - in 2.7 times. The antioxidant activity in juice test samples is much higher compared with juice control and wine samples.

Nous avons élaboré et proposé une technique du jus concentré en polyphénols obtenu de variété Saperavi. Nous avons étudié : échantillon du jus de Saperavi produit par notre procédé (essaie) ; échantillons du jus et du vin rouge sec obtenus de même variété par procédé usuel (références). Nous avons constaté que le somme des acides phénolcarboniques aux échantillons du jus expérimental est de 5,9 fois plus haut par rapport aux échantillons de référence et de 91% plus haut par rapport aux échantillons du vin; le somme de catéchines augmente dans les échantillons expérimentaux du jus 7,6 fois par rapport aux échantillons de référence et 2,7 fois par rapport aux échantillons du vin. Activité antioxydante aux échantillons expérimentaux est beaucoup plus haute tant par rapport aux échantillons de contrôle du jus qu'aux échantillons du vin.

Introduction

Red grape varieties contain the full range of biologically active substances, amongst which the polyphenols are distinguished by various curative features. Numerous research studies

conducted in various countries of the world have confirmed high antioxidant effect of the polyphenols. Free radicals presented in human body accelerate oxidation processes of the cells, whereas polyphenols neutralize the damaging effects of free radicals. Catechins are characterized by P-vitamin activity and proanthocianidins – by antimutagen activity; quercitine, kaemferol, ellagic acid and resveratrol are capable to inhibit the development of malignant tumors; malvidol, p-coumaric and caffeic acids have bactericidal action, and tannin has strong antiviral effect. Phenolic complex of red grape varieties is of universal biological activity and it has curative effect to about 20 various diseases (Durmishidze, Khachidze 1985; Pace-Asciak et al.1995; Miyagi, Miwa, 1997; Flesch, 1998;; Knekt et al. 2000; Bavaresco, 2003; Papadopoulou et al. 2005;).

Recommended daily dose of polyphenols for the prevention and treatment of numerous diseases makes 100-500 mg and red wine contains 0.5 liter of the mentioned dose. The red wines have high antioxidant properties because red grape varieties are characterized by high levels of polyphenols and in the process of alcoholic fermentation their extraction into the wine happens from the hard parts of clusters. At the same time, it has been established that only those red wines which have high polyphenolic content reveal antioxidant effect (Versari , 1999; Servili et. al, 2000; Gomez-Plaza et al., 2001; Verts, Litvak,2001; Verts, Litvak,2003; Papadopoulau et al. 2005; Shalashvili et al. 2007**;**. Roussis et al, 2008**)**.

Due to the fact that wine intake is not recommended for patients and is not allowed for children, the development of technology of making grape juices concentrated with polyphenols from the red grape varieties has acquired more and more importance for the purposes of prevention.

At present the demand for polyphenol-rich products has increased considerably in the world market.

We have developed the technology of making juice concentrated with polyphenols from the Saperavi red grape variety.

The purpose of our research was to study and to compare quantitative indices of phenolcarbonic acids, catechins and antioxidant activity in juice samples prepared by the existing technology from Saperavi grape variety, technology offered by us (concentrated with polyphenols) and in samples of table dry red wine made on the basis of the existing technology from the same variety.

Materials and Methods

The objects of research were:1)Control juice samples prepared (Daskalov et. al 1966) by the existing technology (crushing and squeezing of stemless pulp); 2) Test juice samples prepared on the basis of polyphenols concentrated technology developed by us (Ebelashvili et al. 2009) 3) Dry red wine samples prepared from the same grape variety on the basis of existing technology (Valuiko, 1973).

In research objects the determination of phenolcarbonic acids and catechins was conducted using the high performance liquid chromatography (HPLC) method, on the apparatus Pro Star of the firm Varian with UV detector.

Separation of components was performed on chromatographic column with reversed-phase sorbent Microsorb 100-S C18 (250mm x 4.6 mm x 5.0 mm). Elution was performed in gradient mode at the rate of mobile phase feed equal to 1 ml/min. The following solutions were used: Solution A – water/phosphoric acid (in the ratio of 99.5/0.5); solution B –

acetonitrile/water/phosphoric acid (in the ratio 50/49.5/0.5). The wine samples were diluted five times and juice samples ten times with solution A and filtered through membrane filter (pore diameter 0.22 μm). The solvents and commercial standards used during the analysis were purchased from Sigma-Aldrich (Germany). The detection was performed at wavelengths 280 nm. Identification was conducted by comparison of retention time of standard substances and defined components as well as by using the method of standard substances addition known in special literature (Bonerz et al. 2008)

The antioxidant activity was studied (Yinrong ,Foo , 2001) by stable radical (DPPH). As standard antioxidant, there was used triolox (soluble form of vitamin E)

Results and Discussion

The research was carried six months later.
The obtained results are presented in the Tab. and Figs. 1 and 2.

Table. Phenolcarbonic acids, catechins and antioxidant activity in juice samples prepared from Saperavi grape variety and dry red table wine samples.

Phenolic components (mg/dm^3) and antioxidant activity (%)	Control juice	Test juice	Red wine
Gallic acid	2.946	4.477	20.015
(+)-catechin	25.859	182.867	67.710
Chlorogenic acid	34.767	83.134	0.549
Vanilic acid	4.396	6.290	2.701
Caffeic acid	9.619	38.121	2.495
(-)-epicatechin	36.905	294.135	105.498
p-coumaric acid	1.339	3.094	1.558
Sinapic acid	37.303	361.939	250.157
t-cinnamic acid	1.602	7.498	7.019
Total phenolcarbon acids	91.972	546.022	294.524
Total catechins	62.764	477.002	173.208
Antioxidant activity	30	60	50

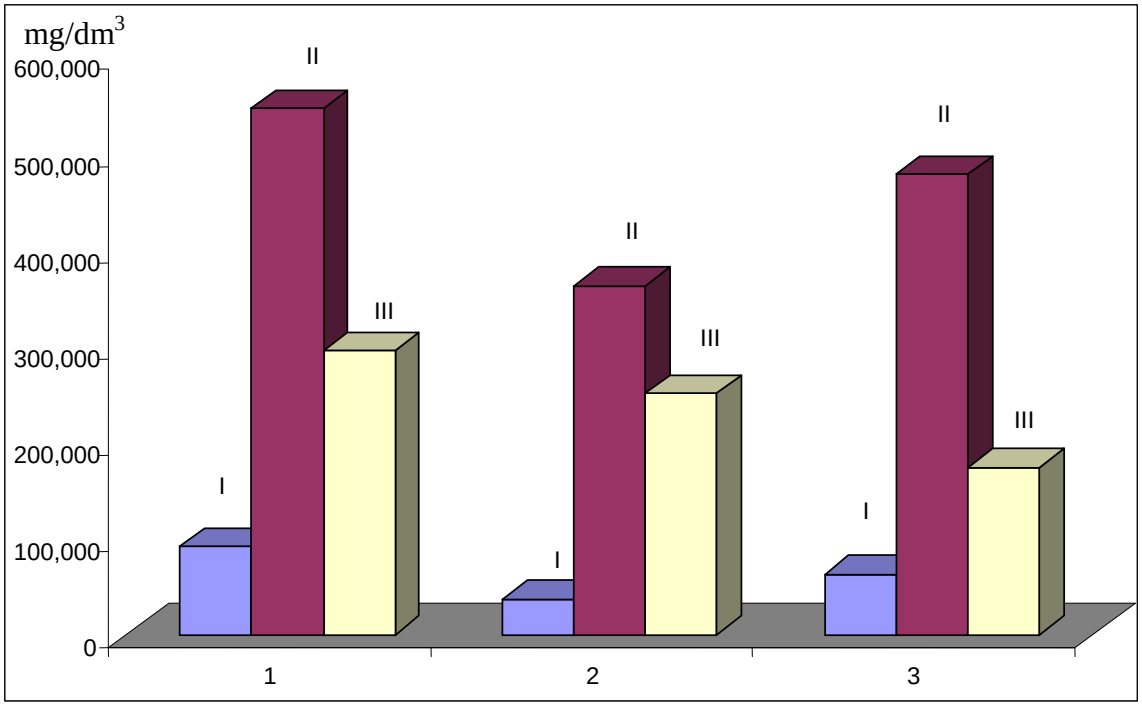

Fig.1.

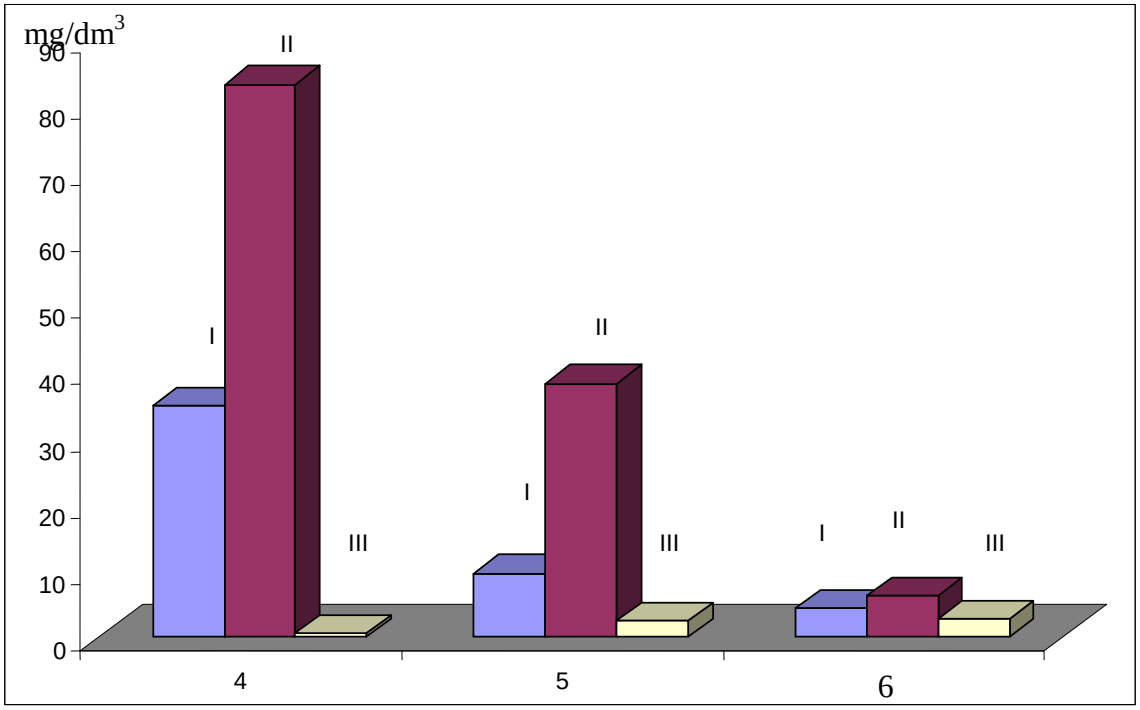

Fig.2.

1. Total of phenolcarbon acids
2. Sinapic acid
3.Total of catechins
4. Chlorogenic acid
5. Caffeic acid
6. Vanillic acid

I – control juice; II – test juice; III – red wine.

In research objects we have identified and quantitatively determined gallic, chlorogenic, vanillic, caffeic, p-coumaric, sinapic, ellagic and cinnamic acids, (+)- catechin and (-) – epicatechin.

The study has shown that quantitatively both in juice and wine samples, sinapic acid is presented most of all, then chlorogenic, caffeic, vanillic acids and (-) epicatechin.

From the obtained data it is seen that the quantity of the mentioned components in juice test samples prepared using the technology developed by us is much higher compared both with control juice samples and dry red wine samples prepared from the same variety according to the existing technology.

The total of phenolcarbonic acids in juice test samples (546.022 mg/dm^3) compared with controls (91.972 mg/dm^3) is 5.9 times higher but in red dry wine samples prepared from the same Saperavi grape variety (294.524 mg/dm^3) using the existing technology is comparatively higher (91%).

Total of catechins in juice test samples (477.002 mg/dm^3) in comparison to controls (62.764 mg/dm^3) increases in 7.6 times and compared to dry red wine samples (173.208 mg/dm^3) grows in 2.7 times.

The total amount of phenolcarbonic acids in juice test samples grows mainly at the expense of quantity-increase of sinapic, chlorogenic, caffeic and vanillic acids. The quantity of the mentioned acids in the juice test samples (correspondingly: 361.939; 83.134;38.121; 6.29 mg/dm^3) was much higher both in comparison to juice control samples (correspondingly: 37.303; 34.767; 9.619; 4.396 mg/dm^3) and dry red wine samples (correspondingly: 250.157; 0.549; 2.495; 2.701 mg/dm^3).

As is known among phenolcarbonic acids, caffeic acid is distinguished with strong antioxidant capacity. At the same time it is also characterized by bacteriocide effects which is so high that its activity is often expressed by a certain unit of penicillin (Valuiko,1973.).

The obtained results have shown correlation dependence between total amounts of phenolcarbonic acids and catechins in samples, and antioxidant activity.

It has been established that antioxidant activity in juice test samples is considerably higher (60%) both in comparison to juice control samples (30%) and in dry red wine samples (50%).

In juice test samples an increase of phenolcarbonic acids and catechins must be explained by using the processing technology developed by us. By means of the proposed technology, the inactivation of oxidation enzymes occurs before crushing the grape which makes possible to maintain monomeric polyphenols content unoxidized in pulp.

At the same time it is known that skin and seeds of red grape varieties also contain acidulated forms of phenolcarbonic acids (Rodopulo, 1983;) The technology proposed by us causes hydrolysis of acidulated forms and maximal extraction of unoxidized polyphenols.

As a result of the above processes nutritive value in grape juice test samples increases.

It should be also noted that pasteurization makes it possible to maintain monomeric polyphenols in unoxidized form. In red wines their amount decrease because they themselves oxidize and protect wine antocyanes from oxidation. At the same time their condensation and polymerization takes place (Daskalov,1069; Valuiko,1973; Rodopulo, 1983;) but in pasteurized juice (Daskalov et. al 1966) these processes proceed with less intensity

.

Conclusions

The obtained results indicate that juices prepared with the help of the technology developed by us have high nutritional value and preventive properties in comparison to juice and red wine samples prepared by existing technology. The offered technology promised to be efficient and prospective.

Acknowledgements

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References

1. Bavaresco L., 2003 Role of viticultural factors on stilbene concentrations of grapes and wine. Drugs Ep.Clin.Res. 29:181-187.
2. Bourzeix, M., D. Weyland, et al. 1986. A study of catechins and procyandins of grape clusters, the wine and other by products of the vine. Bull. OIV 59:1171-1254.
3. Corder R, W. Mullen, N. Q. Khan, S. C. Marks, E. G. Wood, M. J. Carrier and A. Crozier 2006, Oenology: Red wine procyanidins and vascular health. Nature v.444, 566
4. P.Daskalov P., R.Aslanian, P.Tenov, M.Zhivkov, P.Voiadzhiev, (1969), Plodovye i ovoschnye soki. Translated from Bulgarian. Moscow, 421.
5. Durmishidze S., Khachidze O. 1985. Grape Biochemistry. Ed. T.Kezeli. “Metsniereba” , Tbilisi (Georgian).
6. Ebelashvili N., Mujiri L., Chkhartishvil N., Jorjikia N., Asashvili T. 2009. production technology of Grape Juice concentrated with polyphenols. Prior. patent #11180/01. “Georgian patent”.
7. Flesch M., A.Schwarz, M. Bohm, (1998), Effects of red and white wine on endothelium-dependent vasarelaxation of rat aorta and human coronary arteries. AmJPhysiol;275:H;1183-1190.
8. Gomez-Plaza E., et al. 2001 Phenolic Compounds and Color Stability of Red Wines: Effect of Skin Maceration Time. Am.J.Enol.Vitic. Vol.52,#3, 266-270.
9. Knekt P, Isotupa S, Rissanen H, Heliovaara M, Jarvinen R, Hakkinen S, Aromaa A, Reunanen A. Quercetin intake and the incidence of cerebrovascular disease. Eur J Clin Nutr. 2000 May;54(5):415-7.)
10. .Miyagi Y., K.Miwa, (1997), Inhibition of human low-density lipoprotein oxidation by flavonoids in red wine and grape juice. Am J. Cardiol; 80: 1627 – 1631.
11. Pace-Asciak CR et al. (1995), The red wine phenolics trans resveratrol and quercetin block human platelet aggregation and eicosanoid synthesis. Clin Chim Acta; 253: 207-219.
12. Papadopoulou C., Soulti K., Rousssis I.G. 2005. Potential Antimicrobial Activity of Red and White Wine Phenolic extracts against strains of *Staphylococcus aureus, Eschericnhia coli and Candida albicans.* J.Food Technol.Biotechnol., 43 (1), 41-46.
13. Rodopulo A.K. 1983. Foundations of Winemaking Biochemistry. Ed. A.S.Vecher.”Pischevaya promyshlennost” Moscow (Russian).

14. Roussis I.G., Lambroupoulos I. et al. 2008. Antioxidant Activities of some Greek wines and wone phenolic extracts. Journal of Food Composition and Analysis. 21, 614-621.
15. Servili M., Stefano G.De, Piacquadio P., Sciancalepore. 2000. A novel method for Remooving Phenols from Grape Must. Am.J.Enol.Vitic. Vol.51,#4, 357-361.
16. . Shalashvili A, I.Targamadze, N.Zambakhidze, D.Chichua, V.Nareklishvil, D.Ugrekhelidze,(2007),Comparison of Wines of Kakhetian and European Types according to Quantitative Content of Flavonoids and Antiradical Efficiency. Bull.Georg. Natl. Acad.Sci.175, 4.
17. Valuiko G.G. 1973. Biochemistry and technology of red wines. Ed. Drboglav E.C."Pischevaya promyshlennost" Moscow (Russian).
18. Versari A.,Barbanti D., Galassi S 1999. Antocyanin composition of Montepulciano d Abruzzo must during industrial fermentation process. Journal of Wine Research,10,(3) 223-227.
19. .Verts K, V. Litvak (2001) Medicine and alcoholic beverages. J.Winemaking and Viticulture, 1, 34-36, (Russian
20. Verts K., V. Litvak (2003). Wine and diet. J.Winemaking and Viticulture, 5, 2003, 49-51, (Russian).

UTILIZATION OF CONCENTRATED GRAPE JUICE AS A SWEETENER FOR MAKING NATURAL FOOD PRODUCTS

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ABSTRACT

Aim of the research is utilization of concentrated grape juice as a sweetener, specifically in combination with fig fruit for making various natural, processed products, on the basis of improved technologies. Innovation of the current research is preparing of various kinds of food products by using concentrated mixture of grapes and fruit juice, with new technologies and receipts; a secure technology for reduction of acidity in grape concentrate has been developed. It is possible to use the low acid grape concentrate in different ways. Obtained goods have not only nutritional, but also diet functions. Separate object is evaluated as qualitatively, so from the curative and prophylactic points of view. Technical result of the research is diversity of assortment of natural and food products as well as those of functional purpose.

RESUME

Untersuchungszweck war gebrauch den konzentrierten Traubesaft statt Zucker. Zwar auf Grund der verbesserten Technologie im Kombination mit Feigefrucht Annahme den verschiedenartige, verarbeiteten Produkten. Now how der Untersuchung ist das, dass mit gebrauch den konzentrierten Frucht und Traubesaft, nach neue Technologie und Rezept verschiedene Nahrungsmittel hergestellt hergestellt sind, es ist Gefahrlose, sauersreduzierende Technologie der Traubekonzentrat verarbeitet. Gebrauch Niedrigsauerige Traubekonzentrat ist nach verschiedenen Zweck möglich. Bekommene Produkten haben nicht nur Nahrungsmittel, sonder dietische Belastung. Einzelne Produkten sind geschätz wie nach qualitativ, so nach Heil, Verbeugende Ansicht. Technische Ergebnis der Untersuchung ist Vermehrung der Assortiment der natürlichen, nahrungsmittel und funktionalen Produkten

INTRODUCTION

Utilization of sucrose, as sweetener and a concentrate occupies important place in the technologies of making food products. Negative results of its regular utilization is known as well, that urge the scientists on looking for alternative methods and using sweeteners easy of digestion in the face of mono-sugars (glucose + fructose).

Aim of the research is utilization of grape juice concentrate as sweetener; making natural, processed goods of various kinds on the basis of the improved technologies. Under the conditions of concentration of the grape juice, index of the titratable acidity is being increase two and more times. This touches mostly upon those kinds of grapes spread in the western Georgia that worsens quality significantly. For reduction of acidity, utilization of sodium bicarbonate or chalk is suggested (Beridze, 1961; Loladze, 1985); this, in its turn, lowers the worth of the goods.

For reducing acidity of the thickened sweet of grapes we don't use various chemical substances; our goal is achieved by blanching low acid fig fruit into it; mixture-reduction of titratable acidity of concentrated juice.

Fig fruit, which differs in low index of titratable acidity, consists of sugars (12.-28%), proteins (5-6%), organic acids (citric acid, boric acid, ethanoic acid, malic acid and others), mineral substances (Ka, Ca, Cu, P, Mn et al.), food diet fibers, carotenes, amino acid, cellulose, pectin and other easy of digestion bioactive agents, which stipulate their curative features from the point of prophylaxis and curing (Khomizurashvili, 1978; Rublov et al., 2001; Khondorabad, 2004; Vibliani. 2007, 2008a,b; http://www.California.bigs.com (history). Besides, the fruit doesn't need processing with pesticides during vegetation. Consequently, goods made out of it are relatively safe to the human organism. In the current case, we used admixture of grapes and fruit juice (glucose + fructose) for the research object, which are of sweetener's importance as well as of diet-prophylactic one.

Innovation to the research is preparation of food products of various kinds according to the technologies and receipts processed by using concentrated mixture of grape and fruit juice; safe technology for reducing high acidity of grape concentrate is worked out as well. Utilization of low acid grape concentrate is possible in different ways.

MATERIALS AND METHODS

For experiment we selected Georgian native grapevine variety *Rkatsiteli* (*Vitis vinifera* L.), which differs in high content of sugar (18-24%) and such specter of aroma-forming agents, which give it special taste as to fresh products as to processed products (Bagaturia et al., 2006; Kandelaki, 2006; Miqiashvili et al., 2006). We used fruits of specific form of black fig named as "*Shavi Adgilobrivi*". Samples were taken from the regions of eastern Georgia (Kartli, Kakheti). The test was provided on the base of four-year data at the Department of Storage and Processing of Fruit and Berries under the Institute of Horticulture, Viticulture and Oenology.

Following methods have been used for bio-chemical research of initial and final samples: dissolving dry substance – by using refractometer, dry substance – by drying the sample under the temperature 105^0C, titratable acidity – by using solution 0.1nNaOH, pectin substances – by defining calcium pectate, mineral elements – by using atomic absorption spectroscopy, phenol admixtures on high effective liquid chromatograph – total quantity of anthocyanins by using colorimeter method.

RESULTS AND DISCUSSION

At first we prepared concentrated grape juice up to 35-40% of consistence of dry substances (initial data within 18-24%). For blanching under the temperature 65-70^0C, we placed fig fruit in the received juice (correlation 1:1). Boiling was provided during 4-5 minutes and break was lasted until temperature fell down to 65-70^0C. Only 60-70% of total mass of the fig fruit has been separated. Remaining is the juice from the fig fruits, that reduced in some extend acidity index in the grape juice. Subsequently concentration of the grape juice increased again up to 50%. The results showed up, that index of total and titratable acidity is law (0.32-0.28%), while in the concentrated grape juice it is 1.78-1.45%. On account of blanching of the fig fruit, this index lowers to 1.14-0.67% accordingly (Fig. 1).

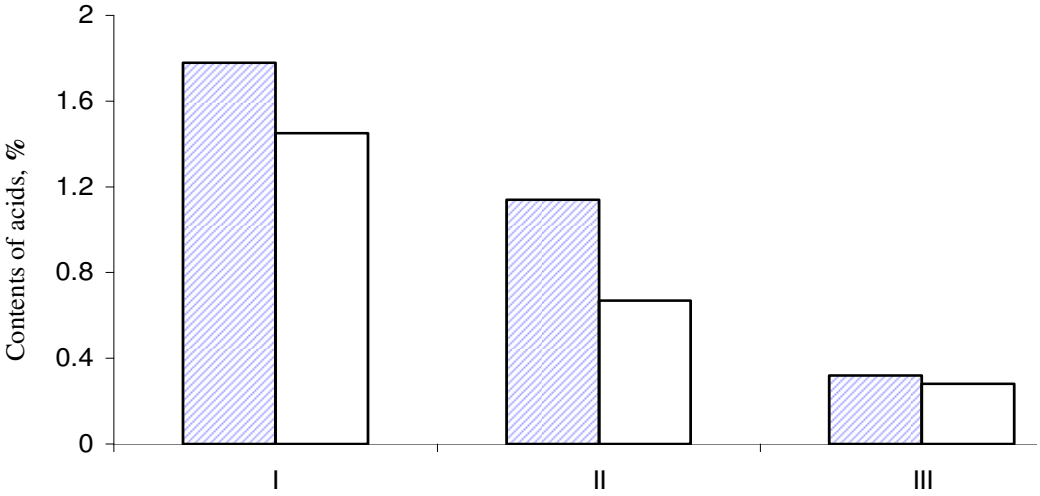

Fig. 1. Acid content in the concentrated juice and in the fig fruit.

I – concentrated grape juice (40%);

II – concentrated grape juice after blanching fig fruit (Correlation 1:1);

III – fig fruit.

▨ - total acidity;

□ - titratable acidity.

Water containing fig fruit transits easily into the concentrated grape juice and increases quality of disociation of the acid growth. Because of augmentation of dilute and low acidity of fig fruit, in the grape juice reduction of titratable acidity is indicated. Concentrate received by admixture of grape juice and fig juice, which differ in high concentration of biologically active substances and mineral elements, have been used by us as sweetener for making various processed food products, which have not only nutritional, but also curative and prophylactical functions.

Utilization of grape and fig juice mixture for various purposes

We used obtained admixture for making *Phelamushi*, *Churchkhela*, and *Sweet pastille*. The obtained products differ in moderate correlation of sugar and acid, less sweet taste and typical flavor. Herewith, dry product is characterized with good physical indexes – soft, pliant, smooth thickness and color.

Churchkhela has been made with the obtained admixture. Known method of preparing it foresees reduction of acidity of grape concentrate by using chemical substances (Beridze, 1961; Georgian Churchkhela, 1979, 1989; Loladze et al., 1985; Chlikadze et al., 1990; http://www.sladka.ru/sweets/orientalsweet/1980). Our method regards changes; privately thickness of concentrated grape juice is achieved by mixing in maize and wheat flour. They fill each other with valuable proteins. For example maize zein consists of amino acids – leucine, isoleucine, fenilalanina, and small quantities of lysine and threonine. Gliadin of wheat differs in consistence of lysine, especially threonine and tryptophan. Herewith, quantity of gluten in the mixture reduces and *Churchkhela* cover becomes relatively less tensile. Correlation is 1.2:1.0, accordingly consistence is 10-15%. Using nut for stuffing was shown preference, as it is cheaper than walnut, furthermore it doesn't differ in special features. Consistence of olein acid and tocopherol is extremely remarkable. Humidity of *Churchkhela* cover is within 16-20%. According to the researches (Tab. 1), dry substance compiles 83.7% including Sugar – 61.3%, while consistence of the rest dissoluble agents is 7.5%. As for

calorie content, *Churchkhela* has quite high index that conditions its important role in the food physiology. Worth of the production is high, containing protein – 2.9%, supported by partial hydrolyze of protopectin in the fig fruit in the blanching process and transition of dissolving protein into the concentrated grape juice.

Tab. 1. Chemical index of *Churchkhela* cover

Dry substance	Dissolving dry substance		Dissolving pectin	Ashes
	Sugar	The rest part		
83,7	61,3	7,5	2,9	1,8

High consistence of ashes (1.8%) is also remarkable. Consists of Ca in large quantity, that role of which is important for metabolism. Physical index of *Churchkhela* prepared, by using new technologies, differs from the existed ones. It is significant, that bioactive substances in the fig fruit enrich and give curative features to it. Storing period is increased as well.

By drying fruit separated after blanching fig into the concentrated grape juice on the 40-45^{0}C, we received dry fig product *Chiri* (Dried fruit).

It is known, that they use sulfurous anhydride in producing dry fruit, as well as blanching in caustic soda solution and processing in sugar syrup (Chavleishvili, 1980; Pkhakadze, 2005), notwithstanding importance of the procedures remarked, they have negative features as well, that loose curative-prophylactic features of the production.

Innovation of our research is that inactivation of enzymes, violation of totality of epidermises and cuticle, growth of concentration of dry dissolving substances may be provided by blanching fruit pulp into the concentrated grape juice. Herewith, mentioned method is used for colored fig fruits. In the obtained product different chemical data have been defined (Tab. 2 and 3).

Tab. 2. Consistence of pectin agent in dry product of the fig fruit

Variety	Before blanching	After planching	Pectine after drying %	
	Dissolving	Dissolving	Dissolving	Dissolving
Shavi Adgilobrivi	1.07	1.23	2.57	1.42

Tab. 3. Chemical data of the fry fig fruit by calculating on absolutely dry mass, %

Variety	Dissolving dry substance	Sugar	Acid	Pectin
Shavi Adgilobrivi	87.8	72.6	1.0	5.5

Dried fruit made by the method offered by us has numbers of priorities – relatively clean product is received, as no sulfurous anhydride and caustic soda is used; quantity of dissolving dry substance increases and accordingly it reduces duration of the labor period. Quantity of the dissolving dry substance increases, basically at the expense of sugars; titratable acidity

and growth of dissolving pectin takes place. The indicated changes improve nutritional, curative, prophylactic and organoleptic features.

CONCLUSIONS

Following conclusions may be done leaning upon findings:

- Secure technology for reduction of high acidity of grape concentrate has been worked out;
- Admixture of grape and fig juice (glucose + fructose) has been received, utilization of which, as sweetener (sucrose), may be provided in various ways;
- New methods for preparing *Phelamushi* materials and *Churchkhela*, that enriched the product with new specter of bioactive agents has been worked out. Design is changed as well;
- The method for making *Chiri* (dried fruit), in which no chemical substances are used, has been changed;
- The obtained products differ in consistence of biologically active agents and mineral elements that give them function of nutritional, curative, diet and prophylactic features;
- Chemical data of the obtained food products has been defined, separate object has been evaluated as qualitatively, so from curative and prophylactic points of view.

BIBLIOGRAPHY

Bagaturia N et al., 2006. White Aromatic Wines of Georgia. *Wine-making and viticulture*, 2: 15. (in Russian)

Beridze G. I., 1961. Technology of Georgian Churchkhela. Tbilisi. Pp. 23. (in Georgian)

Chavleishvili A. 1980., Technology for Drying Fruit and Vegetables. Subsidiary Manual. Tbilisi, Pp. 76-84. (in Georgian)

Chlikadze A. et al., 1990. Invention (11) 300 (1739958). Methods of Producing Georgian National Sweets. 10.04.1990. (in Georgian)

Churchkhelas of Georgia, 1979. Republic Standard of Soviet Socialistic Republic of Georgia 294-79. (in Russian)

Georgian Churchkhela, 1989. vol. 10. Soviet Socialistic Republic of Georgia. 212-89. (in Georgian)

Kandelaki N., 2006. Aromatic Substances in wine-materials of Kakhetian Type after Their Heat Treatments. *Wine-making and viticulture*, 3: 19. (in Russian)

Khandorabad, Gennaia et al., 2004. Translation. News-paper “Metro Plus”.

Khomizurashvili N., 1978. Georgian Fruit-growing. Vol. IV. Drupaceous, nut and subtropical cultures. Tbilisi. Pp. 737-829. (in Georgian)

Loladze G., Mekhuzla N., et al., 1985. Grapes - Source for Valuable Products. Tbilisi. Publishing house “Sabchota Saqartvelo”. 254p. (in Russian)

Mikiashvili M. ar al.; 2006. Research of Essence of Grapes Rkatsiteli. *Georgian Engineer News*, 6: 295-297. (in English)

Pkhakadze I., 2005. Description of Patent on Invention. National Center of Intellectual Property “Saqpatenti”, Tbilisi. 3p. (in Georgian)

Rublev E., Kashman U., Rabinovich V., et al., 2001. Suppressors of Cancer cells – Pitch Weapon (*Ficus caroca* L.): National Production. 264 (7). (in Russian)

Vibliani M., 2007. Method for Preparing Churchkhela. Patent #4557. National Center of Intellectual Property “Saqpatenti”. Tbilisi. 3p.

Vibliani M., 2008a. Method for Preparing Dry Product of Fig Fruit. Identification Number 10549/01. National Center of Intellectual Property “Saqpatenti”. Tbilisi. 3p.

Vibliani M., 2008b. Oddment-free Technologies for Making Spirits and Dry Products by Using Fig Fruit. Monography ISSN 1512-0600, Telavi. 88p.
http://www.California.bigs.com (history). 2007.
http://www.sladka.ru/sweets/orientalsweet/1980.

POLYPHENOL CONTENT AND ANTIOXIDANT ACTIVITY OF RED WINE AND OTHER BEVERAGES CONTAINING GRAPE

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ABSTRACT

Several studies have shown the benefits of the consumption of red wine in reducing the incidence of chronic diseases. These positive activities are mainly associated with flavonoids and phenolic acids. On the other hands, the wine market shows a decreasing trend as a consequence of negative attitude towards alcohol abuse. The lower use of grape in wine production stimulated the development of non-fermented beverage offer, where remaining grape could be used. Grape and mixed juices can represent useful alternative to wine in supplying healthy substances. The aim of this study was the comparison of the antioxidant activity (AOA) and the total polyphenol content (TPC) of different beverages containing grape: red wine, grape juice with or without other red and yellow fruits. Total polyphenol contents in red wines, mixed fruit and full grape juice were in the ratio: 3:2:1. With some exception, the comparison of AOA with the TPC values of each beverage considered shows a significant correlation.

RIASSUNTO

Numerosi studi hanno mostrato i benefici associati al consumo di vino rosso nella riduzione dei fattori di rischio per l'insorgenza di patologie cronico-degenerative. Tali benefici sono principalmente attribuibili ai flavonoidi e agli acidi fenolici. Tuttavia, da alcuni anni, il mercato del vino ha subito una contrazione generalizzata a causa di atteggiamenti negativi nei confronti dell'abuso di alcool. La contrazione nella produzione e nel mercato del vino ha stimolato parallelamente lo sviluppo di bevande non fermentate, in cui potrebbe essere utilizzata l'uva eccedente. Succhi a base di uva e frutta mista sono tra l'altro una possibile alternativa al vino nel fornire al consumatore sostanze con valenza salutistica. Scopo di questo studio è il confronto dell'attività antiossidante (AOA) e del contenuto di polifenoli totali (TPC) in diverse bevande a base di uva: succhi (a base di uva e frutta rossa o gialla), succhi di sola uva e vini rossi. Il rapporto tra il contenuto di polifenoli totali nei campioni di vino rosso, succhi misti e quelli di sola uva è risultato il seguente: 3:2:1. Il confronto tra i valori di TPC e AOA mostra, a parte qualche eccezione, una buona correlazione.

INTRODUCTION

Epidemiological studies suggest that moderate wine consumption can be associated with a protective effect in reducing the risk factors for cardiovascular diseases and cancer (Rimm *et al*., 1996). Most positive effects are due to the content of polyphenols, which are involved in slowing LDL oxidation, inhibiting platelet aggregation and stimulating nitric oxide production (Soleas *et al*., 1997).

More than 500 compounds have been identified in grapes (*Vitis vinifera)* and wine (Schreider, 1979; Rapp, Pretorius, 1989). Water is the main ingredient of wine followed in decreasing order by ethanol, carbon dioxide, glycerol, sugars, polysaccharides, higher alcohols, acids and phenolic compounds (Ough et al. 1972, Singleton 1982, Eglinton et al. 2002).

The phenolic content of the grape berry is distributed as follows: 1% in solid pressed pulp; 5% in juice; 50% in the skins of red grapes and 25% in the skins of white grapes; the remaining 46–69% is in the seeds (Singleton, Esau, 1969). Grape stems also contain significant quantities of phenolic compounds, especially phenolic acids, flavonols and flavanols (Souquet, et al. 2000). Phenolic content ranges between 1732 and 1842 mg/L in red wines and between 209.5 and 285.5 mg/L in white wines (Waterhouse, 2002).

Most of phenolic compounds identified in grapes and red wine are flavonoids. This class includes more than 85% of the phenolic molecules, and in particular flavanols, flavonols and anthocyanins. Wine is rich in flavanols; the concentration of monomeric form (catechin) in wine ranges from 40 to 120 mg/L (Waterhouse, 2002), while oligomeric and polymeric forms (condensed tannins or proanthocyanidins) range in red wine from 500 to 1500 mg/L and in white wine from 10 to 50 mg/L, depending on the maturation (Waterhouse, 2002). Flavonols are present both in grape (skin, rachis and leaves) and in red wine, where the concentration ranges from 50 to 200 mg/L (Waterhouse, 2002). Anthocyanins are found mainly in the grape skin and are responsible for the color of red wine due to their condensation with other flavonoids. In red wine, the concentration of these compounds ranges between 90 and 400 mg/L (Waterhouse, 2002), depending on maturation.

The main source of phenolic compounds in wine is grape, but yeast and oak contribute significantly during winemaking and maturation, respectively. Moreover, the fermentation of must in presence of grape solids allows solubilization of anthocyanins from skins and flavan-3-ols from seeds, skins and stems (Bartolomé *et al*., 2004).

Although several studies suggest that a moderate wine consumption can be healthy, there is no univoque position on the significative difference between wine and alcohol alone or other alcoholic beverages (mainly beer). In the last years, the wine market has shown a decreasing trend due to the frequent abuse of alcoholic beverages also in young people. For this aspect it is extremely important the document published in December 2009 by WHO: *Strategies to reduce the harmful use of alcohol: draft global strategy* (http://apps.who.int/gb/ebwha/pdf_files/EB126/B126_13-en.pdf). On this bases, one of the possible strategies for the grape market is the promotion of non-fermented beverages containing grape juice. Grape, in fact, is a good source of polyphenols with nutraceutical potential.

The aim of the research presented in this paper is the correlation between total phenolic content and the antioxidant activity assayed in red wines and commercial juices containing grape alone or fruit juice mixture.

MATERIALS AND METHODS

Wine samples

The wines assayed in this research were: Chianti classico, Chianti classico riserva DOCG 2006, Red from Tuscany IGT and Chianti 2008.

Full grape juice and mixed fruit juice (containing grape) were purchased in Italian supermarket, and their composition is reported in Table 1.

Table 1- Ingredients and composition of mixed fruit juice and grape juice analyzed

JUICE	INGREDIENTS
Mix juice 1	White grape juice (35%), red grape juice (30%), pomegranate juice (10%), hibiscus extract (0.2 %), L-ascorbic acid
Mix juice 2	Peach and orange pulp and juice, grape juice, sugar, glucose-fructose syrup, maracuja juice, mango pulp and juice, extracts (green tea, white tea and apple) 0.11%, L-ascorbicc acid, vitamin E, beta-carotene.
Mix juice 3	Water, apple pulp and juice, red orange juice, pomegranate and cherry juice, sugar, glucose-fructose syrup, extracts (red grapevine leaves, *Sambucus nigra* and apple) 0.15%, citric acid, L-ascorbic acid and vitamin E.
Mix juice 4	water, apple pulp and juice, red grape juice, sugar, glucose-fructose syrup, bluberrie pulp and juice, eldelberrie juice, citric acid, sodium citrate, extracts (red grapevine leaves, green tea, *Sambucus nigra* and apple) 0.28%, L-ascorbic acid and vitamin E
Grape juice 1	red grape juice (50%), water, carbon dioxide
Grape juice 2	100% red grape juice

Total polyphenol content

Total polyphenols content (TPC) was determined according to the Folin-Ciocalteu method as reported by Singleton and Rossi (Singleton, Rossi, 1965). 300 μL of different dilutions of the samples were mixed in test tubes with: 1.5 mL of Folin-Ciocalteu's reagent (Sigma Aldrich, Germany) diluited 10 times, and 1.2 mL of sodium carbonate (Sigma Aldrich, Germany) 7.5 % w/v. After 30 min, the absorbance was measured at 765 nm in a UV-visible spectrophotometer (Varian Cary 50 SCAN, Palo Alto, California, U.S.A.). Results were expressed as equivalents of gallic acid (GAE) in mg/L.

Antioxidant activity

The antioxidant activity (AOC) of wine and juice samples was determined spectrophotometrically as a measure of radical scavenging using 1,1-diphenyl-2-picryl hydrazyl free radical (DPPH) (Brand-Williams *et al*.; Leong, Shui, 2002). Aliquots of 1 mL of DPPH (Sigma Aldrich) solubilized in methanol (5.9 mg/100 mL) were mixed with 0.5 mL of each samples. The absorbance was measured after 30 min at 517 nm. The free radical inhibition was calculated using the expression:

$$IC\ (\%) = (A_o - As)/A_o \times 100$$

where A_o is the absorbance of the reference liquid without sample, and As is the absorption of solution containing samples. The amount of sample necessary to inhibit by 50% the

absorbance of DPPH is defined as IC50. This value is expressed as microliters of sample for milligram of DPPH. Three measures were performed for each sample. In order to calculate the AOA, IC50 values of samples were compared to IC50 value of gallic acid (Sigma Aldrich, Germany) prepared with the same procedure. The ratio (IC50)GA/(IC50)sample represents the gallic acid equivalent of antioxidant activity of samples.

RESULTS AND DISCUSSION

The total polyphenol content and the antioxidant activity of wines and juices are reported in Table 2.

Table 2 - Total polyphenols content (TPC) and antioxidant activity (AOA) of wine and juice samples (mean ± SD)

	TPC (g GA/L)	IC50 (μL/mg DPPH)	AOA (g GA/L)
Chianti Classico (2006)	2.73 ± 0.01	37.13 ± 1.40	13.48 ± 0.51
Chianti Classico (2006)	2.47 ± 0.11	37.53 ± 0.95	13.33 ± 0.33
Red from Tuscany IGT	2.45 ± 0.12	40.67 ± 2.47	12.32 ± 0.73
Chianti (2008)	3.01 ± 0.08	30.87 ± 0.45	16.20 ± 0.24
Mix juice 1	1.37 ± 0.05	81.27 ± 2.84	6.20 ± 0.69
Mix juice 2	1.09 ± 0.02	118.73 ± 6.20	4.22 ± 0.22
Mix juice 3	1.06 ± 0.02	92.20 ± 9.18	5.18 ± 0.52
Mix juice 4	1.14 ± 0.04	98.47 ± 1.14	5.08 ± 0.06
Grape juice 1	0.45 ± 0.002	123.07 ± 0.90	4.06 ± 0.03
Grape juice 2	0.94 ± 0.01	166.87 ± 5.83	3.00 ± 0.10

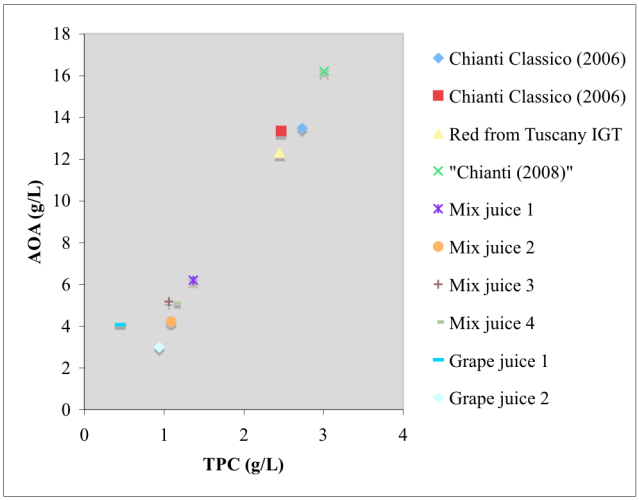
The data presented in Table 2 show that the wines have a polyphenol content ranging between 2.45±0.12 (Red from Tuscany IGT) and 3.01±0.08 g GA/L (Chianti 2008), while in juices this value ranges between 0.45 ± 0.002 g GA/L (Grape juice 1) and 1.37 ± 0.05 g/L (Mix juice 1). The antioxidant activity ranges between 6.09±0.18 g GA/L (Chianti 2006) and 16.20±0.24 g GA/L (Chianti 2008) for wines, and 3.00±0.10 g GA/L (Grape juice 2) and 6.20 g GA/L (Mix juice 1) for juices. In wine samples, the higher values of TPC and AOA, compared to juices, could be due to the phenolic contribution of wood, and to the condensation/polymerization reactions, which take place in wine during maturation (Barolomé *et al.*, 2004). Moreover, it is well known that older wines (2006) have a lower polyphenols content than the younger ones as Chianti 2008. Other factors responsible of this mismatch could be the different grapes variety, and maturation. Generally, the maximum grape antioxidant activity is reached when wine is fully mature. The time of technological maturity was traditionally estabilished for each viticulture area on physical and chemical parameters related to the sweetness/acidity (Bartolomé et *al.*, 2004), but in more recent years new criteria, based on both the phenolic content and the phenolic release from the grape skins have been considered to estabilish the period of maturation (Saint-Cricq de Galuleiac *et al*., 1998). The inclusion of new parameters, such as phenolics/colour characteristics, to evaluate grape maturation may be a new strategy to match grape maturity with the highest antioxidant potential. As regards the juices, Mix juice 1 has the highest TPC and AOA, followed by Mix juice 3, Mix juice 4, Mix juice 2, Grape juice 1 and Grape juice 2. The differences observed are due to the composition of juices. Mix juice 1, in addition to red and white grapes, contains pomegranate juice (10%), which represents an important source of polyphenols, and in

particular anthocyanins (Du *et al*., 1975). Mix juices 2-4 have similar antioxidant activity, thank to the commun presence of vegetable extracts from red grapevine leaves, *Sambucus nigra* and green tea. Mix juice 2 does not contain grape juice and has the lowest value of AOA when compared to other mixed juices.

Figure 1 shows the good correlation between TPC and AOA values measured in different samples (R^2= 0,9546).

Figure 1 - Correlation between total polyphenols content (TPC, g/L) and antioxidant activity (AOA, g/L)

	AOA/TPC
Chianti Classico (2006)	4.94
Chianti Classico (2006)	5.40
Red from Tuscany IGT	5.03
CHIANTI (2008)	5.38
Mix juice 1	4.53
Mix juice 2	3.87
Mix juice 3	4.89
Mix juice 4	4.46
Grape juice 1	9.02
Grape juice 2	3.19



On the basis of these data, it is possible to demonstrate that the antioxidant activity of the beverages analyzed is strictly associated with their polyphenol content.

Grape juice 1 was the only sample whithout good correlation between the two parameters considered; the reasons for this behaviour will be investigated.

CONCLUSIONS

It is known that antioxidant activity of grape-based products is influenced, not only by their content of polyphenols, but also by their phenolic compositions. Both classes of substances are influenced by vintage, grape variety, winemaking techniques, and ageing conditions. We have investigated the total polyphenols content and antioxidant activity of different beverages containing grape: red wine, mixed fruit juices and full grape juices. The total polyphenols content decreased as follows: red wine> mixed fruit juices> full grape juices. Red wine contains phenolic compounds with higher antioxidant activity than those contained in the other samples, and contain higher proportions of non-phenolic antioxidants. Fermentation of wine (see wine 2006 vs wine 2008) may decrease the content of those phenolic derivatives with high antioxidant activity or/and may lead to new vinegar phenolic compounds with lower antioxidant activity than those originally present before maturation (Dàvalos *et al*., 2005). It is also important to note that fruit juices contain other antioxidant molecules, such as vitamins or substances rich in polyphenols as green tea extracts, which contribute to the total antioxidant activity. A good correlation between TPC and AOA was found in sample analyzed. This data could be used for promoting the consumption of these grape juice, alone or with other fruit juices, as sources of polyphenols and other health-promoting substances.

REFERENCES

Bartolomé B., Nunez V., Monagas M., Gomez-Cordoves C., 2004. In vitro antioxidant activity of red grape skins. *Eur Food Res Technol*, 218 :173-177.

Brand-Williams W., Cuvelier M.E., Berset C., 1995. Use of free radical method to evaluate the antioxidant activity. *Food Sci Technol,* 28:25-30.

Dàvalos A., Bartolomè B., Gò mez-Cordovès G., 2005. Antioxidant properties of commercial grape juices and vinegars. *Food chemistry*, 93: 325-330.

Du C.T., Wang, P.L., Francis F.J., 1975. Anthocyanins of pomegranate, Punica granatum. *J. Food Sci*, 40:417-418.

DuPont M.S., Mondin Z., Williamson G., Price K.R., 2000. Effect of variety, processing, and storage on the flavonoid glycoside content and composition of lettuce and endive. *J Agric Food Chem*, 48:3957-64.

Leong, L.P., & Shui, G., 2002. An investigation of antioxidant capacity of fruits in Singapore markets. *Food Chemistry*, 76: 69-75.

Ough C.S., Fong D., Amerine M.A., 1972. Glycerol in wine: determination and factors affecting. *Am J Enol Vitic,* 23: 1-5.

Rapp A, Pretorius PJ., 1989. Flavours and off flavours. *In*: Charalambous, G., (Ed.), Proceedings of the 6th international flavour conference, Rethymnon, Crete, Greece 5-7 July 1989, Elsevier Science Publishers B.V., Amsterdam, pp. 1-21.

Rimm E.B., Klastsky A., Grobbee D., Stampfer M.J., 1996. Review of moderate alcohol consumption and reduced risk of coronary heart disease: is the effect due to beer, wine, or spirits? *Br Med J*, 312:731-735.

Schreier P., 1979. Flavor composition of wines: a review. *CRC Crit Rev Food Sci Nutr*, 12: 59-111.

Singleton, V.L. Esau, P., 1969. Qualitative phenolic composition of grapes and wines. Phenolic substances in grapes and wine and their significance, pp.7-41.

Soleas G.J., Diamandis E.P., Goldberg D.M., 1997. Wine as a biological fluid: history, production, and role in disease prevention. *J Clin Lab Ana,l* 11:287-313.

Souquet J.M., Labarbe B., Le Guernevé C., Cheynier V., Moutounet M., 2000. Phenolic composition of grape stems. *J. Agric.Food Chem,* 48:1076-1080.

Stanley L.L., Mazier M.J.P., 1999. Potential explanations for the French paradox. *Nutr Res,* 19:3-15.

Van de Wiel A., van Golde P.H.M., Hart H.C. 2001. Blessings of the grape**.** *Eur J Int Med,* 12:484-489.

Waterhouse A.L., 2002. Wine phenolics. *Ann N Y Acad Sci*, 957:21-36.

Bioactive Products Made on Basis of Concentrate Grape Juice

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ClAIM

The present paper describes the extension and diversification of grape use. In the processing industry grape juice can be used instead of sugar (sucrose) and in combination with the other fruit it can become the basis for products with bioactive agents.

The concentrate was used for production of bilberry juice, sea buckthorn juice and common fig compote, walnut compote and preserve, sea-buckthorn + pumpkin puree, which were given high organoleptic score.

The aforementioned product content of α-tocopherol, β-Carotene, and - anthocyans, phenolic carbon acides, flavonols, which are very important for the human health. They inhibit free-radical oxidation before it damages cells. They also contain K, Ca, Mg, Fe, Cu, Zn, which play a significant role in the nutrition physiology.

Die Arbeit betrifft die Erweiterung des Verwertunngsspektrums und der Bestimmung von Weintrauben – in der Verarbeitungstechnologie ist auf Grund des Ersatzes von Saccharose durch das WeintraubenKonzentrat und der Kombination mit anderen Obstarten das Produkt erzeugt, das mit bioaktiven Stoffen balanciert ist.

Mit der Benutzung des Konzentrats wurde der Heidelbeersaft, der Sanddornsaft, das Feigenkompott, das Nußkompott und die Nußkonfitüre, die Marmelade – Sanddorn + Kürbis zubereitet. Diese Produkte hatten eine hohe Bewertung in der Degustation.

Die Produkte enthalten α-Tokopherol, ß–Karotin, Anthozyanen, Hydroxycinnamaten, Flavanole. Sie spielen eine besondere Rolle, weil sie im menschlichen Organismus von freien Radikalen initiirte Oxdierungsprozesse anhalten können, bevor die Zelle beschädigt wird. Wichtig sind auch die Angaben von K, Ca, Mg, Fe, Cu, Zn. Ihre Funktion ist in der Physiologie der Nahrung unersetzlich.

Theme title: New products, new technologies, new challenges
Theme subtheme title: Health impact of vitivinicultural products
Type: poster
Language: English
Title: Bioactive Products Made on Basis of Concentrate Grape Juice

INTRODUCTION

In recent years there have been some deep and significant changes in the society's mentality regarding nutrition physiology. New trends have developed initiating modification of the nutrition landscape. These changes are results of the modern medical researches and studies. The nutritive value and therapeutic properties of fruit are determined based on the concentration bioactive components [Beverdige T., et all. 1999; Kähkönen M.P., et all. 2001.]

Grape attracts great interest due to its phytochemical compounds, mainly glucose and fructose as well as aroma and flavor compounds. Many of them have antioxidant effect Grape monosaccharides are important not only in wine production. Grape concentrate can be used as a sweetener in food processing industry taking into account the properties of the raw material and the processed product. Due to the modern viewpoints on sucrose consumption the use of grape concentrate an issue of present interest.

According to the data of the World Health Organization (WHO, 2003) the use of sucrose should be considerably reduced. Sucrose is an effective immunosuppressive substance, which affects mineral metabolism, increases the levels of glucose and insulin, provokes atherosclerosis, facilitates obesity. A total of >50 negative effects are listed. It is believed that free radicals are produced during the sucrose hydrolysis in the human body [http://www.alternative-doctor.com/nutrition/sugar.htm; http://www.forum.zelek. ru/index php?showtopic=4576].

It should be noted that sugar is a source of energy. Its oxidation produces Adenosine-5'-triphosphate (ATP). It has been found that ceasing of sugar intake considerably reduces the normal level of blood glucose (hypoglycemia). The acceptance of natural glucose and fructose is preferable. They are digested with other substances and their absorption is slowed down.

Free radicals cause cardiovascular, pulmonary and other diseases and cancer and compromise immunity [Steinmetz K., Pottr J.D.1996.; Bagchi K., Puri S. 1998; Артемова А. 2001; Kähkönen M.P., et al. 2001; Burdulis D. et all 2007.].

Antioxidants suppress the aforementioned negative processes. Fruits contain antioxidant substances, which inhibit oxidation thus slowing or preventing oxidative damage to the human organism. Such antioxidants are flavonoids, phenol carbonic acids, α-tocopherol, û-carotene and unsaturated fatty acids, namely oleine acids, linol acid and linolenic acid [Kalt K., et al. 1999; Beverdige T., et all. 1999; Артемова А. 2001; Kähkönen M.P.,et al. 2001].

In view of the above it is essential to include in the diet fruit varieties with high contents of the aforementioned substances. In this paper the subjects of research are sea-buckthorn, bilberries, fig and green walnuts.

The purpose of the research is production of processed products from bioactive fruits using grape concentrate instead of sugar.

Research Methods

Sea-buckthorn and bilberry pulpy juices were expressed from berries. Fig compote was produced by blanching. Walnut compote and preserves were made of green walnuts whose exocarp is tough but mesocarp and endocarp are still soft. Fruit was boiled after certain processing. Sea-buckthorn and pumpkin puree were made separately and mixed.

In all the cases sugar was replaced with grape concentrate (soluble solids 65^0B) produced with modern technologies.

Soluble solids and titratable acidity (PH) were measured in all the processed products. In the used raw materials α-tocopherol, û-carotene, anthocyanins, flavonols, phenol carbonic acids were measured with highly-effective Varian Prostar liquid chromatograph; concentration of mineral elements was measured with atomic absorption spectrometer (AANALIST 400) and the ascorbic acid content was determined with Tillman's method; organoleptic analysis was carried out using 18 point scale [Schobinger U.2004].; antioxidant activity was measured using stable free *1,1-diphenyl-2-picrylhydrazyl* radical (DPPH), inhibition index %.

Results

Sea-buckthorn berries are rich in α-tocopherol, ascorbic acid, û-carotene and unsaturated fats (Tabl. 1).It should be noted that sea buckthorn contains unsaturated fatty acids, which increase its antioxidant activity. Besides, sea-buckthorn berries have high acidity, which restricts their use. Addition of sugar lessens the sour taste, but the actual quantity of acids does not change. High acidity has a negative impact on the human nutrition physiology. The titratable acidity of sea-buckthorn berries is 2.9%.

Carrot juice and grape concentrate were added to the sea-buckthorn juice in quantitative proportions of 45:35:20. These additions significantly reduce acidity of the sea-buckthorn juice. Carrot juice has low titratable acidity of < 0.2 g/100g. Besides, mineral elements of carrot react with carboxyl group producing salts (Tabl.1).

Table 1

Phytochemical Compounds of Sea-buckthorn Berries and Juice

Concentrations, g/100g.	**Berry**	**Juice**
Soluble solids	8.0	19.5
Titratable acidity	2.9	1.3
Tocopherol, mg/100	8.7	4.0
Carotene, mg/100	2.7	11.8
Ascorbic acid, mg/100	137.0	47.5
Fat	5.2	2.1
Pectic substances	0.3	0.64
Calcium mg/100	13.3	38.0
pH	2.7	3.5

The antioxidant action of the mixed juice is not reduced due to its carotene concentration of 11.8mg/100gr. Its concentrations of ascorbic acid, tocopherol and fat are in compliance with the proportion of sea-buckthorn juice. Moreover, due to addition of carrot the pectin and calcium concentrations which are very important for human physiology increased in the mixed juice.

The pectic substances have various positive effect on the human health. They can detox endotoxins and exotoxins, prevent radiation damage, lower blood sugar level and inhibit cholesterol penetration from the intestine. Calcium is involved in bone tissue formation, regulates muscle system, including myocardium. Calcium deficiency causes destruction of bone tissues and suppression of immune system.

Based on the organoleptic analysis organoleptic properties of the sea-buckthorn juice were given the score of 16.1 points and assessed as Very Good. Variance is V=9.5%.

The mixed puree of sea-buckthorn and pumpkin with grape concentrate as a sweetener has similar properties. The proportions of the aforementioned raw materials in the puree are 45:40:15 percents or 45:35:20 percents according to the contents of soluble solids in the pumpkin.

There is increasing interest in bilberries which have antioxidant effect due to the anthocyanins and phenolic compounds they contain.. The objective of the research was production of sugar-free bilberry juice using grape concentrate as a sweetener. The quantitative proportions of juice and grape concentrate are 4/1 or 5/1 depending on the amount of soluble solids in the juice. The characteristics of the mixed juice made of plums and bilberries using grape concentrate are shown in Table 2 below.

Table 2

Characteristics of the Plum and Bilberry Mixed Juice with Grape Concentrate

Characteristic	Content
soluble solids, %	15-20%
Titratable acidity, %	0.7-1.0%
pH	3.8-4.0

The contents of flavonols and phenol carbonic acids in the juice were determined. The chromatographic peak is >10. Some of the substances found in comparatively large proportions are (+) catechin, (-) epicatechin, chlorogenic acid, p-coumaric acid and sinapic acid (Tabl. 3)

Table 3

Contents of Flavonols and Phenol Carbonic Acids in Bilberry Juice mg/l

Substance	Contents
(+) catechin	43.04
(-) epicatechin	18.28
Chlorogenic acid	23.80
Caffeic acid	3.4
P-coumaric acid	12.9
Sinapic acid	9.49
Vanillin	4.33

The total concentration of anthocyanins is 3200 mg/l. The bilberry anthocyanins are derivatives of 5 anthocyanidins, namely cyanidin, delphinidin, malvidin, petunidin and peonidin., which have quite high antioxidant capacity. The chromatograph detected a total of 17 anthocyanins. Mineral elements, which are found in juice also contribute to its high quality (Tabl. 4), especially Zn due to its antioxidant properties, as well as K, Ca, Mg and Na, which are important for human health.

Table 4

Mineral Elements Concentrations in Bilberry Juice, mg/l

Mineral Element	Concentration
K	1052
Ca	127
Mg	74
Zn	1.6
Na	21

The antioxidant capacity of bilberry juice is quite high. Its antioxidant activity measured using *1,1-diphenyl-2-picrylhydrazyl* (DPPH) is 55% and Trolox equivalent antioxidant capacity is 56.5%.

The bilberry juice were given organoleptic score of 16.2 points and assessed as Very Good. Variance is V=8.0%.

The bilberry juice produced with the aforementioned technology can be consumed or used as a component in processed products.

The use of figs is limited to their ripening season. As figs contain a wide range of elements essential for human health, it is important to ensure their year-round use. Some of the especially noteworthy components are monosaccharides (glucose and fructose), pectic substances, proteins and high concentrations of mineral elements (K, Ca and Fe). In view of

the aforesaid it is important to extend fig consumption period. It is necessary to produce products and elaborate technologies maintaining or increasing bioactive properties of figs, especially their antioxidant activity.

At this stage we propose production of fig compote. Traditionally it is produced with sucrose syrup. According to the proposed technology the mix of bilberry and grape concentrates will be used instead of syrup. The quantitative proportions of concentrates and juice will be 4/1 or 5/1 depending on the amount of soluble solids in the juice and figs.

Figs are blanched at the temperature of 85-95^{0}C, exposition is 5-7 minutes. Then figs are placed in the container together with bilberry juice, 50-60% of raw materials and 50-40% of solution.

The characteristics of fig compote are shown in Table 5.

Table 5

The Characteristics of Fig Compote

Characteristic	Content
soluble solids, %	18-22%
Titratable acidity, %	0.6-0.53%
pH	3.8-4.1

One of the benefits of fig compote made with bilberry juice and grape concentrate is high content of antioxidant substances, including all anthocyanins present in bilberry juice.

The aforementioned fig compote also contains flavonols and phenol carbonic acids, namely (-) epicatechin – 14.1 mg/l, (+) catechins – 42.8 mg/l, as well as p-coumaric acid, sinapic acid, chlorogenic acid and vanillin in the concentrations of 6.0-4.0-3.0-4.8 mg/l respectively (Tabl. 6).

Table 6

Contents of Antioxidant Components in Fig Compote

Substance	Contents
(+) catechin	42.8
(-) epicatechin	141.6
Chlorogenic acid	3.0
Caffeic acid	0.8
P-coumaric acid	6.0
Sinapic acid	4.0
Vanillin	4.8

Fig compote capacity to inhibit 1,1-*diphenyl-2-picrylhydrazyl* (DPPH) is 45%.

Fig compote was given organoleptic score of 14 points and assessed as Good. Variance is V=22%.This percentage shows that the tasters' assessments varied greatly and some gave 17 points to the product. The variance can be explained by the the novelty of the product.

The production of the aforementioned fig compote is technologically feasible. It also contains bioactive components, which supplement each other and nutritive, preventive and therapeutic qualities of the product.

The technology of producing green walnut preserves is as follows: green walnuts are first placed in water and then soaked in lime water solution. Afterwards they are washed and boiled in the alum solution with 5% sugar. This is the preparation stage. Then the walnuts are boiled in sucrose syrup.

We propose to use grape concentrate instead in sugar for green walnut preserves. It should be dissolved in water to reach the concentration of 25-40%. 2-3 liters of this solution are required for 1 kg of walnuts. The nuts should be boiled 4 times and kept for 5-8 hours.

The green walnut preserves have various important properties. They contain fiber, which is not a source of energy and plastic material for human organism, but facilitates food digestion.

The green walnut preserves are also rich in minerals (Tabl. 7). They contain K (1,451 mg/l) and Ca (1,647 mg/l). It seems that some Ca remains in walnuts after soaking them in lime water solution. Fe, Cu and Zn mineral elements of green walnut preserves (in 0.6-1.9-1.0 mg/l concentrations respectively) have antioxidant effect. Pb concentration in green walnut preserves (0.15 mg/l) is half of the allowed standard. This factor should be taken into account when green walnut preserves are produced as most of the nut trees grow near motorways.

Table 7

Concentrations of Micro- and Macroelements in the Juice of Green Walnut Preserves

Elements	Concentration, mg/l
K	1451.0
Na	47.1
Mg	105.6
Ca	1647.0
Fe	0.6
Cu	1.9
Zn	1.0
Pb	150 microgram/l

The walnut compote was also produced. Its preparation stage is the same as that of walnut preserves. Walnuts are boiled first in water and then grape concentrate is added. The concentration of soluble solids in nuts and juice should vary between 18-22%. Walnut compote is rich in flavonols and phenol carbonic acids (>12).

Walnut compote has high concentrations of (-) epicatechin, vanillic acid and chlorogenic acid (in 13.9-4.1-3.0 mg/l concentrations respectively). The concentrations of catechins and caffeic acid are relatively low (2.4-1.5 mg/l) (Tabl. 8), but they have considerable antioxidant effect.

Table 8

Contents of Flavonols and Phenol carbonic acids in Walnut Compote

Substance	Concentration, mg/l
(+) catechin	2.4
vanillic acid	4.1
Chlorogenic acid	3.0
Caffeic acid	1.5
(-) epicatechin	13.9
Vanillin	0.15
P-coumaric acid	1.3
Sinapic acid	0.07

The total concentration of phenolic compounds in fruit and juice is 1,020 mg/l and the capacity to inhibit *1,1-diphenyl-2-picrylhydrazyl* (DPPH) is 50%. which is a high index.

As a result of the organoleptic analysis organoleptic properties of the walnut compote were given the organoleptic score of 14.2 points and assessed as Good. Variance (V=19%) is quite high. The walnut preserves have the same characteristics.

Conclusions:

Grape concentrate can be used for production of juices, puree, compote and preserves. It gives to the processed product disease preventive and somewhat therapeutic properties. Fructose constitutes 40-45% of the total sugar amount. Fructose metabolism is not regulated by insulin. Besides, grape concentrate contains phenolic compounds.

The concentrations of soluble solids and pH in juice, compote and puree vary between 15-22 and 3.5-3.8. They meet technological, organoleptic and nutrition physiology requirements.

It is well known that sea-buckthorn berries have high contents of bioactive substances. The juice and puree produced with the proposed technology will expand the consumption of this product. They have lower acidity and maintain antioxidant action of berries.

Bilberry juice contains 17 anthocyanins, flavonols like (+) catechin, (-) epicatechin and phenol carbonic acids, like chlorogenic acid, p-coumaric acid and sinapic acid.

The fig compote produced with the aforementioned technology has higher antioxidant action than fig fruits. Due to the use of bilberry juice instead of syrup the fig compote contains all the anthocyanins of the bilberry juice and has high concentrations of flavonols and phenol carbonic acids.

The green walnut preserves and compote contain fiber, high concentrations of mineral elements. They also have considerable concentrations flavonols and phenol carbonic acids.

Lead (Pb) concentration in walnut preserves (0.15 mg/l) is half of the allowed standard. This factor should be taken into account as nut trees often grow near motorways.

Bibliography

1. Artemova A. 2001.Sea Buckthorn Healing and Anti-aging, Saint Petersburg Dilya Publishing House, pg.27-29.

2. Bagchi K., Puri S. 1998. Free radical and antioxidants in health and disease. Eastern mediterranean Health Journal.V4.2. P.350-360.

3. Beverdige T., Li T.S.C., Oomah B.D., Smith A. 1999. Sea Buckthorn Products: manufacture and Composition. J. Agric. Food Chem. 47. P. 380-388.

4. Burdulis D., Ivanauskas L., Dirse V., Karlauskas S., Razukas A.2007. Study of diversity of antocyanin composition in bilberry fruits. Medicina (Kaunas). 43.12

5. Crifford M.N. 2000. Anthocyanins, nature, occurrence and dietary burden. J. SCI Food Agri.80. P.163-172.

6. Kalt K., Forney C.F., Martin A., Prior R.I. 1999. Antioxidant After fresh storage of Small fruits. Agric. Food Chem. 47. P. 4638-4644.

7. Kähkönen M.P., Hopla A. I., Hernonen M. 2001. Berry phenolics and their antioxidant activity. J. Agric. Food Chem. 49.P.476-482.

8. Steinmetz K., Pottr J.D.1996. Vegetables, fruits and cancer prevention: a review. J Am Diet Assoc.96. P.1027-1037

9. Fruit and Vegetable Juices, under the editorship of Schobinger U. 2004, translated from German, 3rd Edition, Saint Petersburg, Professions. 640 p.

10. http://www.alternative-doctor.com/nutrition/sugar.htm

11. http://www.forum.zelek.ru/index php?showtopic=4576

INFLUENCE OF SOME BIOACTIVE PRINCIPLES IN RED WINE UPON CELLULAR OXIDATIVE PHOSPHORYLATION

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ABSTRACT

The *in vitro* influence of a Romanian red wine (Fetească neagră) upon cellular respiration and oxidative phosphorylation process of the batracian muscular and hepatic cells was studied. The oxidative phosphorylation process was appreciated by calculation of P:O ratio (oxidative phosphorylation index) values, the oxygen respiratory cellular consumption by Warburg micromanometric method and inorganic phosphate cellular content by the Bell-Doisy-Briggs method. The red wine induced certain stimulation of cellular respiration and of oxidative phosphorylation process, with amplitudes depending on the wine used concentrations (1–3%) and the cell type. The results evidence the influence of bioactive principles (especially polyphenols) in red wine upon the cellular energetic processes as well as some of theirs useful pharmacological properties.

L'influence *in vitro* d'un vin rouge roumain (Fetească neagră) sur la respiration cellulaire et les processus de phosphorylation oxydative des cellules musculaires et hépatiques batracien a été étudiée. Le processus de phosphorylation oxydative a été appréciée par le calcul de les valeurs P:O ratio (indice de phosphorylation oxydative), la consommation d'oxygène respiratoire cellulaire a été déterminé par la méthode micromanometrique Warburg et le contenu du phosphore inorganique par la méthode Bell-Doisy-Briggs. Le vin rouge induite certaine stimulation de la respiration cellulaire et du processus de phosphorylation oxydative, selon les concentrations du vin utilisé (1–3%) et de le type de cellule étudiée. Les résultats prouvés l'influence des principes bioactifs (en particulier les polyphenols) de le vin rouge sur les processus cellulaires énergétiques ainsi que certains de leurs propriétés pharmacologiques utiles.

INTRODUCTION

It is by now generally accepted the idea that some bioactive compounds of wine, polyphenols especially, evidence a series of properties known as inducing certain beneficial effects, as a result of moderate wine consumption, which also explain – among others – the so called „French paradox“ [Dudley *et al.*,2008].

Consequently, the antiradicalic, antiatherogeneic, vasodilating, anti-inflamatory, hepatoprotecting and others positive properties – in correlation with the positive effects of the bioactive principles from wine (polyphenols especially) – have been evidenced in a series of diseases, such as cardiovascular, hepatic, neurological maladies, cancer etc. [Jackson, 2008, Shan He *et al.*,2008, Cotea *et al.*, 2008, Dudley *et al.*,2008].

Previous investigations of ours [Cotea *et al.*, 2008, Neacşu *et al.*2009,] analyzed several aspects of the influence of some fractions isolated from red wine on cellular respiration and on the properties of the blood vessels of certain laboratory animals.

The present paper analyzes the effects of various concentrations of Romanian Fetească neagră red wine upon the intensity of cell respiration and of oxidative phosphorylation in muscular and hepatic frog cells.

MATERIALS AND METHOD

The experiments, performed *in vitro*, on striated muscular cells and hepatic cells taken over from frog (*Rana ridibunda*, Pall), treated with red wine obtained from Romanian Fetească neagră grapes, following cellular respiration and oxidative phosphorylation process. The studied wine had an alcoholic strength of 10.80 % v/V, FCI of 52 mg/L, TPI of 28.83 mg/L, total antocyans – 211.13 mg/L.

The experiments were performed on batches of biological preparations of sartorius muscle and liver from 10 animals. These preparations were treated with red wine in concentrations of 1, 2 and 3 mL/100mL, in a physiological Ringer solution, comparatively with untreated reference groups, incubated in normal Ringer (NR) solution.

The intensity of cellular consumption of oxygen (μL/g fresh tissue= mm^3/g tissue) was determined by the Warburg micromanometric method, at constant temperature and volume. The process of oxidative phosphorylation was appreciated through values of oxidative phosphorylation index, expressed by the P:O ratio, on the same preparations on which cellular respiration was also determined.

The values of the inorganic phosphate (Pi = μmol/g tissue) were represented by the difference between initial Pi content of the preparations, in the beginning of the respiration period, and the final values, recorded in the end of the experiment. The cellular Pi content was determined by the Bell-Doisy-Briggs method [Nuță, Buşneag, 1977].

The values of the oxidative phosphorylation index were calculated with relation:

$$P:O = \frac{\mu mol\ Pi/g\ tissue}{\mu atomg\ O/g\ tissue}$$

The experiments were performed at room temperature (22 ºC), for 60 minutes.

The obtained data were statistically analysed by Student test.

RESULTS AND DISCUSSION

The data recorded after a 60 minutes experiment show that the red wine obviously influences cellular processes, different values being recorded, *versus* the untreated, reference bath, both as to the intensity of cellular oxygen consumption and level of oxidative phosphorylation, expressed by the values of the P:O ratio.

In the untreated, control batch (Tab. 1), the values recorded in the end of the experimental period, for muscular cells, showed an average value of oxygen consumption of 1.309 μL/g fresh tissue (0.935 μatomg /g tissue), and an average content of inorganic phosphate (Pi) of 2.196 μmol/g fresh tissue, while the index of oxidative phosphorylation (the P:O ratio) took a value of 2.348. In the hepatic cells of the control batch, the respiration oxygen consumption was of 0.861 μL/g fresh tissue (0.615 μatomg /g tissue), the Pi content was of 1.591 μmol/g fresh tissue, and the P:O ratio – 2.587.

The data recorded for the control batch (Tab. 1), as actually those registered wine-treated ones, evidenced obvious differences between the behaviour of the muscular and respectively – hepatic cells. For example, in muscle cells, the values of the oxygen

respiratory consumption and of Pi content are higher than in the hepatic cells, however the index of oxidative phosphorylation records lower values.

Tab. 1

	Muscle cells				Liver cells			
	O_2 consumed		Pi µmol/g	P:O	O_2 consumed		Pi µmol/g	P:O
	µL/g	µatomg/g			µL/g	µatomg/g		
$\overline{X}$	1.309	0.935	2.196	2.348	0.861	0.615	1.591	2.587
ES	0.094		0.244		0.113		0.215	
CV %	17.489		27.330		28.262		22.176	

This situation indicates a more intense respiration of the muscle cells, as well as a higher level of oxidative phosphorylation at the level of hepatic cells – comparatively with the muscle ones – along with a higher energetic yield (of ATP production), which means that state 3 of processes of cellular respiration is prevailing [Brand *et al.*, 1993, Shan He *et al.*, 2008].

Such differences may be explained if considering the different morpho-physiological characteristics of the two types of cells. Thus, the striated muscular cells are of excitable type, with a contractile function, while the hepatic ones are non-excitable and play a metabolic role, being the center of numerous synthesis processes with energetic implications [Alberts *et al.*, 1998, Hăulică, 2007].

The different characteristics of the two types of cells also explain their different reactivity during the treatment with red wine. Consequently, in the cellular batches incubated in a wine-containing physiological solution (Ringer), generally, both cellular respiration and the process of oxidative phosphorylation are stimulated, although the amplitude of the phenomenon is different, as depending on the nature of the processes, type of investigated cells and applied wine concentration (Figs. 1 and 2).

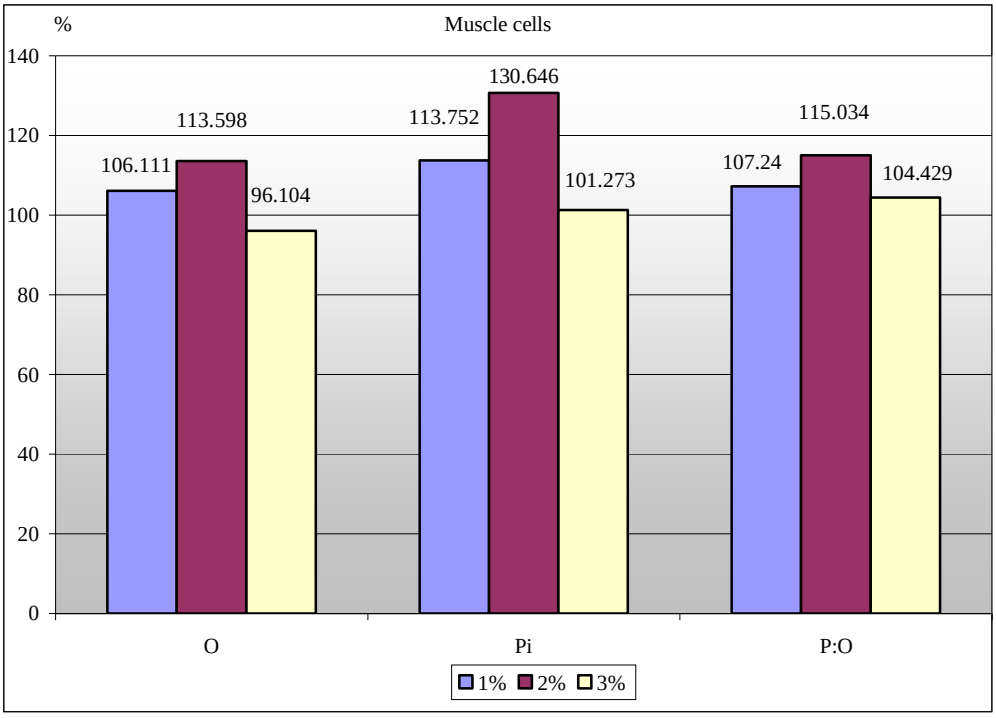

Fig. 1

Similarly with the control batch, the treated ones register – in the muscle cells – higher values of oxygen consumption and content of inorganic phosphate, comparatively with the

values evidenced by hepatic cells, while the oxidative phosphorylation index (P:O) takes lower values.

An 1 % red wine concentration induces, in muscular cells (Fig. 1), an increase of the respiration oxygen consumption up to 106.111 % *versus* the value of the reference batch (100 %), along with an increase of the Pi content up to 113.752 %, as well as a higher value of P:O ratio, up to 107.240 %. In hepatic cells (Fig. 2), an increase of the values of such parameters should be also noticed, the oxygen consumption increasing up to 110.337 %, the Pi content – up to 115.713 %, and the P:O ratio – up to 104.948 %, comparatively with the values registered in the control batch.

On may therefore observe that, at an 1 % wine concentration, oxidative phosphorylation is more intense in the hepatic (P:O = 2.715) than in the muscle (P:O = 2.518) cells, yet, comparatively with the values of the reference batch, the efficiency of the process is lower (with 2.292 %) in the hepatic than in the muscle cells, although the values oxygen consumption and of Pi are higher than those of the muscle cells.

The treatment with 2% red wine concentration evidences the strongest stimulation action of the cellular processes under investigation, when the highest values of the parameter involved are recorded (Figs. 1 and 2). Thus, comparatively with the values of the reference group, the muscular cells recorded an increase of respiratory oxygen consumption up to 113.598 %, of the Pi content – up to 130.646 %, and the P:O ratio, respectively, up to 115.034 %. In the hepatic cells, higher values than in the reference batch are also recorded, up to 137.282 % (respiratory oxygen), 148.837 % (Pi), and 108.465 %, respectively (P:O ratio).

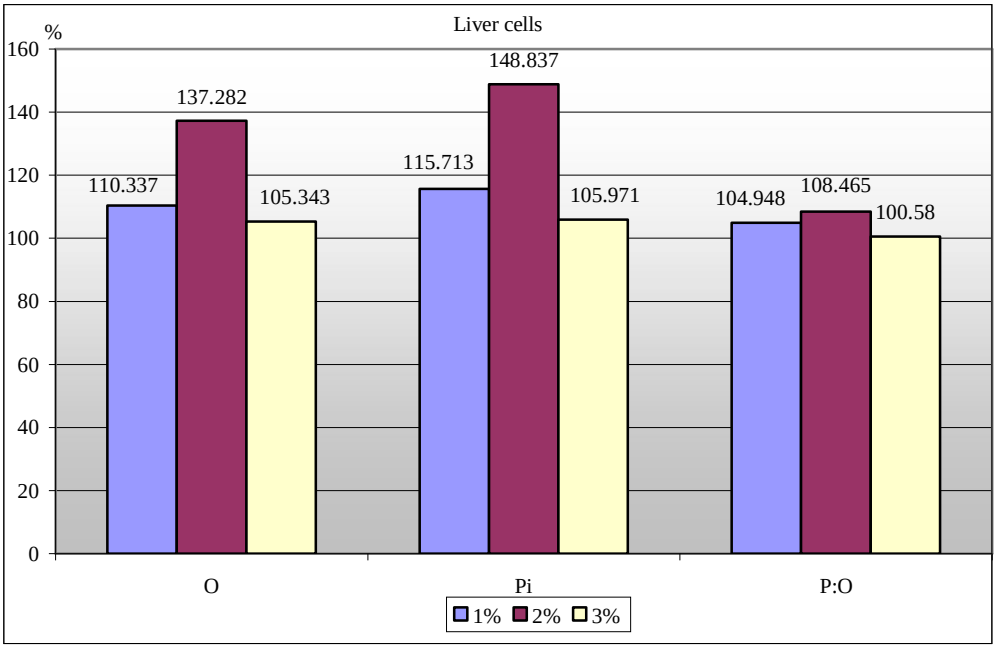

Fig. 2

Similarly with the treatment with 1 % red wine concentration, in the hepatic cells, the respiratory oxygen consumption and Pi values are lower than in the muscular cells, and the increase of the P:O ratio is also more reduced than in the muscular cells (108.465% compared with 115.034 %), which indicate a weaker stimulation of oxidative phosphorylation by 2 % wine concentration.

A 3 % concentration of red wine (Figs. 1 and 2) generally induces weaker effects than those determined by lower concentrations, which is probably due to a higher ethanol ratio

in the incubation medium, known as having its own physiological effects of oxidative phosphorylation depression [Cunningham, Bailey, 2001].

In muscular cells (Fig 1), under the action of such a concentration, respiratory oxygen consumption attains a lower value than in the reference batch (96.104 %), the Pi content remains practically unchanged (100.273 %), while the P:O ratio registers only a slight increase (104.273 %), comparatively with the control. The hepatic cells (Fig. 2) evidence higher values than the muscular ones, yet much more reduced than those attained with lower wine concentrations, as follows: respiratory oxygen consumption – 105.343 % *versus* the control, Pi – 105.971 %, and the P:O ratio – 100.580 %.

As generally known, the intensity of cellular respiration is directly correlated with the mitochondrial processes characterized by respiratory oxygen consumption and release of energy, deposited in the macroergic structure of the ATP molecules [Alberts *et al.*, 1998, Lehninger, 1987]. Production of ATP at mitochondrial level involves phosphorylation of cellular ADP ($ADP + Pi \rightarrow ATP$), a process correlated with the chain of electron carriers and with the mitochondrial enzymatic systems within the Krebs cycle, the final electron acceptor being the oxygen taken over through breathing. Within such processes, for 1 mol of glucose, which represent the metabolic substrate, 3 moles of ATP are generated – if the first carrier of electron chain is NADH, or 2 moles of ATP if the succinate, oxidated through $FADH_2$ intervenes, and only 1 mol ATP if cytochrome c belonging to the chain of electron carriers is oxidated. Therefore, such processes involve the P:O ratio, which represent the index of oxidative phosphorylation expressing the coupling degree of ADP phosphorylation with the oxygen consumed through respiration, taking part in oxido-reduction processes from which ATP results [Alberts *et al.*, 1998, Lehninger, 1987]. At a maximum phosphorylation degree, the respiration processes occurs in state 3, at a P:O ratio of 3 value, complete oxidation of glucose thus occurring; on the contrary, at a minimum (zero) phosphorylation, cellular respiration is in state 4, glucose being incompletely oxidated [Brand *et al.*1993, Lehninger, 1987], and the P:O ratio records its minimum value.

An important observation to be made is that the active principles from wine (polyphenols, especially) cause an increase in the enzymatic activity of the mitochondrial complexes, thus stimulating oxidative phosphorylation and ATP synthesis in cardiac cells, inducing cardioprotecting effects [Dudley *et al.*,2008].Various investigations also evidenced the antiradicalic properties of wine (the red wine, mainly) and the positive effects in other affections, characterized by disorders of the cellular oxidative and energetic processes [Burda, Oleszek, 2001Chiriță *et al.*, 2009, Cotea, 2008, Jackson, 2008, Shan He *et al.*, 2008].

The results of the present study, evidencing that red wine leads to high values, close to 3, of the P:O ratio, indicate a high degree of oxidative phosphorylation, with production of ATP and no generation of free radicals, which highlights the antiradicalic properties of the Fetească neagră wine, already evidenced in some other researches [Chiriță *et al.*, 2009], and also its energetic effects.

CONCLUSIONS

The Fetească neagră red wine stimulates cellular aerobe respiration and oxidative phosphorylation in both muscular and hepatic cells, the amplitude of its effect being dependent on the wine concentration applied and on the cell type.

Respiratory oxygen consumption is more intensely stimulated in hepatic cells, while the index of oxidative phosphorylation records higher values in muscular cells.

The obtained results attest the positive influence of the bioactive principles in red wine upon cellular energetic processes, and evidence some useful pharmacological properties of these principles.

BIBLIOGRAPHY

Alberts B., K., Bray D., Johnson A., Lewis J., Raff M., Roberts K., Walter P., 1998**.** Essential Cell Biology, Garland Publishing Inc., New York, London

Brand M.D., Harper M.E., Taylor H.C., 1993. Control of the effective P/O ratio of oxidative phosphorylation in liver mitochondria and hepatocytes, *Biochem J.*, 291, 739-748

Burda S., Oleszek W., 2001. Antioxidant and antiradical activities of flavonoids, *J. Agric. Food. Chem.*, 49, 2774–2779

Chiriță Otilia, Tudose-Sandu-Ville Șt., Cotea V.V., Niculaua M., Neacşu I., Odăgeriu Gh., 2009. Étude comparative des l'activité antiradicalaire des vins rouges Merlot et Fetească neagră obtenus dans les vignoble Iaşi par differents technologiques des fermentation maceration, *XXXII*nd *World Congress of Vine and Wine*, Zagreb, Croatia, 6 pp., electronic version - CD

Cotea Irina, Neacşu I., Colibaba Cintia, Cotea V.V., 2008. Influence of some white and red wines on the oxidative stress induced by experimental atheromatosis, *Bulletin de l' OIV*, vol. 81, nr. 923–925, 57–64

Cotea V. V., Neacşu I., Niculaua M., Bild W., 2008. Respiratory and vascular effects of some fractions isolated from red wine, *Cercetări Agronomice în Moldova*, XLI, vol. 2 (134), 51–60

Cunningham C.C., Bailey S.M., 2001. Ethanol consumption and liver mitochondria function, *Biol. Signals Recept.*, 10, (3-4), 271–282

DudleyJ.I.,Lekli I., Mukherjee S., Das M., Bertelli A.A.A., 2008. Does white wines qualify for French Paradox ? Comparison of the cardioprotective effects of red and whitw wines and their constituents: resveratrol, tyrosol, and hydroxytyrosol, *J. Agric. Food Chem.*, 56 (20), 9362-9373

Hăulică I., 2007. Fiziologie umană, ediția a III-a, Ed. Medicală, Bucureşti

Jackson S.R., 2008. Wine Science, Principles and Applications. Third edition, Acad. Press, Elsevier Inc., Burlington, London, San Diego

Lehninger A. L., 1987. Biochimie, vol. II, Ed. Tehnică, Bucureşti

Neacşu I., Cotea V.V., Niculaua M., Chiriță Otilia, Mogârzan Cristina, Cioroiu B., Lăcureanu G., 2009. In vitro effects of some fractions isolated from red wine upon cellular respiration. *XXXII*nd *World Congress of Vine and Wine*, Zagreb, Croatia, 6 pp., electronic version- CD (Abstracts volume, p.178)

Nuță G., Bușneag C., 1977. Investigații biochimice, Ed. Didactică şi Pedagogică, Bucureşti

Shan He, Cuirong Sun, Yuangjiang Pan, 2008. Red wine polyphenols for cancer prevention, *Int. J. Mol. Sci.*, 9, 842-853.

FIVS-Assure

Presented by Bennett Caplan

Head of Secretariat, FIVS

www.fivs.org

June, 2010

Background

- The alcohol beverage industry faces differing and often competing views of the proper place of alcohol beverages in society due to the many risks and benefits associated with the consumption and marketing of alcohol beverages.

FIVS – ASSURE

Background

FIVS Vision

"A successful global beverage alcohol industry, operating on the principles of corporate social responsibility, sustainability, and focus on consumer interests, in an environment free from trade-distorting factors of all kinds."

Guiding Principles for the Responsible Advertising and Marketing of Alcoholic Beverages

FIVS

GUIDING PRINCIPLES FOR ADVERTISING AND MARKETING PRACTICES FOR ALCOHOL BEVERAGES

Background

FIVS is a world-wide organization designed to represent all sectors of the wine, spirits and beer industries. It includes producers, distributors, importers, exporters, and trade associations, and it interfaces with many international organizations. FIVS was founded in July 1951, and has its headquarters in Paris, France.

In 2004, the members of FIVS adopted a strategic plan for the organization which sets its direction and purpose for the future. The vision of FIVS, set forth in that strategic plan, is as follows:

A successful global beverage alcohol industry, operating on the principles of corporate social responsibility, sustainability, and focus on consumer interests, in an environment free from trade-distorting factors of all kinds.

In keeping with its vision, FIVS has developed a set of guiding principles for the advertising and marketing of alcohol beverages. Many codes have appeared over recent years and in different countries to provide guidance in this area. Some have been produced by individual companies, some by trade associations, and some by other groups.

It is desirable to have the greatest degree of similarity possible between these codes, while acknowledging that national and cultural norms, together with differing national regulatory frameworks, require variances in some areas. In order to promote this goal, FIVS decided to produce a document outlining the major principles that should be considered in producing codes of best practice for the advertising and marketing of alcohol beverages.

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FIVS-Assure (Social Aspects)

FIVS-Assure Website

This strategic initiative takes the form of a social aspects website, called FIVS-Assure, that provides FIVS members and other interested parties with current and relevant information on social aspects programs in the alcohol beverage industry. This guide draws upon exemplary practices by companies, trade associations and other entities from around the world in the social aspects arena.

Members of the alcohol beverage industry face differing and often competing views of the proper place of their products in society. While scientific evidence pointing to the healthful benefits of moderate consumption continues to mount, FIVS also recognizes that excessive consumption can be harmful. As a result, industry representatives have searched for ways to combat the myriad of societal issues that arise out of excessive alcohol consumption.

With the copious and creative initiatives arising out of the industry itself, FIVS began developing FIVS-Assure, a reference guide that touches upon the many societal aspects related to alcohol beverages, ranging from drinking and driving to the responsible marketing of alcohol beverages. FIVS-Assure will provide guidance as to how associations and companies around the world might handle the many social impacts of over-consumption of alcohol.

FIVS-Assure Website

Members Only

Link to FIVS-Alive Workspace

FIVS members who are involved in this Strategic Initiative's Working Group may access the collaborative online workspace on

FIVS-Assure Initiative

- A resource for users to develop and/or improve their social aspects programs.
- Presents current approaches.
- Draws from:
  - Trade associations
  - Companies
  - Other global entities

FIVS-Assure Website

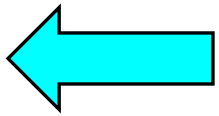
- The website is organized in the following manner:
  - Drawing from global sources
  - Selective
  - Guided and mapped out
  - Easy to use
  - Free and publicly available

Website Structure

- Main Topic Page
  - Description of Issue
  - Sub-category Titles and Descriptions
    - Sub-Page
      - **Examples**

FIVS-Assure: An Industry Resource for Social Aspects

FIVS-Assure is a strategic initiative of FIVS that provides a guide for resources on social aspects programs in the alcohol beverage industry. This guide draws upon exemplary practices by companies, trade associations, and other entities from around the world. These "Useful Approaches" are presented in relation to the topics outlined below.



- Young People & Alcohol
- Drinking & Driving*
- Responsible Drinking*
- Marketing & Advertising

** These sections of the site are still under development.*

Back to FIVS Home Page

Young People & Alcohol

Table of Contents

Young people's drinking causes concern due to this population's relative social, emotional, and physical immaturity. Furthermore, the lessons they learn at this period in their lives will shape their relationship with and attitude towards alcohol as adults. As priorities in addressing young people and alcohol differ by country and by region, a number of approaches to this issue exist, as described below. These initiatives will generally target young people between 12 and 25 years of age.

For a table detailing the minimum legal drinking and purchase ages worldwide, see the International Center for Alcohol Policy (ICAP)'s website.

Alcohol Awareness

Educating young people about alcohol helps prevent underage drinking, reduce harmful drinking patterns, and prepare youths for responsible drinking as adults, if they chose to drink

- Independent learning for young people: Learning about alcohol and its effects outside of any formalized setting
- School-based initiatives: Incorporating alcohol and alcohol prevention into school curriculum
- Organized events and activities: Educating through events and activities aimed at shaping young people's attitude towards alcohol

Parents and Family

Strong family bonds and certain qualities such as resilience and high self-esteem in young people help delay alcohol initiation and promote responsible drinking

- Independent learning for young people: Learning about alcohol and its effects outside of any formalized setting
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Parents and Family

Strong family bonds and certain qualities such as resilience and high self-esteem in young people help delay alcohol initiation and promote responsible drinking attitudes.

- Conversations about alcohol:Encouraging parent-child communication and effective parenting
- Strong young people and families: Building high self esteem in youth and promoting strong family bonds

Illegal Access to Alcohol

Some underage youth are able to obtain alcohol from retailers, friends, adults, or their parents. In some countries, retailers are expected to make reasonable efforts to verify that an individual purchasing alcohol is legally allowed to do so. A number of elements are involved in this process of empowering the vendor to verify the customer's age. Other efforts focus on preventing parents and other adults from providing alcohol to minors.

- Training: Reinforcing the necessity of age verification and teaching techniques to do so
- Identification cards: Developing and implementing tools to verify customers' age
- Campaigns and Signage: Reminding salespeople and youth of their role in preventing illegal access to alcohol
- Evaluation: Measuring age verification compliance rates and punishing retailers who make illegal sales
- By proxy: Preventing parents and other adults from providing alcohol to underage youth

Excessive Drinking

In many countries, concerns have arisen over dangerous consumption patterns – sometimes called binge drinking – that are particularly prevalent among young people. This issue is addressed in the responsible drinking section of the website.

Marketing and Advertising

Alcohol marketing and advertising that is perceived to target youth has prompted calls in certain countries for severe restrictions. Producers and affiliated industry-sponsored organizations have comprehensive codes of practice aimed at reducing youth exposure to alcohol advertisements and marketing. Some trade organizations have review panels to ensure that when violations occur, action is taken. Such practices are covered in the marketing & advertising section of the website.

Prévention du risque alcool

INFORMATIONS, OUTILS ET CAMPAGNES DÉDIÉS À LA PRÉVENTION DU RISQUE ALCOOL

L'alcool & vous

le risque alcool

alcool & route

alcool & travail

alcool & grossesse

alcool & parents

Prévention du risque alcool

"Infos Alcool Parents" : un site internet dédié aux parents sur les risques liés à la consommation d'alcool de leurs adolescents

infos &Alcool parents

Paris, le 6 juillet 2009 - Afin d'aider les parents à prévenir d'éventuelles situations à risque liées à la consommation d'alcool de leurs adolescents, Entreprise & Prévention lance le site Internet « Infos Alcool Parents » en collaboration avec le Professeur Daniel Bailly, pédopsychiatre. Accessible à l'adresse www.alcooletparents.com, cet outil propose informations et conseils sur la manière d'aborder le sujet de l'alcool avec les jeunes.

Lire la suite...

Les producteurs de boissons alcoolisées souhaitent pouvoir faire de la prévention dans leurs publicités: plus de 8 français sur 10 y sont favorables

Rechercher

Tapez vos mots clés GO

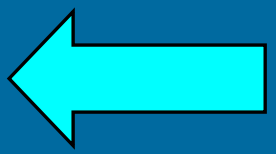
Responsible Drinking

This section of the FIVS-Assure website is under development and will be available in the future.

Back

Future Development

- Drinking and Driving
  - Designated Driver Initiatives
  - Breathalyzers
  - Demonstrations
  - Marketing
- Responsible Drinking
  - Alcoholism
  - Binge Drinking
  - Moderate Consumption
  - Women and Pregnancy



WineinModeration.eu

Art de Vivre

Stakeholder Brochure

Questions?

Thank you for your attention.