CONSERVATION, CHARACTERISATION AND MANAGEMENT OF GRAPEVINE GENETIC RESOURCES: THE EUROPEAN PROJECT GRAPEGEN06

Bacilieri R.[1], Maghradze D.[2], Grando S.[3], I. Pejic[4], Maul E.[5], Munoz G.[6], Eiras-Dias J.[7], Schneider A.[8], Boselli M.[9], This P.[1]

[1]INRA – Diveristé Génétique et Génomique Vigne - Bat 21. UMR DIA-PC. 2, Place P. Viala, 34060. Montpellier, France. bacilieri@supagro.inra.fr
[2]Institute of Horticulture, Viticulture and Oenology. 6 Marshal Gelovani Ave. 0159. Tbilisi, Georgia.
[3]Istituto Agrario di San Michele all'Adige (IASMA) Fondazione Edmund Mach Research Centre. Via Mach 1 38010 Trento, Italy
[4]Agronomski fakultet Sveučilišta u Zagrebu Svetošimunska cesta 25, 10000 Zagreb, Hrvatska
[5]Julius Kühn-Institut - Federal Ressearch Centre for Cultivated Plants (JKI), Institute for Grapevine Breeding Geilweilerhof, 76833 Siebeldingen, Germany.
[6]Instituto Madrileño de Investigación y Desarrollo Rural, Agrario y Alimentario (IMIDRA), Finca El Encín, Alcalá de Henares, Spain
[7]Estacao Vitivinicola Nacional, EVN – INIAP, 2565-191, Dois Portos, Portugal
[8]Istituto di Virologia Vegetale, Consiglio Nazionale delle Ricerche, CNR-IVV, 10135 Grugliasco Torino, Italy
[9]Dipartimento di Scienze, Tecnologie e Mercati della Vite e del Vino, Università di Verona, via della Pieve 70, 37029 San Floriano, Italy

ABSTRACT

Since 2007, research institutes and grapevine collections from 17 countries of Europe, Caucasus and North Africa are working together for the conservation, characterization and management of grapevine genetic resources. The initial work consisted in inventorying and describing the varieties in the partners' collections, using standardized morphological, agronomic, and molecular descriptors, and to link the information into a unique European web-database. The growing database contains today passport data from 25.000 living accessions, of which 2.500 are linked to photos and 1.600 are characterized for 48 OIV descriptors and 9 microsatellite markers.

The project is also working on 448 old autochthonous varieties that have presented a regional interest in the past but are not clearly identified. Comparing their microsatellite fingerprint with the total database will enable us to find if they are original resources and to uniquely define their traits.

The database website is open for consultation, with different levels of access that allows in-dept searches but also confidentiality, according to the user needs. At the end of 2010, the fingerprint data will also be online, so that other collections or professionals can check their own varieties against the referenced varieties to verify names and true-to-typeness.

The final objective of the European project is to promote an optimized conservation scheme of the *Vitis* germplasm, involving ex-situ, cryo- and on-farm conservation, so that the resources are permanently maintained, easily accessible and field-tested in a pertinent agricultural context. This network of resources will also provide plant material as a basis for biotechnology, genomic and breeding research.

ABSTRACT (Italian)

Conservazione, caratterizzazione e gestione delle risorse genetiche della vite: il progetto europeo GrapeGen06

Dal 2007, gli istituti di ricerca e le collezioni di vite di 17 paesi d'Europa, del Caucaso e del Nord-Africa collaborano per la conservazione, la caratterizzazione e la gestione delle risorse genetiche della vite. Inizialmente, il lavoro ha consistito nell'inventario e la descrizione delle varietà delle collezioni partner, utilizzando descrittori morfologici, agronomici e molecolari standardizzati, le cui informazioni sono poi inserite in un unico database Europeo. Il database sta crescendo e contiene oggi i dati passaporto di 25.000 varietà viventi, di cui 2.500 sono descritte da foto e 1.600 da 48 descrittori OIV e 9 marcatori microsatelliti.
Il progetto sta inoltre lavorando su 448 antiche varietà autoctone che hanno presentato un interesse regionale nel passato, ma che sono mal identificate. Comparare il loro profilo genetico con il database permetterà di determinare la loro originalità e di definire inequivocabilmente le loro caratteristiche.
Il database e' accessibile al pubblico, e prevede diversi livelli di accesso permettendo una ricerca approfondita e allo stesso tempo la confidentialità, secondo i bisogni degli utilizzatori. Alla fine del 2010, la base dei marcatori molecolari sarà ugualmente aperta, permettendo cosi' ai professionisti di verificare la conformità delle loro varietà.
L'obiettivo finale del progetto europeo e' di organizzare uno schema ottimale di conservazione delle risorse di *Vitis*, facendo ricorso a metodi ex-situ, di crio-preservazione e conservazione in fattoria, permettendo la perenizzazione delle risorse, rendendole facilmente accessibili e analizzandole nel contesto agricolo. Questa rete di collaborazione potrà anche provvedere materiale di base per la ricerca in biotecnologia, genomica e selezione.

ABSTRACT (French)

Conservation, caractérisation et gestion des ressources génétiques de vigne : le projet européen GrapeGen06

Depuis 2007, les institutes de recherche et les collections de vigne de 17 pays d'Europe, du Caucase et d'Afrique du Nord collaborent pour la conservation, la caractérisation et la gestion des ressources génétiques de vigne. Le travail initial a consisté à inventorier et décrire les variétés des partenaires, en utilisant des descripteurs morphologiques, agronomiques et moléculaires standard, et liant l'information dans une base de données européenne unique. La base de données grandissante contient aujourd'hui les données passeport de 25.000 accessions vivantes, dont 2.500 sont décrites par des photos et 1.600 sont caractérisées pour 48 descripteurs OIV et 9 marqueurs microsatellite.
Le projet travaille également sur 448 variétés autochtones ayant présenté un intérêt régional dans le passé mais mal identifiées. Comparer leur profile microsatellite avec l'ensemble des données nous permettra de déterminer leur originalité et de définir de manière non-équivoque leurs caractéristiques.
La base de données est ouverte au public, et prévoit des niveaux d'accès différents, pour permettre les recherches approfondies ainsi que la confidentialité, selon les besoins de l'utilisateur. Fin 2010, les données moléculaires seront également publiques, permettant aux professionnels de vérifier la conformité de leurs variétés.

L'objectif final du projet européen est de promouvoir un schéma optimal de conservation des ressources de vigne, impliquant conservation ex-situ, la cryo-preservation et la conservation à la ferme, pour pérenniser les ressources de vigne, les rendre facilement accessibles et les tester dans leur contexte agricole. Ce réseau de ressources fournira également le matériel de base pour la recherché en biotechnologie, génomique et sélection

INTRODUCTION

Genetic resources for cultivated plants and their wild relatives have recently received considerable attention. Genetic resources are indeed indispensable to meet future demands for the crops, such as for example, being a reservoir of biotic and abiotic resistance genes or of new qualitative traits. After considerable genetic losses due to selection of performing cultivars, mankind is increasingly worried about its germplasm heritage. Worldwide measures and actions are undertaken for safeguarding the still existing diversity (Bioversity, Global Plan of Action, ECPGR, European Union initiatives) and to promote its description and documentation. The recent discovery of resistance genes against powdery mildew in autochthonous *Vitis vinifera* accessions (Coleman et al, 2009) opens up new interests in germplasm screening and utilization.

Diversity of grape (*Vitis vinifera* L.) is quite large since the number of cultivars worldwide is estimated from 6000 to 11000 (Maul et al. 2008). Due to the long history of grape cultivation (McGovern 2003), the possibility of vegetative propagation and the intense movement of material between countries around the world, the exact number of cultivars is quite difficult to ascertain; this is also due to the large number of homonymies and synonymies. The knowledge of the situation for grape wild relatives (*Vitis* species as well as the subspecies *sylvestris*, the wild compartment of *Vitis vinifera)* is not better due to the lack of information on the relative importance of the wild individuals in Europe.

Considering the commercial, historical and human importance of grape for Europe, the European Union supported a research project launched in 2008 for 4 years (Grapegen06, http://www1.montpellier.inra.fr/grapegen06/accueil.php) aimed at the inventory, characterization, conservation and management of grapevine genetic resources at an European scale. Research institutes and germplasm collections from 17 countries from EU as well as Eastern Europe are working together in order to monitor and describe *Vitis* European germplasm, implement true-to-type identification, promote an overall conservation scheme for safe preservation of grapevine germplasm and ensure accessibility of the data and the living resources in particular through a centralized database (http://www.eu-vitis.de).

GrapeGen06 project is organized into 7 work packages:

- Molecular analysis of accessions throughout the collections (WP1)
- Acquisition of primary and secondary descriptors of autochthonous varieties (WP2)
- On-farm evaluation of agronomic features of autochthonous varieties (WP3)
- Inventory and characterization of *Vitis sylvestris* populations in Europe (WP4)
- Search and elucidation of synonymies, homonymies and unknown accessions throughout the collections (WP5)
- Upgrading and enrichment of the database (WP6)
- Development of alternative conservation methods such as cryopreservation and elaboration of long-term conservation plan (WP7).

Up to date results of this network are presented in this manuscript.

MATERIALS AND METHODS:

WP1: Molecular characterization was performed using the 6 SSR markers and the common standardized protocol of allele scoring defined during previous Generes081 project (This et al, 2004). In order to increase the identification power of the system, 3 extra SSR loci (VVMD25, VVMD28, VVMD32) were included in the analysis.

WP2: The ampelographic description of old, autochthonous and neglected varieties was done according to 32 primary and 16 secondary descriptors recently harmonized by UPOV, IPGRI and OIV (OIV, 2007). Pictures of shoots, leaves, bunch and berries from the varieties described were also taken in order to get a better identification. Control of the sanitary status of the analysed cultivars was carried out by ELISA technique (Clark and Adams, 1977). Viruses analysed were those considered in European legislation like Leafroll (GLRaV-1 and GLRaV-3), Fanleaf (GFLV) and Arabis mosaic (ArMV). Data and pictures have been uploaded into the European database.

WP3: A uniform protocol and comparable final analysis were also defined for the on-farm analysis including wine making and wine quality evaluation.

WP4: A 'Protocol for the inventory of *V. vinifera* subsp. *sylvestris*' was defined and applied for the inventory of *V. sylvestris* wild sites in Europe and the inventory of *V. sylvestris* wild plants. Some important population characters were taken into account: the number of plants in the population, the sex of these plants, the presence in the proximity of other *Vitis* which could intercross with the wild population, the status of preservation, the risk of loss of the populations., and the owner of the land.

WP5: Sorting of homonyms and synonyms and identification of unknown or mislabelled accessions was carried out through the comparison of microsatellite profiles from questionable accessions using the 9 loci. Reference molecular profiles were provided by partners, so that when one identity was found, it was often corroborated by more than one laboratory. An allele size coding system developed within the project assisted in the comparison of profiles obtained by different laboratories. Moreover, ampelographic descriptions and images of "questionables" vines under test (shoot, adult leaf and bunch) provided a further tool for confirming variety correct identification.

WP6: The website database adopted common data standards, which is a prerequisite for searching for data. With respect to passport data, the FAO / IPGRI Multi-crop Passport Descriptor (MCPD) format used by EURISCO (Alercia et al 2001) has been adopted. Standardization of expressions used for various criteria (e.g. *Vitis* species, berry colour, utilization) has been carried out. Descriptors of the OIV descriptor list for grapevine varieties and species (OIV 2009) have been used to establish a standardized format. Genetic fingerprint is based on the 6 markers of Genres081, plus 3 new most common and polymorphic markers. The 6 markers of Genres081 had been recommended as a standard set for grapevine genotyping and are hence widely applied and published (This et al. 2004).

In terms of technical infrastructure, the working tools for the development of the applications are (1) MySql as a relational database management system, (2) PHP as the internet programming language, (3) Cascading Style Sheets (CSS), (4) the operating system LINUX and (5) the HTTP web server APACHE.

GrapeGen06 partners checked the database for functionality and made suggestions for improvements.

WP7: Protocols for cryopreservation of pollen, seeds and dormant buds are under development. They will allow the long-term conservation of genes by mean of cryoconservation of pollen and seeds, or of global genotypes by conservation of buds. In parallel, field safety duplication and introduction of wild accessions were performed.

RESULTS

Molecular data on varieties

Until now, 2624 coded profiles were obtained. A preliminary population analysis was performed based on the coded data produced during the project by all the partners at 9 microsatellite loci, including identical genotypes (Tab. 1) . The number of different alleles (Na) found within the 47 reference cultivars (cv-refer in Table 1, similar to those analyzed by This et al, 2004) was compared with the number of different alleles found in the population of new and old genotyped accessions. Observed heterozygosity (Ho) was compared with expected heterozygosity (He) at the same loci.

Table 1. Summary of SSR data available in the project

Locus	**N (cv-refer)**	**N (wp1)**	**Na (cv-refer)**	**Na (wp1)**	**Ho (cv-refer)**	**Ho (wp1)**	**He (cv-refer)**	**He (wp1)**
VVMD5	47	2568	17,000	23,000	0,894	0,835	0,907	0,853
VVMD7	47	2595	17,000	24,000	0,809	0,818	0,861	0,816
VVMD25	47	2488	14,000	24,000	0,851	0,796	0,847	0,791
VVMD27	47	2624	23,000	28,000	0,979	0,841	0,898	0,840
VVMD28	47	2492	18,000	34,000	0,979	0,872	0,923	0,895
VVMD32	47	2441	14,000	40,000	0,511	0,803	0,883	0,832
VVS2	47	2586	19,000	27,000	0,894	0,792	0,889	0,818
VrZAG62	47	2580	19,000	25,000	0,872	0,833	0,885	0,827
VrZAG79	47	2545	13,000	19,000	0,787	0,814	0,882	0,861

Na = Number of alleles; Ho = observed heterozygosity, He = expected heterozygosity = diversity measure

Morphological data on varieties

The growing database contains 1600 accessions characterized for 48 OIV descriptors (699 accessions described in GrapeGen06). Some key characters have been chosen to show the variability among varieties and regions (Tab. 2).

Berry colour has a high incidence on wine organoleptic characteristics. The most frequent berry colour is green-yellow (50 %) followed by black or violet colour (41 %) and other minor colours like rose or green. But the situation is different among regions being dark colours predominant in accessions studied by partners from Italy and Germany meanwhile green-yellow colours are predominant in accessions from Austria, Portugal and all the Eastern Countries.

Bunch length is an indicator of bunch size and weight. The most common length is medium (55 %), but the low length is more frequent in accessions studied by France and Eastern Countries (except Hungary) meanwhile bunches of higher length are clearly more frequent in accessions from Germany.

The time of bud burst is an indicator of resistance to spring frost. Although many varieties have the bud burst in a medium time, there are also many of them, which do it in early time (mainly in France, Austria, most Eastern Countries, and some partners from Italy).

Table 2. Accessions described by each partner and frequencies of some key characters.

Institute	City, Country	Number of Accessions (GrapeGen06)	Berry color	Bunch length	Time of bud burst	Number of lobes in leaves	Density prostrate hairs on leaves
INRA	Montpellier, France	64	47 % g* 48 % b	42 % l 5 % h	41 % e 10 % t	58 % l 42 % h	64 % l 5 % h
BAZ – IRZ	Siebeldingen, Germany	35	37 % g 46 % b	11 % l 43 % h	9 % e 3 % t	51 % l 49 % h	63 % l 6 % h
HBLAuBA	Vienna, Austria	24	71 % g 29 % b	4 % l 25 % h	42 % e 29 % t	29 % l 71 % h	50 % l 21 % h
IMIDRA	Madrid, Spain	46	48 % g 52 % b	22 % l 11 % h	9 % e 28 % t	11 % l 89 % h	35 % l 17 % h
EVN - INIAP	Dois Portos, Portugal	57	58 % g 42 % b	23 % l 5 % h	- -	35 % l 65 % h	16 % l 11 % h
CRA – ISV	Conegliano, Italy	25	52 % g 48 % b	12 % l 16 % h	28 % e 8 % t	8 % l 92 % h	52 % l 0 % h
CNR-IVV	Torino, Italy	92	20 % g 70 % b	7 % l 12 % h	10 % e 9 % t	51 % l 49 % h	49 % l 17 % h
IASMA	S.Michele, Italy	22	36 % g 55 % b	27 % l 36 % h	41 % e 14 % t	23 % l 77 % h	55 % l 23 % h
DISAA - UNIUD	Udine, Italy	31	48 % g 39 % b	10 % l 32 % h	10 % e 10 % t	58 % l 42 % h	55 % l 10 % h
DISTEMEV-UNIVR	Verona, Italy	4	0 % g 100 % b	25 % l 25 % h	0 % e 25 % t	0 % l 100 % h	75 % l 25 % h
PTE SZBKI	Pecs, Hungary	106	66 % g 16 % b	6 % l 36 % h	32 % e 0 % t	36 % l 64 % h	25 % l 35 % h
RICP	Ruzyne, Czech Rep.	30	70 % g 17 % b	63 % l 10 % h	87 % e 3 % t	0 % l 100 % h	63 % l 13 % h
ARI	Nicosia, Cyprus	10	40 % g 40 % b	0 % l 0 % h	10 % e 0 % t	0 % l 100 % h	50 % l 10 % h
VŠSVVM	Modra, Slovakia	12	58 % g 17 % b	33 % l 8 % h	0 % e 0 % t	8 % l 92 % h	42 % l 8 % h
FAZ	Zagreb, Croatia	100	55 % g 40 % b	47 % l 11 % h	12 % e 7 % t	17 % l 83 % h	35 % l 8 % h
Institute of Botany	Tbilisi, Georgia	13	46 % g 46 % b	54 % l 0 % h	100 % e 0 % t	46 % l 54 % h	0 % l 100 % h
IHVO	Tbilisi, Georgia	27	70 % g 22 % b	- -	- -	33 % l 67 % h	- -
Total		**699**	**50 % g 41 % b 9 % o**	**26 % l 19 % h 55 % m**	**34 % e 11 % t 55 % m**	**33 % l 67 % h**	**49 % l 19 % h 32 % m**

*g: green-yellow; b: black-violet; h: high; l: low; e: early; t: late; o: others; m: medium

A high percentage of the accessions studied have five ore more lobes in leaves (67 %). These types of leaves are frequent in accessions from Austria, Spain, Portugal, Eastern Countries and some partners from Italy. On the other hand, leaves with 3 lobes or without them are more frequent in accessions from France and Udine (Italy).
Leaves with low density of prostrate hairs have the highest frequency. This is common in most of the countries. Only a higher number of accessions from Hungary and Georgia have a higher density of prostrate hairs.
It appears that a different morphological tendency exists between varieties from the East and varieties from the West in Europe. This tendency supports the belief that the spread of viticulture from Eastern to Western Europe involved the dissemination of domesticated varieties in different times (Bouquet, 1982).
Although a lot of synonyms have been found together with other work packages (WP1, WP5), this work shows the great diversity in old, neglected European cultivars.

On farm analysis of cultivars

With 16 partners we were able to acquire data for 68 neglected autochtonous cultivars grown on farm. Up to now, complete information i.e. historical data, vineyards description, ampelography, agronomy, enology and virus data, were obtained for 14 cultivars and the work is in progress. Wines will be ready in 2010 for evaluations.

Inventory of populations of *V. vinifera sylvestris*

Currently, inventories were performed by 10 partners in 8 countries. We consider as population a site with more than one *sylvestris* plant. A total of 189 sites were identified (Tab. 3) and their positioning obtained by GPS mapping. Each population has a mean of 8,2 plants per population.
In these sites, about 1 553 plants have been identified; among them 615 were identified as dioecious plants (303 female plants and 312 male plants). For the others 938 plants the analysis of flower sexual type was not possible (plant not flowering, flowers inaccessible).

Table 3. location of the 189 populations identified so far

Austria:	05
France:	34
Georgia:	59
Germany:	01
Italy:	05
Portugal:	12
Slovakia	12
Spain:	61

True to type analysis

Around 450 accessions from 13 European collections are currently under examination: results achieved up to now concern around 200 accessions, while the analysis of the remaining 250 “questionables” is under way. More than a quarter of the first set of unknown accessions were most likely truly identified, as their genetic profiles definitely match with those of the reference cultivars at an appropriate number of markers (at least 7/7, more frequently 9/9) (Fig. 1). For another 15 % of the analyzed "questionables", a possible identity hypothesis was found with 4-5 common microsatellite loci, but the assumption requires now to be validated, besides vine morphology, by an higher number of markers.

Table 4 gives an example of the results achieved from the identification analysis carried on several samples. Minor discrepancies shown by molecular values mainly depended on data rounding and must be thus ignored. Data coding system definitely provided a reliable tool for microsatellite profile comparison among laboratories, allowing the development of a reference international molecular database on *Vitis*. Thanks to such analyses, cultivars requiring identification often since long time, such as Bonarda di Chieri faux (=false), Malvasia Candia faux, or having a local name (Garnacha francesa, Maria lunga) were clearly identified. For others, such as the Portuguese Sarigo, the synonym Jaén (blanco) from Spain was established.

Some misnomer examples are also shown in Tab. 1. The so called Trentham black in ESP080 collection and Trentham black faux (=false) in FR139 is clearly Bibiola, a cultivar rather widespread once in Piedmont (Italy) and currently neglected; the true Trentham black is a distinct variety, once grown as table grape in the British green houses (Barron, 1892). Mathiasz Janoš is an evident misnomer for the well know Müller-Thurgau, the wrong name referring to the famous Hungarian breeder, who, however, did not create this grape obtained instead by professor Hermann Müller.

Around half of the investigated grape varieties remain un-identified (Fig. 1) or, in several cases, just require more common markers to be identified. Nearly a third of them, however, were locally known and locally documented through traditional cultivation and historical references: the question to be asked, for these cultivars, was if they were only regional/local varieties or were also grown elsewhere. If no other possible synonyms are found, we could assume it is likely these cultivars are distinctive genotypes and have a limited, local importance, thus representing unique genetic resources, valuable for conservation, description, and documentation.

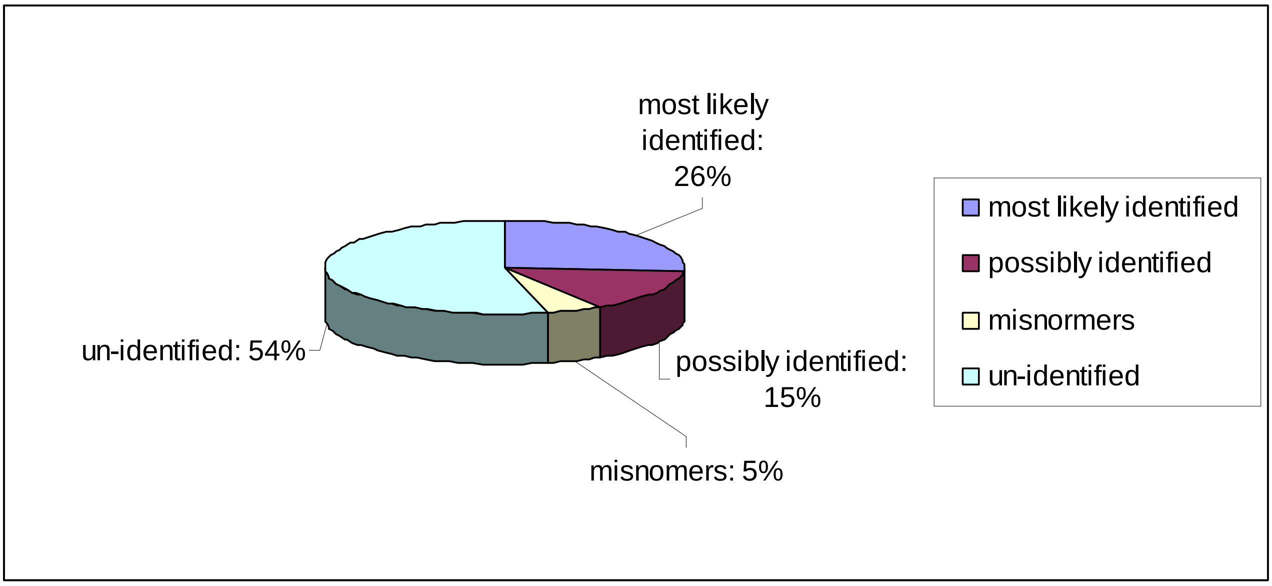

Fig. 1 – Results of the identification process carried on 200 accessions requiring correct identification ("questionable" accessions).

Tab. 4 –Some results of the identification process trough the comparison of coded molecular profiles (discrepancies in marker size are in italic).

Istitution/ Laboratory	**"Questionable" accession (italic)/ ”true to type” (below)**	**Remarks on the “true to type”**	**VVS2**		**VVMD5**		**VVMD7**		**VVMD25**		**VVMD27**		**VVMD28**		**VVMD32**		**ZAG62**		**ZAG79**	
FRA139	*Bonarda di Chieri faux*		N+10	N+20	N+4	N+18	N+22	N+32	N+6	N+20	N+10	N+14	N+18	N+44	N+5	N+17			N+6	N+14
ITA360	Durasa	Durasa is a local cultivar from Piedmont (Italy)	N+10	N+20	N+4	N+18	N+22	N+32	N+6	N+20	N+10	N+14	N+18	N+44	N+5	N+17	N+18	N+20	N+6	N+14
FRA139	*Malvasia di Candia faux*	Moscatello selvatico is a regional cultivar from Apulia (Italy)	N+26	N+28	N+10	N+10	N+18	N+22	N+14	N+28	N+4	N+19	N+28	N+32	N+29	N+37	N+12	N+26	N+14	N+18
ITA360	Moscatello selvatico		N+26	N+28	N+10	N+10	N+18	N+22	N+14	N+28	N+4	*N+20*	N+28	N+32			N+12	N+26	N+14	N+18
ITA371	Moscatello selvatico		N+26	N+28	N+10	N+10	N+18	N+22	N+14	N+28	N+4	N+19	N+28	N+32	N+29	N+37	N+12	N+26	N+14	N+18
AUT024	*unknown*	Interspécific hybrid Gamay X Riparia	N+10	N+36	N+10	N+42	N+8	N+22			N+14	N+42	N+4	N+22			N+14	N+20	N+8	N+20
FRA139	Oberlin noir = Oberlin 595		N+10	N+36	N+12	*N+44*	N+8	N+22	N+4	N+32	N+14	N+42	*N+2*	*N+20*	N+5	N+5	N+14	N+20	N+8	N+20
ESP080	*Garnacha francesa*	Prime name: Plant droit	N+28	N+28	N+4	N+14	N+2	N+18	N+14	N+28	N+10	N+10	N+32	N+42	N+5	N+17	N+26	N+30	N+10	N+16
FRA139	Plant droit		N+28	N+28	N+4	N+14	N+2	N+18	N+14	N+28	N+10	*N+19*	N+32	N+42	N+5	N+17	N+26	N+30	N+10	N+16
PRT051	*Sarigo*		N+14	N+22	N+12	N+14	N+12	N+18			N+6	N+6					N+14	N+30	N+10	N+10
FRA139	Jaen blanc	Prime name: Jaén	N+14	N+22	N+12	N+14	N+12	N+18	N+6	N+20	N+6	N+6	N+18	N+32	N+17	N+21				
ESP080	Jaén blanco		N+14	N+22	N+12	N+14	N+12	N+18	N+6	N+20	N+6	N+6	*N+32*	N+32	*N+21*	N+21	N+14	N+30	N+10	N+10
ITA360	*Bibiola*	local ancient cultivar from Piedmont (Italy)	N+10	N+28	N+4	N+10	N+16	N+18	N+4	N+14	N+4	N+14	N+12	N+28	N+17	N+27	N+12	N+26	N+8	N+22
FRA139	Trentham black faux		N+10	N+28	N+4	N+10	N+16	N+18	N+4	N+14	N+4	N+14	N+12	N+28	N+17	N+27				
ESP080	Trentham black	Trentham black is a misnomer	N+10	N+28	N+4	N+10	N+16	N+18	N+4	N+14	N+4	N+14	N+12	N+28	N+17	N+27				
ITA360	*Maria lunga*		N+10	N+22	N+10	N+10	N+12	N+18	N+6	N+6	N+10	*N+20*	N+18	N+20	N+15	N+27	N+14	N+24	N+14	N+14
FRA139	Dorona di Venezia		N+10	N+22	N+10	N+10	N+12	N+18	N+6	N+6	N+10	N+19	N+18	N+20	N+15	N+27				
ESP080	Dorona di Venezia		N+10	N+22	N+10	N+10	N+12	N+18	N+6	N+6	N+10	N+19	N+18	N+20	N+15	N+27				
ITA371	Dorona di Venezia		N+10	N+22	N+10	N+10	N+12	*N+19*			N+10	N+19	N+18	N+20	N+15	N+27	*N+15*	*N+26*	N+14	N+14
CZE041	*Mathiasz Janoš*	Mathiasz Janoš is a misnomer	N+20	N+28	N+4	N+6	N+16	N+26	N+14	N+20	N+6	N+6	N+18	N+28	N+17	N+17	N+20	N+20	N+6	N+8
FRA139	Müller-Thurgau		N+20	N+28	N+4	N+6	N+16	N+26	N+14	N+20	N+6	N+6	N+18	N+28	N+17	N+17	N+20	N+20	N+6	N+8
ESP080	Müller-Thurgau		N+20	N+28	N+4	N+6	N+16	N+26	N+14	N+20	N+6	N+6	N+18	N+28	N+17	N+17				
ITA371	Müller-Thurgau		N+20	N+28	N+4	N+6	N+16	N+26			N+6	N+6	N+18	N+28	N+17	N+17	N+20	N+20	N+6	N+8
FRA274	*Jaen*	Jaen is a misnomer	N+22	N+28	N+4	N+14	N+18	N+26	N+6	N+14	N+6	N+14	N+20	N+20	N+17	N+21	N+14	N+20	N+10	N+14
FRA139	Mencia	Prime name: Mencía	N+22	N+28	N+4	N+14	N+18	N+26	N+6	N+14	N+6	N+14	N+20	N+20	N+17	N+21				
ESP080	Mencía		N+22	N+28	N+4	N+14	N+18	N+26	N+6	N+14	N+6	N+14	N+20	N+20	*N+21*	N+21	N+14	N+20	N+10	N+14

Note : in yellow the discrepancies found

European database improvement and enrichment

The European *Vitis* Database was designed in a way that the initiated activities can continue even after termination of the project. Collection curators, internal or external to the project, have now the possibility to manage their own data, since the basis was laid to enable partners of GrapeGen06 to upload and modify their data. Import programs for all kind of data and photos have been developed, as well as an interactive modification system for MCPD-data. This was the most innovating aspect and has not been implemented in any other crop database before.

<u>Database design</u>

The European *Vitis* Database offers three access levels:

- *Public access*: search options encompass MCPD data, characterization data and photos.
- *All partner access*: in addition to the public access search options, SSR-marker data, which are protected by a confidentiality agreement among the GrapeGen06 partners, are available. Export of MCPD data, characterization data, SSR-marker data, virus data, on-farm evaluation data and *Vitis sylvestris* germplasm data is possible.
- *Partner specific access*: this area is designated for upload, deletion and interactive modification of data and photo. Export of data is possible.

Further options are:

- Download of MCPD, OIV descriptors.
- Export of file formats for MCPD data, characterisation and SSR-marker data, virus data, on-farm evaluation and *Vitis sylvestris* germplasm.
- Retrieval of institute codes of the worldwide existing grapevine collections. They are used by the partners e.g. as holding institution, donor, breeder. Contact data of institute codes are provided.
- Download of descriptions of autochthonous varieties (text and photographs).
- Download of a handbook, illustrating search options and results in detail, see the top of the homepage.

<u>Database contents</u>

The European *Vitis* Database encompasses data of the two European Projects GrapeGen06 (2007-2010), the former project Genres081 and MCPD-data of the Black Sea-project (Maghradze 2009). In total 31 collections located in 21 countries are registered. Altogether they maintain 31.864 grapevine accessions. The following accession specific data have been imported: (1) characterisation data from 1.956 accessions, (2) SSR-marker data from 2.485 accessions, (3) virus data from 200 accessions and (4) 2.777 photographs of shoot tips, leaves and clusters.

<u>Database benefits</u>

The following user groups are addressed:

- *Curators of grapevine germplasm repositories*:

  Trueness to type assessment via genetic fingerprint and ampelography assists in sorting out the collections through identification of misnomers, synonymy and homonymy. The utilization of the variety code number of the *Vitis* International Variety Catalogue (www.vivc.de) was agreed by all partners to tag identical accessions. This is a first step towards a true European inventory of the existing germplasm. The objective is the identification of endangered genotypes existing only once in grapevine collections, the determination of gaps to be filled and the organisation of double conservation of the most important genetic resources.

For plant material exchange virus status information is crucial. The addition of this information has started.

- *Wine growers*:
  For growers interested in planting rare historical cultivars, adapted to the local or regional growing conditions, agronomic features and wine characteristics of autochthonous cultivars are to be found via on-farm evaluation or advanced search. Short one-page descriptions can be downloaded.
- *Breeders, researchers and industry*:
  Availability of germplasm, its description by ampelography and genetic fingerprint are useful to check trueness to type, to select appropriate material for crossings or investigations and supports the assessment of genetic diversity on a morphologic and genetic level.

Conclusion

The objective of Grapegen06 project was to consolidate a network of scientists and germplasm collections in Europe, in the objective to optimize the conservation of genetic resources. In this regards, the project was very successful, since much work was performed within the project.

Its main achievements until now certainly comes from the integration of several methodologies leading to the monitoring of true-to-type genetic resources. Besides the establishment of the tools monitoring the long term preservation of grapevine germplasm and providing professionals with cultivar specific data, the main aim was to put into practice a "sustainable" database which is constantly maintained by the curators of grapevine collections and that should become available to the general public.

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Plastid DNA sequence diversity in a worldwide set of grapevine cultivars (*Vitis vinifera* L. subsp. *vinifera*)

B.Schaal[1], J.Beck[1], S.-C.Hsu[1], T.Beridze[2,3], M.Gamkrelidze[2], M.Gogniashvili[2], I.Pipia[2], V.Tabidze[2], P.This[4], R.Bacilieri[4], V.Gotsiridze[5], M.Glonti[5]

[1]Washington University in St Louis, One Brookings Drive, Campus Box 1054, St. Louis, MO 63130-4899, USA, schaal@biology2.wustl.edu
[2]Durmishidze Institute of Biochemistry and Biotechnology, D.Agmashenebeli Al., 10th km, 0159, Tbilisi, Georgia, tgberidze@yahoo.com
[3]Ilia State University, 3/5, Cholokashvili Ave.Tbilisi, Georgia, 0162, tengiz_beridze@iliauni.edu.ge
[4]Institut National de la Recherche Agronomique (INRA) 2, Place P. Viala, 34060 Montpelllier- France, roberto.bacilieri@supagro.inra.fr
[5]Institute of Horticulture, Viticulture and Oenology, 6 Marshal Gelovani Ave., 0159, Tbilisi, Georgia, agotsiridze@yahoo.com

ABSTRACT

DNA sequence diversity was investigated at two plastid regions (the *trnH-psbA* intergenic spacer and the *rpl16* intron) in a geographically diverse group of 113 cultivated grape samples. This group included 40 samples from the Republic of Georgia, home to over 500 grape cultivars and the earliest archaeological evidence of grape domestication. The greater Caucasus region in which Georgia lies is widely believed to be the area in which grape domestication began, and the study of genetic diversity in this region is viewed as key to understanding grape domestication in general. Four plastid haplotypes are evident in the 113 samples, and are designated by their character-states at each of the 3 polymorphic positions: (AAA)–23 samples, (ATT)–29 samples, (GTA)–26 samples, and (ATA)–35 samples. The AAA haplotype was only observed in Georgian samples. The observation that the Georgian cultivars exhibited both unique plastid DNA variation (the AAA haplotype) and all other observed plastid haplotypes is consistent with previous studies that have observed both unique and high levels of genetic variation in wild grape (*V. vinifera* subsp. *sylvestris*) in the greater Caucasus region.

La diversité des séquences d'ADN a été étudiée au niveau de deux régions chloroplastiques (la région intergénique *trnH-psbA* et l'intron *rpl16*) dans un groupe géographiquement diversifié de 113 échantillons de vignes cultivées. Ce groupe comprend entre autres 40 échantillons provenant de la République de Géorgie, qui abrite plus de 500 cultivars de vigne et les plus anciennes traces archéologiques de sa domestication. La région du Caucase où s'étend la Géorgie est en effet considérée être le lieu où la domestication de la vigne a débuté, et l'étude de la diversité génétique dans cette région est considérée comme étant essentielle à la compréhension de la domestication de la vigne en général. Quatre haplotypes plastidiaux sont ressortis de ces 113 échantillons : (AAA) présents dans 23 cultivars, (ATT) présents dans 29 cultivars, (GTA) présents dans 26 cultivars, et (ATA) présents dans 35 cultivars. L'haplotype (AAA) a été observé uniquement dans les cultivars Géorgiens. L'observation selon laquelle les cultivars Géorgiens montrent à la fois les quatre haplotypes plastidiaux, dont certains spécifiques à des régions géographiques (comme l'haploype AAA) confirme les études précédentes qui ont observé un haut niveau de variation

génétique chez la vigne sauvage (*V. vinifera* subsp. *sylvestris*) dans la région du grand Caucase.

INTRODUCTION

Plant and animal domestication has been called "…the most important development in the past 13,000 years of human history," (Diamond, 2002). Among domesticated plants, the domestication of grapevine (*Vitis vinifera* L.) for food and drink is an event of tremendous cultural and economic importance. Ensuring the long-term value of cultivated plants like grape, particularly in regards to trait improvements such as disease resistance, requires the retention of genetic variability in the form of seed and rootstock collections (Tanksley, McCouch, 1997). Like many other domesticated plants, cultivated grape (*Vitis vinifera* L. subsp. *vinifera*) has experienced recent large-scale demographic events that affect the level of genetic variability available (reviewed in Miller, Schaal, 2006). Both the Phylloxera and mildew epidemics of the 19th century and the growing dominance of a small number of commercial wine varieties in the 20th century are believed to have caused a major reduction in overall genetic variability in cultivated grape (This et al. 2006; Santiago et al. 2008).

Fortunately, recent molecular studies have shown that considerable genetic variation exists within the estimated 6000 grape cultivars both at global (Aradhya et al. 2003; Arroyo-Garcia et al. 2006; De Mattia et al. 2008) and local scales (Bulgaria–Dzhabmazova et al. 2009; Crete–Lefort, Roubelakis-Angelakis 2002; Croatia–Maletic et al. 1999; France–Bowers et al. 1999; Hungary–Jahnke et al. 2008; Israel–Klein et al. 2008; Portugal–Lopes et al. 1999; Almadanim et al. 2007; Spain/Greece–Arroyo Garcia et al. 2002; Tunisia–Snoussi et al. 2004; Turkey–Karatas et al. 2007; Ukraine-Huertz et al. 2008). These studies also indicated that a portion of the genetic variation in cultivated grape is geographically restricted, leading to the suggestion that wild grape (*Vitis vinifera* L. subsp. *sylvestris* (C.C. Gmel.) Hegi) could have been taken into cultivation in independently in multiple areas, therefore capturing different subsets of the total variation of the wild ancestor (Arroyo-Garcia et al. 2006).

In this study we examine plastid DNA sequence variation in a geographically diverse group of *V. vinifera* cultivars. To date no study has broadly assessed DNA sequence variation in this way. In addition, this study includes a large number of traditional cultivars from the Republic of Georgia, home to over 500 grape cultivars and the earliest archaeological evidence of grape domestication (Ketskhoveli et al., 1960; McGovern 2003). The greater Caucasus region in which Georgia lies is widely believed to be the area in which grape domestication began (Negrul, 1946; Unwin 1991; McGovern et al. 1996; Phillips 2000), and the study of genetic diversity in this region is viewed as key to understanding grape domestication in general (This et al. 2006).

MATERIALS AND METHODS

Tissues for DNA samples came from three sources. We received 9 DNA solutions and 59 cuttings of grape cultivars from the Institut National de la Recherche Agronomique (INRA) Montpellier, France. Dried leaves of 30 grape cultivars were received from National Clonal Germplasm Repository at the University of California, Davis. Finally, 40 Georgian cultivars were received as cuttings from the Georgian Institute of Horticulture, Viniculture, and Oenology, Tbilisi. Variable success with DNA isolation resulted in a final set of 113 samples (62 INRA, 11 UC-Davis, 40 Georgian Institute of Horticulture Viniculture and Oenology) (Table 1). Samples received as cuttings were either grown in water at room temperature until leaves appeared, or the cuttings themselves were homogenized using a pencil sharpener. DNA isolation was carried out according to the CTAB protocol presented in Lodhi et al. (1994) or with a Plant Genomic DNA Extraction Miniprep System (Viogene

U.S.A., Sunnyvale, CA). When necessary, extracted DNAs were purified with GenElute columns (Sigma-Aldrich, St. Louis, MO).

A preliminary sample set comprising seven Georgian and two French cultivars were investigated by sequencing three non-coding plastid DNA regions (the *trnH-psbA* intergenic spacer, the *rpl16* intron, and the *accD-psaI* intergenic spacer). The *trnH-psbA* intergenic spacer was amplified with the primers "trn H" and "psb A" (Hamilton, 1999). The *accD-psaI* intergenic spacer was amplified with the primers "ACCD-769F" and "PSAI-75R" (Barkman, Simpson, 2002). The *rpl16* intron was amplified with the primers "rpl16_5'" (5' TGTTGTTTACGAAATCTGGTTC 3') and "rpl16_3'" (5' ATGCTTAGTGTGTGACTGGT 3') (this study).

After evaluating the SNPs present in these three plastid regions for the 9-sample preliminary data set (see results), the entire *trnH-psbA* intergenic spacer and a 367 bp portion of the *rpl16* intron was sequenced for the remaining 104 samples. The new target *rpl16* intron region was sequenced with the primers "rpl16 internalF" (5' GAATAATACACTGAATCG 3') and "rpl16 internalR" (5' ATTGAGTGGGATGGCGGA 3') (this study). PCR conditions included denaturing at 94° C (1 minute), 30 cycles of 94° C denaturing (1 minute), 55° C annealing (1 minute), and 72° C extension (2 minutes), followed by a final extension step at 72° C (5 minutes). PCR products were purified with GenElute PCR Clean-Up Kits (Sigma-Aldrich, St. Louis, MO), dye-labeled using a Big Dye Terminator Kit (Applied Biosystems, Foster City, CA) and analyzed on either Applied Biosystems 3100 or 3700 genetic analyzers at either the Biology Department of Washington University (St Louis, MO) or the Laboratory Services Division of the University of Guelph, (ON, Canada). Sequences were manually aligned in Se-Al (Rambaut, 2002), and haplotype networks were generated using TCS 1.18 (Clement et al., 2000).

RESULTS AND DISCUSSION

Five polymorphisms (not including polynucleotide length variation) were detected among the nine samples in the preliminary data set, one in the *trnH-psbA* intergenic spacer, and two in both the *accD-psaI* intergenic spacer and *rpl16* intron. Since the substitutions present at both *trnH-psbA* and *accD-psaI* divided the cultivars into the same groups, only *trnH-psbA* and the internal portion of *rpl16* were sequenced for the total 113-sample set. The *trnH-psbA* intergenic spacer was 335 bp in 109 samples. Four samples were 334 bp, owing to either one less nucleotide in a poly-A region (Gouais blanc and Saperavi Meskh) or a poly-T region (Alphons Lavalee and Yugoslavia 360). The targeted portion of the *rpl16* intron was 367 bp in all samples. The haplotype network derived from the 113-sample *trnH-psbA*/*rpl16* dataset is shown in Fig. 1. Four plastid haplotypes are evident in Fig. 1, and are designated by their character-states at each of the 3 polymorphic positions: (AAA)–23 samples, (ATT)–29 samples, (GTA)–26 samples, and (ATA)–35 samples (Table 1).

Plastid DNA sequence variation was observed in our geographically diverse set of *V. vinifera* susp. *vinifera* samples, consistent with previous studies of microsatellite and isozyme variation (see introduction). Interestingly, the (AAA) plastid haplotype was found only in the cultivars from the republic of Georgia. Twenty-five (63%) of the 40 included Georgian cultivars exhibited this haplotype, and these "Rkatsiteli" group cultivars all originate from eastern Georgia. Contrast this group with the nine cultivars (23%) of the "Chkhaveri-Pinot noir" group (GTA), most of which are cultivated in western Georgia near the Black Sea coast. Another six of the Georgian cultivars exhibited the "Saperavi-Cabernet Sauvignon" (ATT) haplotype. Among these is the well known cultivar Saperavi, which is now mainly distribured in eastern Georgia, but is believed to have originated in south-west Georgia. Only two Georgian cultivars exhibited the "Chardonnay-Mtsvane Meskhuri" group haplotype (ATA), as this group comprises mainly French cultivars.

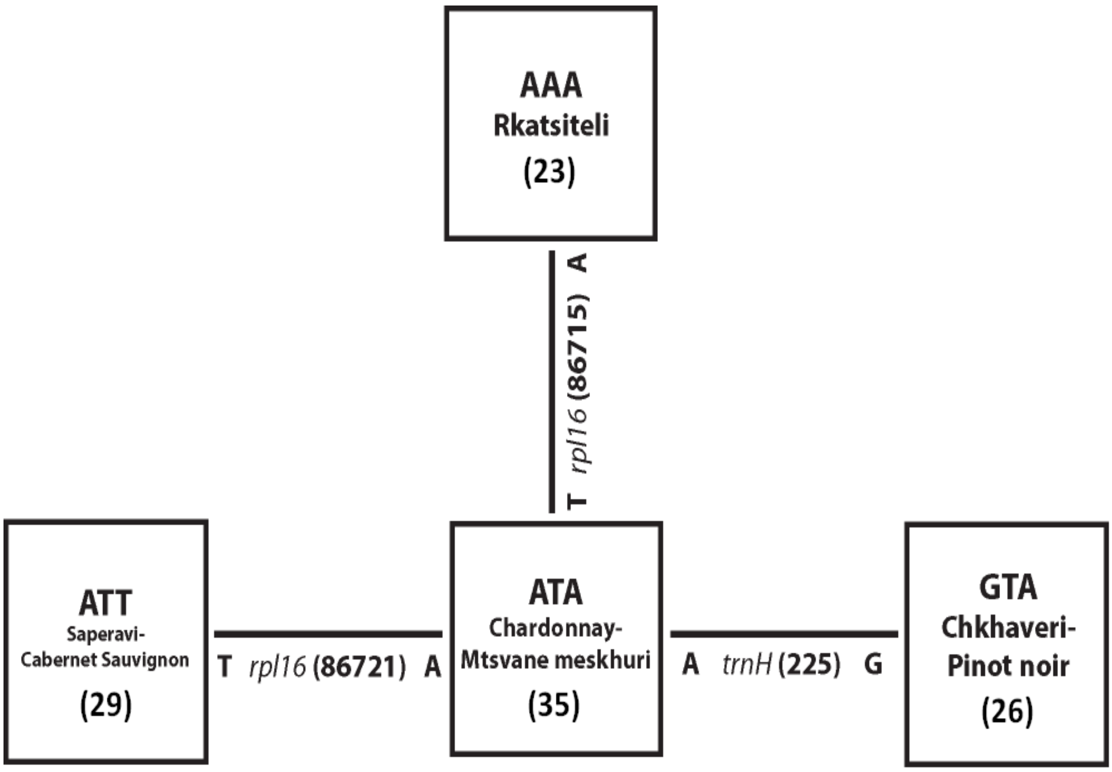

Fig. 1 Haplotype network derived from substitutions observed in the 113-sample *trnH-psbA*/*rpl16* dataset. Each haplotype is designated with a three-letter acronym, an informal group name, and the number of samples exhibiting the haplotype. Each branch between haplotypes represents a single mutational step. The substitution type and position in the published *V. vinifera* chloroplast genome (Jansen et al., 2006) are indicated along each branch.

Table 1. Sample information for the 113 sequenced samples. GIHVO = Georgian Institute of Horticulture, Viniculture, and Oenology; INRA = Institut National de la Recherche Agronomique ; UC Davis = National Clonal Germplasm Repository at the University of California, Davis.

Sequence group AAA			**Sequence group ATA**		
Cultivar	Country of origin	Tissue source	Cultivar	Country of origin	Tissue source
Akhardani	Georgia	GIHVO	Mtsv. Mesk.	Georgia	GIHVO
Aleksandrouli	Georgia	GIHVO	Saper. mesk.	Georgia	GIHVO
Almura shavi	Georgia	GIHVO	Aligote		INRA
Budeshuri tetri	Georgia	GIHVO	Ahmeur bou Ahmeur		INRA
Chinuri	Georgia	GIHVO	Alphonse Lavallee		INRA
Chitiskv. Mesk.	Georgia	GIHVO	Chardonnay		INRA
Chkapa	Georgia	GIHVO	Cinsaut		INRA
Gldanula	Georgia	GIHVO	Clairette		INRA
Gorula	Georgia	GIHVO	Colombard		INRA
Mekrenchkhi	Georgia	GIHVO	Cot		INRA
Meskhuri shavi	Georgia	GIHVO	Fahri		UC Davis
Mtsvane Goruli	Georgia	GIHVO	Ferral Izalva		UC Davis
Ojaleshi	Georgia	GIHVO	Folle blanch.		INRA
Rkatsiteli	Georgia	GIHVO	Gamay		INRA
Sazuravi	Georgia	GIHVO	Gamay de Bouze		INRA
Shaba	Georgia	GIHVO	Gamay de Chaudenay		INRA
Shavi asuretuli	Georgia	GIHVO	Gamay Freaux		INRA
Shavkapito	Georgia	GIHVO	Gouais blanc		INRA
Sqelkana adr.	Georgia	GIHVO	Kali Sahehi		UC Davis
Tavkveri	Georgia	GIHVO	Kurtelaska		UC Davis
Thethri Gomis	Georgia	GIHVO	Marsonne		INRA
Tkhelkana adr.	Georgia	GIHVO	Mauzac		INRA
Tkvlapha shavi	Georgia	GIHVO	Melon		INRA
			Merlot		INRA
			Monbadon		INRA
			Muscat d'Alexandrie		INRA
			Piquepoul blanc		INRA
			Reine des Vignes		INRA
			Romorantin		INRA
			Roussanne		INRA
			Sahebi		UC Davis
			Sultanine		INRA
			Terret gris		INRA
			Valdiguie		INRA
			Yugosl. 360		UC Davis

Sequence group ATT			Sequence group GTA		
Cultivar	Country of origin	Tissue source	Cultivar	Country of origin	Tissue source
Chitisthvala meskh.	Georgia	GIHVO	Aladasturi	Georgia	GIHVO
Sabatono	Georgia	GIHVO	Chkhaveri	Georgia	GIHVO
Saperavi	Georgia	GIHVO	Kachichi	Georgia	GIHVO
Tsigizi	Georgia	GIHVO	Kamuri shavi	Georgia	GIHVO
Kharisthvala qartlis	Georgia	GIHVO	Krakhuna	Georgia	GIHVO
Kharistvala meskh.	Georgia	GIHVO	Pirgebuli	Georgia	GIHVO
Aramon		INRA	Shonuri	Georgia	GIHVO
Cabernet franc		INRA	Tsitska	Georgia	GIHVO
Cabernet Sauvign.		INRA	Tsolikauri	Georgia	GIHVO
Carmenere		INRA	Alvarelhao		INRA
Chasselas		INRA	Carignan		INRA
Chenin		INRA	Dattier Beyr.		INRA
Emperor		INRA	Grenache		INRA
Gewürtztraminer		INRA	Himrisnky		UCDavis
Itonychi Mavro		UC Davis	Macabeu		INRA
Muscadelle		INRA	Meunier		INRA
Muscat Hambourg		INRA	Mourvedre		INRA
Muscat petits blanc		INRA	Müeller-Thur.		INRA
Nicolas Horthy #39		UC Davis	Perlette		INRA
Sauvignon		INRA	Pinot noir		INRA
Savagnin blanc		INRA	Pinot gris		INRA
Semillon		INRA	Pinot blanc		INRA
Stambulari		UC Davis	Riesling		INRA
Tallian		UC Davis	Sauvignonasse		INRA
Traminer rot RG		INRA	Servant		INRA
Tannat		INRA	Syrah		INRA
Ugni blanc		INRA			
Veltliner rot		INRA			
Viognier		INRA			

The observation that the Georgian cultivars exhibited unique plastid DNA variation (AAA haplotype) and all other observed plastid haplotypes is consistent with previous studies that have observed both unique and high levels of genetic variation in wild grape (*V. vinifera* subsp. *sylvestris*) in the greater Caucasus region (Arroyo-Garcia et al., 2006; Grassi et al., 2006, 2008; De Mattia et al., 2008).

ACKNOWLEDGEMENTS

CRDF-GRDF Georgian-U.S.Bilateral grants Program, 2004 (GEB2-3326-TB-03) for T.B.
Science and Technology Center in Ukraine, Grant N4666, 2008
Georgian National Science Scholars (NSS) Program for I.P. NSS N01-07 (2007)

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Identification of Armenian and Georgian *Vitis* spp cultivars by SSR markers and molecular relationships with European grapevines

Identificazione di varietà di *Vitis* ssp dell'Armenia e della Georgia con marcatori SSR e rapporti con cultivar europee

E. Frare[(1)], A. Costacurta[(2)], S. Giannetto[(1)], S. Meneghetti[(1)], M. Crespan[(1)]

[(1)]C.R.A. Centro di ricerca per la viticoltura, Via Casoni 13/a, 31058 Susegana (TV), Italy
manna.crespan@entecra.it

[(2)]Accademia italiana della vite e del vino

ABSTRACT

Thirty-six grapevine accessions have been collected, 22 from Armenia and 14 from Georgia, totalling 36 samples. They have been analysed with 11 SSR markers and 27 microsatellite profiles have been obtained. After comparison with the CRA-VIT database and also with data available in literature, only 33% of these genotypes was identified. Homonymy and synonymy cases found inside the accessions sampled in the present work are discussed, and also in respect to what already published. Our results show that there is some confusion in the correct identification of local cultivars, even those better known and most spread. The genetic variability inside the varieties found has been evaluated with Identity software. Being aware that Transcaucasia is the first domestication area of grapevine, Armenian and Georgian genotypes have been compared with 271 varieties, mainly European, 174 of which are from Italy, searching for possible genetic similarities. To this aim "Structure" and "Genetix" softwares have been used. The obtained results are discussed.

RIASSUNTO

Sono state raccolte 22 accessioni di *Vitis* ssp provenienti dall'Armenia e 14 dalla Georgia, per un totale di 36 campioni. Questi materiali sono stati analizzati con 11 marcatori SSR, ottenendo 27 profili molecolari. Dopo il confronto con il database del CRA-VIT e con i dati di letteratura, solo il 33% dei genotipi è stato identificato. Vengono discussi i casi di omonimia e di sinonimia individuati entro le accessioni campionate nel presente lavoro e rispetto a quanto già pubblicato. I nostri risultati evidenziano che vi è una certa confusione nella corretta identificazione delle varietà locali, anche delle più note e diffuse. E' stata valutata la variabilità genetica dei genotipi individuati con il software Identity. Sapendo che la regione transcaucasica, rappresenta la principale zona di origine della vite coltivata, i genotipi di Armenia e Georgia sono stati messi a confronto con 271 varietà, prevalentemente europee, di cui 174 italiane, per individuare eventuali vicinanze genetiche, usando i software "Structure" e "Genetix". Vengono discussi i risultati ottenuti.

INTRODUCTION

The aim of our work was to investigate if varieties coming from Armenia or Georgia are nowadays growing in Europe and particulary in Italy. Moreover we were interested in evaluate the genetic relationships among Near Est and European cvs, because Armenia and

Georgia are thought to be the most important centre of origin of *Vitis vinifera*, together with Azerbaijan.

MATERIALS AND METHODS

Vegetal material. Twenty-two accessions of *Vitis spp* coming from Armenia and 14 from Georgia have been sampled, totalling 36 (Tab. 1).

Tab. 1 - List of accessions analysed and related provenance.

DNA No	Accession name	Provenance
1,09	ARARATI	Armenia
4,09	ARENI	Armenia
36,09	ARMENIA 4	Armenia, Ararat Valley
38,09	HACIABASH 8	Armenia
30,09	ITSANPTUK 8	Armenia
7,09	ITZAPTUK	Armenia
8,09	KAKHET	Armenia
37,09	KHALIZ 1	Armenia, Ararat Valley
10,09	MSKHALI	Armenia
2,09	MSKHALI	Armenia
39,09	MUSKAT 3	Armenia, Ararat Valley
34,09	NAZELI	Armenia
9,09	QISHMISH	Armenia
111,09	QISHMISH RED 6	Armenia, Ararat Valley
35,09	QISHMISH WHITE 5	Armenia, Ararat Valley
31,09	SHAHUMYAN 7	Armenia
29,09	TABLE WHITE 9	Armenia, Ararat Valley
3,09	TAIFU	Armenia, Ararat Valley
6,09	TOZOD	Armenia
32,09	VARDAGUYN YERVANI	Armenia, from Institute
5,09	VOSKEHAT	Armenia, Areni village
33,09	VOSKEHAT	Armenia, from Institute
28,09	BUDASCIURI 13	Georgia
113,09	IZABELLA BIANCO 11	Georgia
112,09	IZABELLA NERO 10	Georgia
27,09	PINOT NERO 9	Georgia
11,09	RKAZITELI n. 1	Georgia
13,09	RKAZITELI n. 7	Georgia
18,09	SAPERAVI n. 3	Georgia
20,09	SAPERAVI n. 5	Georgia
12,09	SAPERAVI n. 8	Georgia
17,09	SCONOSCIUTO n. 12	Georgia
15,09	SCONOSCIUTO n. 14	Georgia
16,09	SCONOSCIUTO n. 6	Georgia
14,09	TITA n. 4	Georgia
19,09	TITA SHAVI n. 2	Georgia

These materials have been analyzed with the 11 SSRs routinely used at CRA-VIT for grape variety identification. Six of them belong to the international set recommended in the frame of the European project GenRes081 and are therefore useful for comparison purposes with other databases.

Genomic DNA extraction and PCR amplification. Genomic DNA was isolated from young leaves ground using Tissue Lyser (Qiagen) according to the protocol for DNeasy Plant Mini Kit (Qiagen). Eleven nuclear microsatellites were studied in one multiplex: VVS2 (Thomas and Scott 1993), VVMD5, VVMD7, VVMD27, VVMD28 (Bowers *et al*. 1996, 1999), ssrVrZAG62, ssrVrZAG79 (Sefc *et al*. 1999), ISV2, ISV3, ISV4 and VMCNG4b9 (Crespan 2003). Primer concentrations have been modulated in order to balance signal intensity for each locus. The cycling program was: 2 min at 94 °C, followed by 30 cycles of 45 s at 94 °C, 1 min 30 sec at 55 °C, 1 min at 65 °C, and a final extension step of 45 min at 65 °C. Electrophoresis was carried out in an ABI 3130xl Genetic Analyzer (Applied Biosystems) and the fragments were sized with GeneMapper 4.0 using GeneScan 500 LIZ size standard (Applied Biosystems) as an internal marker (Applied Biosystems).
Allele calling was performed using a specific Bins Set built for variety identification; our Bins Set is made by 53 reference data representing almost all the alleles present in our database.
Mutants for berry color have been analyzed with the protocol described in Giannetto *et al.* 2008, using the primer pair VvmybA1 (3).

Statistics on molecular data and genotype correlations evaluation.
The genetic variability inside the varieties found has been evaluated with Identity 1.0 free software, i.e. number of alleles/locus, allele frequencies, expected and observed heterozygosity, probability of null alleles, and probability of identity (PI).
Using the SSR database of CRA-VIT, the molecular profiles of Armenian and Georgian samples were compared with those of 271 genotypes, mainly European varieties, 174 of which were from Italy, searching for possible genetic similarities. Three different softwares were used: NTSYS, GENETIX and STRUCTURE 2.3.1. In the first case, genetic similarity (GS) estimates between individuals were calculated using the band similarity coefficient of Lynch (1990) for SSR data in diploid genomes. The cluster analysis was performed according to the unweighted pair-group arithmetic average method (UPGMA) and the dendrogram of all individuals was constructed from the symmetrical GS matrix using NTSYS-pc 2.10 software.The Analysis of Functional Correspondence (AFC) was performed with GENETIX software (Belkhir *et al*. 2003), available free at http://www.univ-montp2.fr/%7Egenetix/genetix/genetix.htm. As regards STRUCTURE elaborations, we set the following parameters: length of burn-in period 10,000 generations and 10^5 Markov chain Monte Carlo replications; we used the admixture model (Pritchard *et al*. 2000) and run the analyses with correlated allele frequencies (Falush *et al*. 2003). No information was given on the presumed origin of the individuals (USEPOPINFO=0). Structure was run for *K* values ranging from 1 to 20.

RESULTS AND DISCUSSION

Twenty-seven molecular profiles have been obtained (Tab. 2) and they were 15 for Armenian and 12 for Georgian samples. The SSR profiles were compared with the CRA-VIT database and with data available from literature. Only 33% of genotypes were identified, whilst most of the varieties showed to be different from all those analyzed until now.

Among the Armenian accessions we found Areni (1 accession), Askeri (1 accession), Khusaine belyi *alias* Husaine blanc (3 accessions), Mskhali (3 accessions) and Sultanina (3 accessions).

Tab. 2 - SSR analysis results.

provenance	DNA No	Accession Name	comparison with Vouillamoz et al. (2006)	Identity
Armenia	4,09	ARENI	Areni Chernyi_3	**Areni**
	34,09	NAZELI		**Askeri**
	7,09	ITZAPTUK		**Khusaine belyi (Husaine blanc)**
	30,09	ITSANPTUK 8		
	31,09	SHAHUMYAN 7		
	1,09	ARARATI	Mskhali	**Mskhali**
	10,09	MSKHALI		
	29,09	TABLE WHITE 9		
	35,09	QISHMISH WHITE 5		**Sultanina**
	111,09	QISHMISH RED 6		
	9,09	QISHMISH		
	36,09	ARMENIA 4		Unknown G7
	8,09	KAKHET		Unknown G11
	37,09	KHALIZ 1		Unknown G12
	2,09	MSKHALI		Unknown G8
	38,09	HACIABASH 8		
	39,09	MUSKAT 3		Unknown G5
	3,09	TAIFU		Unknown G9
	6,09	TOZOD		Unknown G2
	32,09	VARDAGUYN YERVANI		Unknown G3
	5,09	VOSKEHAT	Khardjzhi	Unknown G10
	33,09	VOSKEHAT		Unknown G4
Georgia	18,09	SAPERAVI n. 3		**Isabella**
	112,09	IZABELLA NERO 10		
	14,09	TITA n. 4		**Taifj rosovi**
	20,09	SAPERAVI n. 5	Dondglabi, Kapistoni Im., Gorula and Tavkara	Unknown G13
	11,09	RKAZITELI n. 1	Rkatsiteli	**Rkatsiteli**
	13,09	RKAZITELI n. 7		
	28,09	BUDASCIURI 13		Unknown G21
	113,09	IZABELLA BIANCO 11		Unknown G19
	27,09	PINOT NERO 9		Unknown G17
	12,09	SAPERAVI n. 8		Unknown G14
	17,09	SCONOSCIUTO n. 12		Unknown G20
	15,09	SCONOSCIUTO n. 14		Unknown G15
	16,09	SCONOSCIUTO n. 6		Unknown G18
	19,09	TITA SHAVI n. 2		Unknown G16

In this last case, the variant with red berries (DNA No 111.09) has been distinguished using the diagnostic method pointed out by Giannetto *et al*. (2008) (Fig. 1).

Among the Georgian samples four genotypes are hybrids or presumable hybrids due to particularities in the profile, such as alleles present only in the rootstocks and absent in *V. vinifera* or multiple signals at VMCNG4b9 locus (Crespan *et al*. 2009): they are Isabella (2 accessions) and other three unidentified samples (113.09, 27.09 and 15.09). We found Taifj rosovi (1 accession) and Rkatsiteli (2 accessions); the remaining six samples were not identified.

The two Armenian accessions named Voskheat were different each other and also in respect to the sample analysed by Vouillamoz *et al*. (2006). It comes out that the correct identity of this variety is not well defined and it is important to highlight the question because it plays a major role among the wine grape cultivars autochthonous of Armenia.

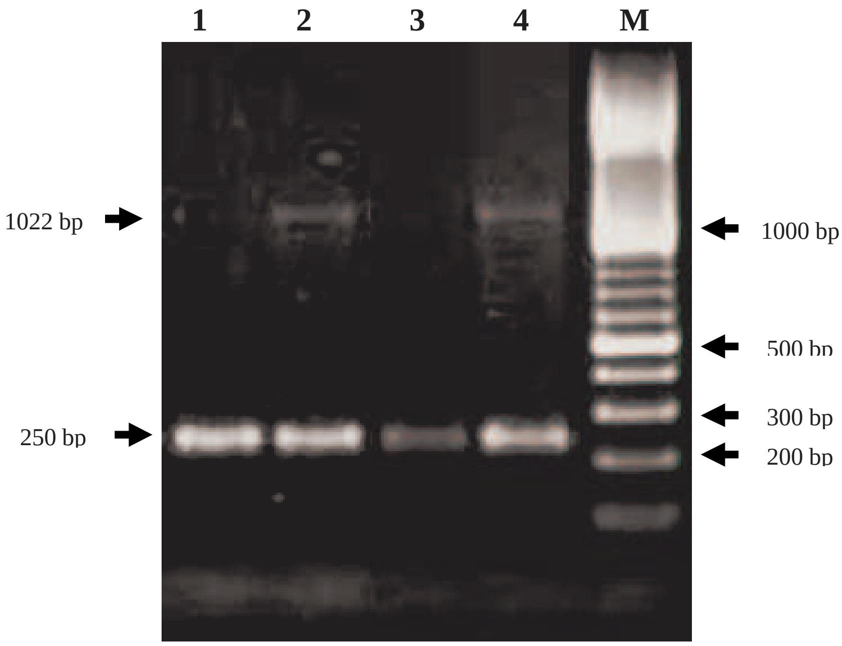

Fig. 1 Discrimination between white and red Qishmish accessions analyzing *VvmybA1* gene. Lanes: 1. reference white Sultanina; 2. reference pink Sultanina; 3. Qishmish white; 4. Qishmish red; M is the molecular ladder 100 bp DNA step ladder from Promega.

An analogous case came out for Saperavi which is, together with Rkatsiteli, one of the most known indigenous wine cultivars of Georgia. The three Saperavi samples we analyzed showed three different SSR profiles and one of them has been identified as Isabella, which is a known hybrid of *Vitis labrusca* x *V. vinifera*. Moreover none of the other two (DNA No 12.09 and 20.09) matches the profile of Saperavi analyzed by Vouillamoz *et al*. (2006).

We found also additional alleles at the very highly polymorphic VVMD28 locus, one of 236 and the other of 238 bp. They are in the range of *V. vinifera* and integrate the stair of alleles available until now.

The genetic variability inside the genotypes found has been evaluated with Identity software, computing the number of alleles, the heterozygosity observed and expected, the probability of identity, and disregarding the four hybrids. We found 98 alleles with an average of 8.9 alleles by locus. The average He was 0.77 and the observed one 0.80; the total probability of identity1.41e-11, which is significantly low.

Knowing that the Near Est and particularly the Transcaucasian region is the principal centre of origin of cultivated grapevine, Georgian and Armenian genotypes were compared with 271 *Vitis vinifera* varieties, mostly European, and among them 174 were from Italy, in the effort to find genetic relationships, using three different approaches.

The cluster analysis produced a very complex tree, in which 8 genotypes were spread up-down in different and very distant groups (data not shown). Nevertheless, referring to Tab. 2 for sample identification, the other 19 varieties were grouped in a well defined part of the tree, at the bottom (Fig. 2): a part encompasses Sultanina related genotypes, another Husaine blanc and the last cv Rkaziteli. G19 genotype remains clearly apart, supporting the hypothesis that it could be a hybrid.

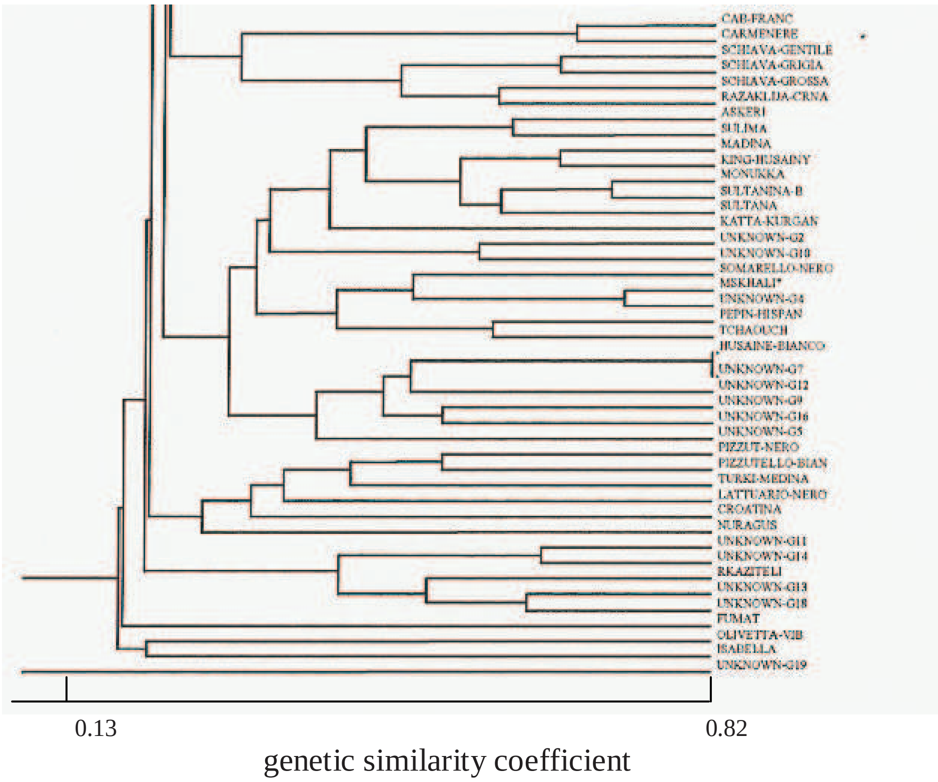

Fig. 2 Bottom part of the dendrogram produced using Band coefficient and UPGMA clustering.

Genetix results. A first elaboration comprising all 298 individuals produced an AFC graphic crushing 296 samples in an unique group and putting G17 and G19 largely aside. Since we supposed that these two genotypes could be hybrids, we discarded them from the Genetix analysis and produced another elaboration using 296 individuals. This time 295 genotypes were tightly grouped and Isabella remained alone. Knowing that it is an interspecific hybrid, it was excluded from the analysis, reducing the comparison to 295 samples (Fig. 3). Many interesting information came out from AFC graphic. First at all, even if the number of Armenian and Georgian samples is poor, they occupy decidedly the right part of the graph and are highly spread all over the three dimensional space, much more than all other cultivars. This is not surprising, giving that Armenia and Georgia are the first centre of origin of *Vitis vinifera* and therefore the greatest genetic variability is expected here. The Italian varieties are spread along the horizontal axis and some of them remain alone on the left side of the graph: they are ancient minor cultivars of North Est Italy such as Corbina, Raboso Piave, Schioppettino, Ciavalgian, Berzamino and also the most known Refosco's and Lambrusco's. It is very interesting to underline that the Lambrusco's are considered very close to *sylvestris* grapes, and it will be an intriguing topic to deep inside this preliminary indication, since this part of Italy could be another centre of origin of *sativa* grape. Another surprising observation was to find Garnacha tinta at the right side of the graph, together with many Sardinian varieties, such as Girò, Nuragus and Monica.

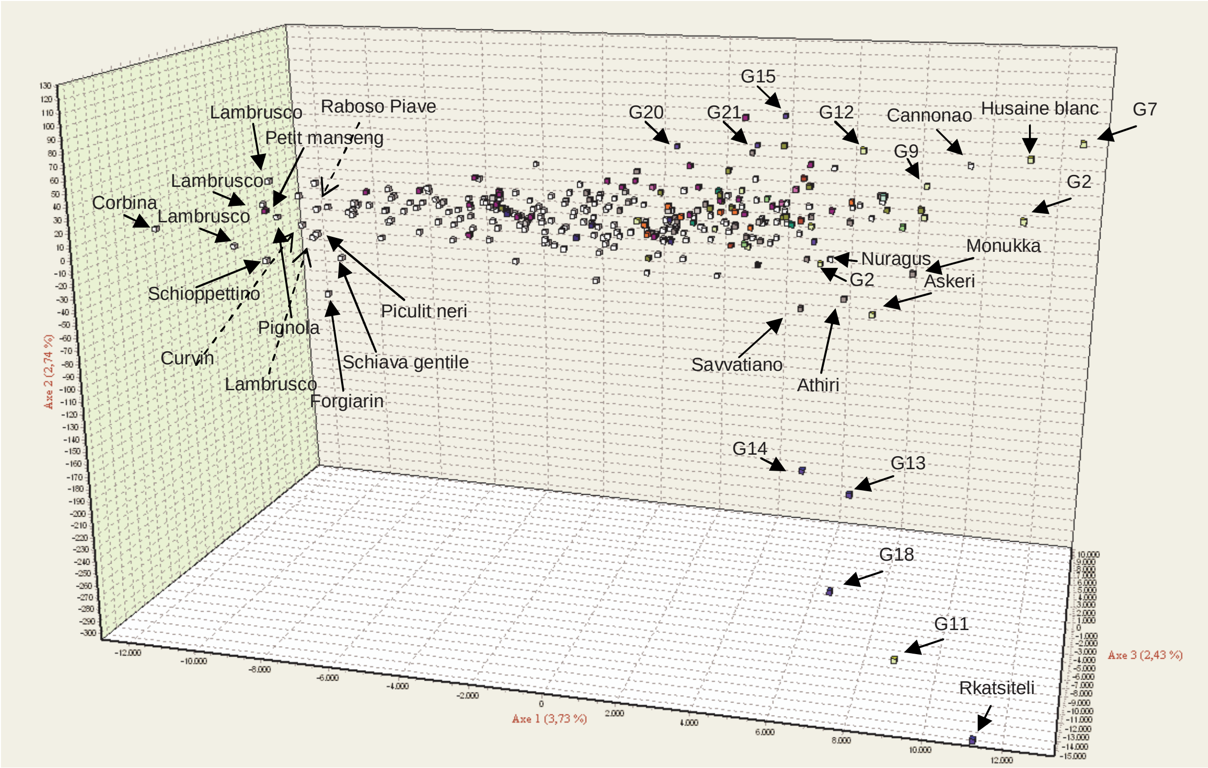

Fig. 3 AFC graph. The names of the samples refer to Tab. 2 identification results.

The Spanish origin of Garnacha tinta is disputed in favour of a Sardinian origin (Lovicu 2006). In our work Garnacha tinta groups with many Sardinian varieties; besides, they showed to be more close to Armenian and Georgian cultivars than other Italian varieties and this fact is very intriguing because Sardinia was hypothesized as a second centre of origin for cultivated grape (Grassi *et al.*, 2003). As expected, Greek cultivars are also close to the Armenian and Georgian ones. On the contrary, Muscat blanc à petits grains is in the middle. We know that it is a very ancient variety, parent of a lot of cultivars with muscat flavour (Crespan and Milani, 2001), and its position resembles a bridge from Est to West. Rkatsiteli is the most distant one, localized at the right side in the bottom.

The analysis performed with STRUCTURE to infer K, i.e. the number of genetic clusters or gene pools in all genotypes, failed to reveal any maximum value of *ln P(D)* over the range of K values from 1 to 20, which would allow the assignment of the true K value. Therefore the 298 cultivars could not be divided into sub-populations (data not shown).

CONCLUSIONS

Our results show that there is some confusion in the correct identification of local cultivars, even those better known and most spread as Voskheat and Saperavi.

The results of the analysis of the genetic relationships among Armenian, Georgian and European cultivars show that many Sardinian (Italy) varieties are very close to Armenian samples, whilst Lambrusco's and other minor cultivars of North Est Italy group apart. Many interesting indication emerged from AFC analysis, which need to be deepened in order to get additional insights into the spreading and the evolution of grapevine genetic resources.

ACKNOWLEDGMENTS

The authors thank Vadim OGANESSYAN (Tbilisi, Georgia), Hratch Kaspar, (Jerivan, Armenia) and Sergio Sanson (Conegliano-TV, Italy) for providing Georgian and Armenian samples.

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GEORGIAN *VITIS* GERMPLASM: USAGE, CONSERVATION AND INVESTIGATION

D. Maghradze[(1)], O. Failla[(2)], R. Bacilieri[(3)], S. Imazio[(2)], L. Vashakidze[(1)], R. Chipashvili[(1)], I. Mdinaradze[(1)], N. Chkhartishvili[(1)], P.This[(3)], A. Scienza[(2)]

[(1)] Institute of Horticulture, Viticulture and Oenology. 6 Marshal Gelovani Ave. 0159. Tbilisi, Georgia.
d_maghradze@geo.net.ge, larisa.vashakidze@yahoo.com, i_mdinaradze@yahoo.com

[(2)] Department of Crop Science. University of Milan. Via Celoria 2, 20133. Milano, Italy.
osvaldo.failla@unimi.it, serena.imazio@unimi.it, attilio.scienza@unimi.it

[(3)] INRA - Génétique de la Vigne - Bat 21. UMR DIA-PC. 2, Place P. Viala, 34060. Montpellier, France.
roberto.bacilieri@supagro.inra.fr, patice.this@supagro.inra.fr

ABSTRACT

Georgian grapevine germplasm (*V. vinifera* L.) originated in diverse regions of the country over a long historical period. During the XX century bred varieties enriched it. Georgia is also a place where *V. vinifera sylvestris* spread in large numbers, provided an important initial impulse to the domestication of grapevine. The main cultivated varieties in Georgia are autochthonous varieties, having high-market value – best of those are also cultivated in East Europe and Middle Asia. Conservation initiatives for Georgian *Vitis* germplasm started since XIX century and entered into XXI century with some difficulties. However, Georgia was able to establish new field collections and collaborative works, in the framework of international projects and local initiatives. This germplasm is the object of intensive investigations; it attracts international collaborations because of its genetic diversity. Investigations based on SSR fingerprinting and ampelographic methods are used for classification of varieties and understanding their phylogenic relationships.

RIASSUNTO

Il germoplasma di vite georgiano (*V. vinifera* L.) si è originato in differenti aree del paese nel corso di un lungo periodo storico. Durante il XX sec. nuovi vitigni ottenuti per incrocio lo hanno arricchito. La Georgia è un luogo ove è diffusa la *V. vinifera sylvestris*; si ritiene quindi che le antiche civiltà locali abbiano dato un importante contributo alla domesticazione della vite. Le principali cultivar sono autoctone; quelle più apprezzate sono coltivate anche in Europa orientale e Asia Centrale. In Georgia la consapevolezza dell'importanza della conservazione del germoplasma risale al XIX sec. Entrò in difficoltà per i problemi economici nel XXI sec. Comunque la Georgia è stata in grado di costituire nuove collezioni e attività di collaborazione, internazionali e locali. Il germoplasma georgiano è oggetto di intense attività di ricerca anche perché, a causa della sua diversità genetica suscita interesse internazionale. I marcatori SSR e le moderne tecniche ampelografiche sono utilizzate per la caratterizzazione varietale, la comprensione della relativa struttura genetica e delle relazioni filogenetiche.

INTRODUCTION

The germplasm of Georgian grapevine varieties (*V. vinifera* ssp *sativa* DC.) originated in diverse geographic regions of the country over a long historical period, and includes 525 autochthonous varieties. A.M. Negrul (1946) in his famous ampelographic classification groups our varieties mainly in the Proles *pontica*, subproles *Georgica* Negr., and comparatively few number - in the Proles *orientalis,* subproles *Caspica* Negr. During the XX century new bred varieties enriched this germplasm. Georgia, a country of the South Caucasus, is also a place where *Vitis vinifera* ssp *sylvestris* Gmel. strives and is found in large numbers (Ramishvili, 1988); this is believed to have provided an important initial impulse to the domestication of grapevine, by the old civilisations here.

The main cultivated varieties in Georgia are autochthonous varieties, having high-market value (Census, 2004). The best varieties like 'Rkatsiteli', 'Saperavi', 'Tavkveri' and others are cultivated in East Europe, Middle Asia and other countries of the Caucasus.

Awareness of importance and conservation initiatives for Georgian *Vitis* germplasm started since XIX century (Staroselskii, 1893), passed the best period in XX century and entered into XXI century with some difficulties linked to the economic situation of the Country. However, Georgia was available to establish new field collections and collaborative works, in the framework of international projects and local initiatives. Besides, some of the autochthonous varieties are preserved in foreign collections, bringing a reciprocal interest between local and foreign researchers.

Beside the conservation, the grapevine germplasm is the object of intensive investigations in various scientific fields; it attracts international collaborations because of its genetic diversity (Vouillamoz et. al. 2006; Maghradze et al. 2009b). Investigations based on SSR fingerprinting and modern ampelographic methods are used for classification of varieties, for understanding their genetic structure and the phylogenic relationships with the World's germplasm.

The aim of this work was a multidisciplinary characterisation and evaluation of Georgian grapevine germplasm including autochthonous varieties and wild vines, demonstration of its importance for domestic and foreign viticulture and winemaking.

Particular target of this work is to give a description of the Georgian germplasm platform through a molecular approach based on SSR profiling of most interesting wine/table cultivated grapes and representatives of wild populations. Two different types of approaches were carried out: i) analysis and description of the genetic structure of Georgian grapevines basing on the characterization of wild and cultivated collected samples; ii) Comparison of data obtained in the previous investigation (Maghradze *et al.* 2009a) with data obtained by genotyping at 20 SSR loci the 2305 grapevines conserved in the Institut National de la Recherche Agronomique (INRA, France) grape germplasm repository of Domaine de Vassal (INRA Domain de Vassal, 2004). The combined used of these two approaches were considered valuable in describing in a complete way both the structure of Georgian viticulture and its spread in other Countries.

MATERIALS AND METHODS

Plant materials: Georgian autochthonous grapevine varieties (*V. vinifrera* ssp *sativa* DC.) from 5 field collections (Vashlijvari, Skra, Telavi (Georgia), Gorizia (Italy) and Vassal (France) were included in our research. One hundred and thirty nine cultivars were selected for DNA investigation as representatives of supposed Georgian native grape germplasm. At the same time, in this research, 22 wild grapevine (*V. vinifera* ssp *sylvestris* Gmel.) were also added.

Ampelography and Ampelometry: Harmonized ampelographic descriptors of OIV (1983, 2007), IPGRI (1997), GENRES 081 and UPOV (1999) were used for ampelographic, agronomic and cytologal characterization of vine organs. Eighty one parameters of leaves, 3 parameters of bunch, 2 parameters of berries and 3 parameters of seeds were obtained by the software "SuperAmpelo" (Soldavini *et al.* 2007).

Phenology: One hundred thirty four varieties were tested in a field collection, located in Gorizia. Phenological timing, according to Baggiolini's scale (in Coombe, 1995), and technological ripening profiling, in the three year period 2004-2006 has been recorded. Seasonal weather courses were representative of local climate (Maghradze *et al*. 2010).

Anthocyanin Analysis: Eighty nine colored accessions from Gorizia collection and 10 European cultivars was analyzed by HPLC technique according to Mattivi *et al.* (1990).

Cytological analysis: The Karnua's solution 3:1 (ethanol: acetic acid) was used for fixation of cells. Common methods for cytological analyses (Pausheva, 1970; Chkhartishvili *et al.* 2006) were used for investigation of pollen and stigma morphology, structure and parameters. Thirty cultivars and clones of grapevine were discovered in this work. The analysis was conducted at the Institute of Horticulture, Viticulture and Oenology by L. Vashakidze (2006).

SSR genotyping: Samples of young leaves were collected for each accession during the active growing seasons or from woody cuttings green house grown. The DNA was extracted using a commercial kit (Qiagen DNeasy Plant Kit). Twenty nuclear SSR loci were chosen basing on the work of Doligez and coworkers (2006). These loci, selected for their quality and distribution across the 19 grapevine chromosomes, were detected on an automated ABI Prism® 310 Genetic Analyzer (Applied Biosystems – Life Technologies, Foster City, CA, USA). Alleles were scored by eye using GeneMapper 3.10 (Applied Biosystems – Life Technologies) and allele sizes were recorded in bp with two decimal precision allele sizes were standardized to the sizes of reference varieties.

For structure and paternity analysis, the data of the Georgian varieties and wild genotypes were compared against the SSR dataset of the Vassal collection of INRA (France). These data, obtained by Lacou et al. (in prep.) on the same set of 20 SSR, and presented by Le Cunff *et al*. (2008), comprised 2262 cultivars. A number of common reference varieties presenting alleles spanning all the diversity was used, following the method of This *et al*. (2004), to adjust allele length between the dataset obtained in the two laboratories (University of Milan and INRA), so to make the two dataset harmonized and comparable.

Data analysis:

Analysis of the genetic structure of Georgian grapevine germplasm: The apparent isolation of Georgian germplasm makes this viticulture particularly interesting to be investigated, for this reason we used the software *Identity* (Wagner and Sefc, 1999) to calculate the number of alleles (n), the expected (H_e) and observed (H_o) heterozygosity, the estimated frequency of null alleles (r) and the probability of identity (P.I.). To describe the structure of these samples and to perform a study on allele frequencies and characterization, Genetic distances were calculated by the Nei's (1978) genetic distance using the *Microsat* software (Minch *et al.* 1997).

Comparison between Georgian samples and Vassal germplasm collection and Parentage analysis: To verify the contribution of Georgian germplasm platform to viticulture a parentage analysis was carried out with the software FaMoZ adapted to grape (Gerber *et al*. 2003; Di Vecchi Staraz *et al*. 2007). Possible parents determined by logarithm of odds (LOD) scores and significance thresholds were probed among the 2305 cultivars previously characterized with the set of 20 SSR markers. Likelyhood ratios were calculated according to Bowers and Meredith (1997) from alleles frequencies based on this set of data. To verify the kind of the most probable relationships among the Georgian material and between this and the Vassal germplasm collection ML-Relate software was used (Kalinowski *et al*. 2006) . This is a computer program calculating maximum likelihood estimates of relatedness and relationship for codominant genetic data. ML-Relate is designed for microsatellite loci, and can accommodate null alleles. *ML-Relate* is useful for discriminating among four common pedigree relationships: unrelated (U), half-siblings (HS), full-siblings (FS), and parent-offspring (PO). For each of these kind of relationships the likelihood is given helping in categorize the analyzed accessions.

Statistical Analysis: The obtained data were processed by uni- and multivariate statistical analysis as described in the Results section. Data were analyzed using SPSS (V. 14.0) statistical program.

The period of investigation: ranged from 2003 to 2009.

RESULTS AND DISCUSSION

Usage: Main direction of Georgian viticulture is winemaking. The list of standard wine cultivars in Georgia contains 34 ones (from total number of 48 cultivars), including 27 old autochthonous varieties (Law, 1998). They cover most of vineyards within country and can produce high quality wines, highly rated by wine-tasting panels in different countries of the world. Important Georgian varieties are 'Rkatsiteli', 'Saperavi', 'Tavkveri', 'Mtsvane Kakhuri' 'Tsolikouri', 'Ojaleshi', 'Krakhuna', 'Alexandrouli', 'Chinuri' and others.

During XX century Georgian autochthonous varieties of grapevine were involved in breeding programs in Georgia and other countries abroad. As a result, the successful realization of 193 new bread varieties from 13 Georgian native varieties in 16 different countries was achieved (Vakhtangadze *et al.* 2010).

Conservation: at the beginning of XXI century the conservation of Georgian native germplasm had some progress after the regress of the 90th of the XX century: Vashlijvari collection (Tbilisi, Georgia) with 300 accessions was established in the framework of the international project "Conservation and Sustainable use of grapevine genetic resources in the Caucasus and Northern Black Sea region" in 2003-2005. Based on the materials of this collection the Institute of Horticulture, Viticulture and Oenology was able to establish other three field collections in Telavi (573 accessions), Skra (440) and Vachebi (312) in 2008. Other three new collections were set up by Saguramo "Centre for Grapevine and Fruit Tree Planting Material Propagation" (about 400 accessions), "Kindzmarauli" winery and "Shumi" winery (149). Two new collections were established in Italy by the University of Milan.

Except this positive tendency, viticulture in Georgia is threatened by genetic erosion: only a few numbers of local varieties are still cultivated in spite of the available; wide number of indigenous breeding varieties not yet included in germplasm collections or on the brink of extinction due to preservation only in a single collection. Few wild vines are preserved in collections. This is the reason why proper conservation management and activities for Georgian grapevine germplasm is still actual.

Investigation:

Ampelography: Ampelographic cards based on IPGRI and OIV descriptors for grapevine were prepared for varieties 'Gorula' (138 descriptors), 'Gorula clone No23' (138), 'Tavkveri' (114) and 'Satsuravi' (96). 150 ampelographic cards with 56 characters were completed in the Gorizia collection. Local varieties were described in Vashlijvari (216 varieties, 14 descriptors), Telavi (27 var., 17 descript.) and Skra (28 var., 20 descript.) collections.

Based on the inventory of Georgian grape collections a database of native varieties was established in 2004. Eight hundred seventy-five accessions available in the collections were described by the IPGRI-FAO Multicrop Passport Descriptors and data were published in the *Vitis* International Variety Catalogue (http://www.vivc.bafz.de/index.php). In this year the publication of ampelographic descriptors with photos was also started in this database.

A new ampelography of Georgian native varieties was published in Italian language (Del Zan, Failla, Scienza, 2004, 2009). The book was printed in two editions, including complete description of 45 varieties and pictures of 105 varieties.

Phenology: Average multi-year phenological data demonstrates that the timing of bud break and flowering was slightly later in respect to Western European varieties. Veraison timing was clearly later showing a very wide range. Also the ripening time was on average late. Ripening profiling was ample. Georgian varieties according to time of maturation can be grouped in four main clusters: the earlier group, harvested in 269-278 Julian days (September), includes only 9 varieties while other ones mainly were harvested in 280-300 Julian days (October). Maximum sugar accumulation was 25.93 Brix (19.4 in average), minimum total acidity 3.5 g/l (8.9 in average) and PH was between 2.8-3.5 (3.1 in average).

Antocianins: Statistical data processing allowed classifying the sampled varieties into four main groups, out of which two were sub-divided into sub-groups, for a total of ten groups (Rossoni et al. 2007; Maghradze et al, 2009a). We found very high level of total anthocyanins (>3000 mg/kg of berry) in four accessions ('Otskhanuri Sapere', 'Ojaleshi', 'Saperavi Grdzelmtevana' and 'Argvetula'). Through a cluster analysis it was possible to classify all the samples in classes of phenotypic proximity. In particular, 'Rkatsiteli Vardisperi' showed the only presence of delphinidin 3-monoglucoside free and represent an isolate class of particular interest for pharmacological application of this compound.

Cytology: The chromosome number of somatic cells basically are diploid (2n=38), but among those five tetraploid, triploid and diploid-tetraploid clones of 'Tsolikouri', 'Rkatsiteli', 'Saperavi' and 'Gorula' varieties were discovered. The process of mitosis proceeds normally with sufficient high activity of cell division (4.8-8.6%). The frequency of abortive cells varies according to varieties (0.3-2.3%) and presents as in the pre-synthetic (G_1), as in the post-synthetic (G_2) phases of Mitosis.

The pollen parameters of hermaphrodite varieties are: length of air-dry pollen is 21.1-38.8 µm, width is 14.7-23.0 µm, and diameter of colored in carmine grains is 8.1-27.9 µm. The pollen basically has three pores. Pollen of female varieties is pore-less, but infrequently, three-pores pollen grains have been also discovered (0.4-2.5%). Hermaphrodite varieties are high fertile (69.2-98.9%). Single number of fertile pollen (0.6±0.4%) was reported among pollen of female varieties. Pollen germination varies 40-90% and their vitality is 7-10 days. The number of stoma is 135-227 per 1mm^2 of leaf. Their parameters are: length 191-295 µm, width 16.3-21.5 µm, and the number of chloroplasts per stoma is 27.8-21.5.

Microsatellite analysis (genetic structure of Georgian germplasm): The 135 accessions produced 130 distinct profiles when analysed at the 20 considered SSR loci. As already recorded for grapevine species the probability of having different individuals with the same profile at all loci is quite low (P.I.=2.95×10^{-7}), so identical genotypes over all loci can be considered identical by descent and not by chance. Same considerations can be extended to other genetic parameters such as the allele number (n), allele size range, expected (H_e) and observed (Ho) heterozygosity and probability of identity (P.I.).

The IDENTITY software was also used to define synonyms; 4 pairs of redundant genotypes were found in this work at the 20 investigated loci.

Basing on SSR markers Genetic distances among analysed samples were computed and results show, confirming the considerations made basing on observed heteozigosity and allele frequencies, the good level of genetic richness observed in the frame of the selected samples.

This result is quite interesting because this part of Caucasus is considered a cradle of grapevine domestication and it has been for a long time isolated from commercial and plant exchanges with elsewhere especially with the most important European Countries in grape growing and wine making. Despite isolation, and maybe thanks to this, Georgian grapevine germplasm seems to avoid the loss of genetic assortment, thus it seems to be particularly interesting in the frame of genetic and agronomic studies. On the other side, a strict connection of Georgian grapes and the *Terroir*, is clearly evidenced by genetic distances results; basing on these, in fact, we can observe a marked separation of all analysed material in two different groups. More interesting is the observation that the samples are grouped basing on their geographical belongings (eastern or western Georgia). The correlation between genetic and geographic distances is well known and already explored and discussed in grapevine European assortments and is one of the evidence for the existence of different areas of domestication. Twenty three wild accessions were also inserted in the analysis and, as already seen in the viticulture of other European Regions, also in this case the wild accessions are well distinguished from the cultivated compartment. Even if in this case the separation seems not to be as great as the one identified in other Countries.

On the wild samples side, the molecular fingerprint has revealed very few mistakes or inter-specific crossings in the pool of collected samples and no cases of parent offspring relationship among the two (wild and cultivated) compartments.

Comparison between Georgian samples and Vassal germplasm collection and Parentage analysis: The second part of our work was devoted to the comparison of Georgian and other Countries viticultures. In this context we have decided to consider the INRA Grapevine germplasm repository established in Vassal, because of the high number of samples from several *Vitis* species worldwide distributed.

For this reason Famoz and ML-relate softwares were used to evaluate the probability of existing relationships (parent/offspring, full siblings, half siblings, unrelated) among Georgian material and other grapevine accessions collected in Vassal.

The results of this huge research confirmed that the most interesting putative relationships were the ones involving Georgian samples and the Countries belonging to the former Soviet Union or neighbour Countries. Almost none of the most important cultivated grapes seem to have a direct relationship with Georgian material. This is also confirmed by the PCA. As shown in the PCA representation Georgian material is quite distant, under the genetic point of view, from other samples present in Vassal collection. They definitely present a number of allelic novelties (allelic forms) not found elsewhere in the grapevine genepool.

This result seems quite surprising considering the importance of Georgian viticultural heritage which is very well documented under the historic point of view; in fact we have to consider that since grapevine domestication lots of breeding events have occurred in all the grapevine assortments, modifying the genetic structure of plants. This, combined with the isolation of Georgia especially during the Soviet Union, is probably the reason why we have a consistent distance among Georgia and other viticultures. We must underline that these difference make the Georgian genetic platform even more interesting, because we could probably find in this context new genetic traits interesting for genetic improvement in other grapevine varieties.

CONCLUSIONS

Recent investigation one times more confirmed genetic richness and particularity of Georgian grapevine germplasm. Cultivated varieties of Georgia are grouped in two distinct clusters corresponding to west and east parts of the country. Wild accessions are well distinguished from the cultivated compartments and no cases of parent-offspring relationship among wild and cultivated accessions has revealed. Almost none of the most important cultivated grapes seem to have a direct relationship with Georgian material. Management of this germplasm is always important. Due to significance of this germplasm for local viticulture and winemaking activities for cultivation should be stimulated.

ACKNOWLEDGMENTS

This multidisciplinary investigation was supported and realised by: Institute of Horticulture, Viticulture and Oenology (Georgia), Department of Crop Science of the University of Milan (Italy), INRA-Montpellier (France); ERSA agricola Gorizia (Italy); the project "Conservation and sustainable use of grapevine genetic resources in the Caucasus and Northern Black Sea region" with financial support of the government of Luxembourg and realised by the Bioversity International (Maghradze et al. 2009b), "ECO-Net project" with financial support of the EJIDE, France; EU "GrapeGen06 – management and conservation of grapevine genetic resources" project (Bacilieri, 2008), the World Bank Project "Agricultural Research-Extension and Training".

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International Importance of Georgian Vine Genetic Resources

Vazha Gotsiridze[(1)], Georgian Institute of Horticulture, Viticulture and Oenology (IHVO), 6 Marshal Gelovani Avenue, 0159, Tbilisi, Georgia. vajagotsiridze@yahoo.com

Merab Ardzenadze[(2)], Research Institute of Agrarian Technologies of Shota Rustaveli Satet University. 35 Ninoshvili Str. Batumi Town, Georgia. vajagotsiridze@yahoo.com

RReezo Jabnidze[(3)] Research Institute of Agrarian Technologies of Shota Rustaveli Satet University. 35 Ninoshvili Str. Batumi Town, Georgia. vajagotsiridze@yahoo.com

ABSTRACT

The present paper reviews aboriginal Georgian vine varieties and their selection and economic potential and recommends the testing of certain varieties in some vine growing regions of the world.

Dans un article il a exprime le variété des espèces aborigènes de la vigne géorgienne et le potentiel d' utiliser selection et économique. Le recomendation donne un examen de ces races dans le different région mondiale de la vinge.

INTRODUCTION

The Caucasus and the Black Sea basin are known as the genetic centre of Eurasian cultured vine varieties. The scientists found two hearths (centres of origin) of vine species in Georgia. These centres are in Kolkheti and Alazani (Negrul A. "The Soviet Union Ampelography", 1964, Vol.1).

Kolkheti hearth covered not only the western part of Georgia, but also the southern coast of the Black Sea and Socha-Tuapse region (which is now part of the Russian Federation), inhabited with people of Iberian-Caucasian race.

The traces of kvevri (giant clay vessels for wine) with the remains of aboriginal vine varieties have been found in Ajara, Guria, Imereti, Abkhazia, Samegrelo and Racha-Lechkhumi regions of West Georgia and Kartli, Kakheti, Samtskhe-Javakheti and Tao-Klarjeti regions of East Georgia.

At present the numbers of cultured vine varieties by microcenters of their origin are as follows: Ajara- 48, Guria – 58, Samegrelo – 48, Racha-Lechkhumi – 66, Imereti – 84, Abkhazia – 51, Kartli – 68, Kakheti – 89. Meskhet-Jvakheti – 18, Saingilo – 5.

Historic and literary sources mention a total of 525 vine varieties in Georgia. The USSR Ampeleography gives short and long ampelographic description of 414 varieties (Ampeleography of the USSR, 1946-1965, Moscow, 10 Volumes).

The regions of Meskhet-Javakheti, Tao-Klarjeti, Saingilo and Abkhazia are comparatively less studied. With the support of international organizations scientific conditions can be sent to these regions to collect and study the cultured and wild vine varieties. This research should be conducted in the nearest five years to avoid the loss of genetic resources.

The ampelographic study of Georgian vine varieties show that they have all the properties of Vitis Vinifera (Common Grape Vine), namely leaves are deeply, medium or lightly veined, heavily or medium pubescent or glabrous; grape clusters are medium or large; grape berries are small, medium or large; grape colors are black, red or white. In West Georgia late or very late ripening varieties are dominant and in East Georgia –medium and late ripening varieties.

In Georgia most of the industrial vines, i.e. 66-98% are wine grape varieties and 33-7% are table grape varieties. As it is shown in Table No1 the divergence range of the Georgian vine varieties by microcenters of their origin is similar to that of Vitis Vinifera, which is spread throughout the world. As a result of the disease resistance study the vine varieties with high, medium and low resistance to mildew and anthracnose were determined. The disease resistant vine varieties grow in microcenters where the total quantity of precipitation during the vegetation period is relatively high. These microcenters listed in descending order are as follows: Ajara, Guria, Samegrelo, Abkhazia, Imereti, Racha-Lechkhumi, Kakheti and Kartli. It should also be noted that there are some rot resistant vine varieties in humid regions of Georgia, namely Chkhaveri, Ojaleshi, Kachichi, Aladasturi, Chvitiluri, etc.

The technological potential of Georgian vine varieties in the agroecological conditions of Georgia allows production of all types of wine and grapes which are currently produced worldwide.

The Georgian vine varieties Rkatsiteli, Mtsvane, Khikhvikisi, Tsolikauri, Tsitska, Tsulukidzis Tetra, Krakhuna are used for white wine production and Saperavi, Kabistoni Shavi (black), Shavkapreti, Asuruli Shavi, Ojaleshi, Chkhaveri, Kchichi, Atvizhi, Aleksandrouli, Mujuretuli and Tavkveri are used for red wine production. Saperavi and Rkatsiteli are the main vine varieties in the Caucasus , Eastern Europe (Moldova, Ukraine, Romania, Bulgaria) and Russia.

The Georgian vine varieties are used worldwide as initial varieties for new hybrids. Tens of new varieties have been derived from the Georgian varieties of Saperavi, Rkatsiteli, Tavkveri and Mtsvane. However, the genetic resources of Georgian vine are not fully utilized. This is especially true for the following varieties growing in subtropical zone:

1) Chkhaveri has red grapes, a vegetation period of more than 220 days and the total active temperature is 4000^0C. This variety has high sugar accumulation capacity (20-25%) together with high acid level. Several kinds of wine are made from Chkhaveri grapes, namely white, red, dry, natural semisweet and sparkling wines;

2) Ojaleshi variety needs the same ecological conditions as Chkhaveri. Ojaleshi is used for production of dark, dry and naturally semisweet tables wines. Ojaleshi wine produced by Prince Achille Murat in Martvili District (Samegrelo Region) won the gold medal at Paris Wine Exhibition.

We believe that introduction and large-scale tests of the aforementioned Georgian varieties should be conducted in the vine growing regions of the American Continents, China, Australia and New Zealand.

High quality white wines are produced from the Georgian vine varieties of Mtsvane, Khikhvi, Kisi Tsitska and Tsolikauri. The vegetation period of these varieties is 180-200 days and the total active temperature is $3000\text{-}3500^0$C. Testing of the aforementioned varieties in various ecological environments will enrich the industrial vine assortment of vine growing countries.

Table No 1. Botanical and Economic Characteristics of the Georgian Vine Varieties by Microcenters of Their Origin

Microcenters of Kolkheti Center	Leaf Veins			Leaf Pubescence			Grape Cluster Size			Grape Berry Size			Grape Berry Shape			Grape Berry Colour			Ripening Period			Purpose		
	Lightly veined	Medium veined	Deeply veined	Glabrous	Medium pubescent	Heavily pubescent	Small	Medium	Large	Small	Medium	Large	Round	Round oval	Oval	Black	Red	White	Middle	Late	Very late	Wine	Wine Table	Table
Abkhazia	-	98	-	-	44.4	42.2	6.7	75.6	17.8	-	84.4	16.4	64.4	20.0	25	66.7	15.6	17.8	-	11.2	88	82	17	2
Ajara-Guria	96	-	-	-	-	74	-	66.0	34	-	89	-	20	80	-	43.5	19	30	-	3	97	76	20	4
Imereti	47.7	41.5	-	9.2	16.9	73.8	21.5	61.5	16.9	11.5	89.2	-	69.2	18.5	12.5	50.8	9.2	36.5	24.1	73.8	-	87.7	7.7	5
Racha-Lechkhumi	53.7	40	7.4	7.4	37.0	55.6	37	59.3	3.7	7.4	90.7	2.0	-	96	3.7	30.0	35.2	35.2	75.9	24.0	-	98	2.0	-
Kartli	24.6	55.6	20	35.4	37	30	3.0	66.2	31	7.7	71	20	52.3	2.0	26	28	13	60	63	35	-	60	34	6
Kakheti	30	47	22	14.5	49	36	14.0	57.0	29	-	84	16	44	24	32	27	13	59	92	79	-	67	19	13

In view of the above we can make the following conclusions:

1. Georgian vine varieties are characterized with botanical and economic-technological polymorphism;
2. Some Georgian varieties like Saperavi, Rkatsiteli, Mtsvane are the main varieties in vine growing regions of Eastern Europe, Caucasus and Russian Federation;
3. Georgian vine varieties are widely used for creation of hybrids;
4. Despite the aforementioned the genetic and economic potential of Georgian vine varieties is not fully utilized. Therefore we recommend testing of the Georgian vine varieties which have high plasticity and great technological potential in America, Southern Europe, China, Australia and New Zealand. These varieties are Saperavi, Chkhaveri, Ojaleshi, Kachichi, Usakhelouri, Mujuretuli (red varieties) and Rkatsiteli, Mtsvane, Khikhvi, Kisi, Tsolikauri, Tsitska, Krakhuna (white);
5. The objective of genetic research of Georgian vine varieties is to identify the possible relations between the genetic center of the Kolkheti ecological-geographical group varieties and the vine varieties of other groups. Italian, French, American and Georgian scientists are exploring this issue.

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Marker assisted selection (MAS) as a new tool for developing high quality cultivars with sustainable resistance

Eibach, R., Hausmann, L. and Töpfer, R.

Federal Research Centre for Cultivated Plants – Julius Kuehn-Institute
Institute for Grapevine Breeding Geilweilerhof
D-76833 Siebeldingen
E-Mail: rudolf.eibach@jki.bund.de

Abstract English

Since the introduction of phylloxera and the mildew diseases from North America to Europe in the second part of the 19th century, grape breeders around the world are engaged to introduce resistance characteristics existing in wild American and Asian species into the gene pool of the European susceptible quality cultivars. Meanwhile these activities led to remarkable success resulting in cultivars with a high wine quality and a high degree of resistance against Downy Mildew as well as Powdery Mildew, allowing a considerable reduction of plant protection measurements.

In recent years substantial progress has been achieved in understanding the genetics of important traits including mildew resistance. Various research groups generated genetic maps with different genetic backgrounds which allowed the identification of different loci carrying resistance genes. The use of marker assisted selection (MAS) allows not only to monitor the segregation pattern of these resistance loci in the progeny but also identifying the genotypes with multiple resistance loci. This pyramiding by MAS should lead to an increased sustainability of the resistances. Moreover, MAS allows an accelerated introgression of desired traits from wild species into the gene pool of *Vitis vinifera*. As an example this procedure is demonstrated for the resistance against phylloxera on roots which is found within genotypes of *Vitis cinerea*.

The newly available breeding strategies will mark the paradigm shift from empirical to knowledge based breeding.

Abstract German

Seit Einschleppung der Reblaus und der Mehltaukrankheiten von Nordamerika nach Europa in der zweiten Hälfte des 19. Jahrhunderts bemühen sich Rebenzüchter in vielen Ländern, Resistenzeigenschaften aus amerikanischen und asiatischen Wildarten in den Genpool der europäischen qualitätsbetonten Rebsorten einzukreuzen. Zwischenzeitlich wurden auf diesem Weg beachtliche Erfolge erzielt und es konnten neue Qualitätsrebsorten entwickelt werden, die eine erhebliche Reduktion des Pflanzenschutzaufwandes ermöglichen.

In jüngerer Zeit gab es beträchtliche Fortschritte auf dem Gebiet der genetischen Identifizierung und Vererbung wichtiger Eigenschaften der Weinrebe einschließlich der Mehltauresistenzen. Verschiedene Forschergruppen entwickelten genetische Karten in verschiedenen genetischen Hintergründen und es wurden mehrere Genorte mit Resistenzmerkmalen identifiziert. Der Einsatz der markergestützten Selektion (MAS) ermöglicht nicht nur die Verfolgung der Vererbung von Genorten mit Resistenzeigenschaften, sondern auch die Identifizierung von Genotypen, die mehrere Resistenz-Genorte kombiniert aufweisen. Es ist zu erwarten, dass diese Genotypen mit pyramidisierten Resistenzeigenschaften eine stabilere Resistenz aufweisen. Darüber hinaus erlaubt die MAS eine beschleunigte Einkreuzung interessanter Merkmale von Wildarten in den Genpool von *Vitis vinifera*. Beispielhaft ist dies für die Resis-

tenz gegenüber der Reblaus demonstriert, die in dem genetischen Hintergrund von *Vitis cinerea* zu finden ist.
Es wird aufgezeigt, dass sich durch die neu verfügbaren Zuchtmethoden ein Paradigmen-Wechsel von der empirischen zur wissensbasierten Züchtung vollzieht.

Introduction

Since the introduction of phylloxera and the mildew diseases from North America to Europe in the second part of the 19th century, grape breeders around the world are engaged to introduce resistance characteristics existing in wild American and Asian species into the gene pool of the European susceptible quality cultivars. Meanwhile these activities led to remarkable success resulting in cultivars with a high wine quality and a high degree of resistance against Downy Mildew as well as Powdery Mildew. In several countries like Hungary, Austria or Germany new cultivars derived from these breeding programs are registered and their cultivation allows a considerable reduction of fungicide plant protection measurements. Nevertheless there is still a strong interest to improve the degree of resistance as well as to assure the sustainability of the resistance on a long term view.
Tremendous progress in understanding the genetics of important traits including mildew resistance in recent years offers new promising perspectives in achieving this goal. Various research groups generated genetic maps with different genetic background which allowed the identification of different loci carrying resistance genes (Fischer et al. 2004, Doligez et al. 2006, Di Gaspero et al. 2007, Welter et al. 2007, Riaz et al. 2008, Vezzulli et al. 2008, Bellin et al. 2009). A range of molecular markers which are tightly linked to these loci could be identified and by the application of marker assisted selection (MAS) the segregation pattern of these resistance loci in the offspring of a targeted crossing combination can be monitored (Dalbo et al. 2001, Riaz et al. 2006, Akkurt et al. 2007, Eibach et al. 2007, Kim et al. 2008). Moreover within offspring of crossing combinations with different resistance loci in the female and the male parent those individuals carrying the resistance loci of both parents can be identified by the application of MAS. It can be expected that this kind of pyramiding resistance loci should lead to an increased sustainability of the resistances.
The introgression of new additional resistance sources from wild species into the gene pool of *Vitis vinifera* is of vital interest for resistance breeding but it is extremely time consuming and by utilizing traditional breeding techniques it lasts several decades. The application of MAS offers new strategies to shorten this procedure distinctly. The procedure is described by the example of the introgression of the resistance against phylloxera on roots from a selection of *Vitis cinerea* into the gene pool of *Vitis vinifera*.

Material and Methods

Based on a seedling population out of the cross Gf.V3125 *x 'Boerner'* Zhang et al. (2009) identified a quantitative trait locus (QTL) on linkage group 13 for phylloxera resistance on roots (Rpv1). Gf.V3125 exhibits no resistance against phylloxera on roots and is a cross between *'Schiava Grossa' x 'Riesling'*. 'Boerner' is a cross between selections of *Vitis cinerea x Vitis riparia*. It shows resistance against phylloxera on roots which was inherited by *Vitis cinerea*. Three selections (Gf.1998-42-9, Gf.1998-42-55, Gf.1998-42-109) out of the progeny *Gf.V3125 x 'Boerner'* which showed phylloxera resistance were used for a pseudo backcross (pBC) programme. They were used as male parents in a cross with 'Pinot blanc' to create a pBC1-generation. In total 644 seedlings derived from these three crosses. DNA of these seedlings was isolated from young healthy leaves using DNeasy Plant Mini Kit (Quiagen, Hilden, Germany). PCR for SSR loci were performed in standard reactions of 10 µl. Primer pairs were labelled with ABI fluorescent dyes and analyzed by capillary electrophoresis on an ABI 3100 Genetic Analyzer. Marker assisted selection for the target locus was carried out with SSR-markers tightly flanking the resistance locus on both sides. In a second step all seedlings exhibiting the resistance related alleles were screened with a set of SSR-markers equally distributed across the whole genome in order to identify those genotypes with a surpassing percentage alleles inherited from the *Vitis vinifera* parent '*Pinot blanc'*.

Results and Discussion

For checking the Rpv1-locus two SSR-markers above and two SSR-markers below this locus were used for screening. Results in Table 1 indicate that 116 seedlings showing the marker profile given in column 1 carry the resistance correlated alleles. Thus, these seedlings conduct the entire Rpv1-locus. The group of 17 seedlings designated in the next column carry also the markers directly flanking the Rpv1-locus for phylloxera resistance. The absence of the resistance correlated allele on the marker downstream (Gf.13-11 at 69,5 cM) suggest that a crossover between Gf.13-09 and Gf.13-11 has occurred and therefore this region of the chromosome can be traced back to *Vitis vinifera*. Within a pseudo backcross breeding programme with the goal to keep the target locus and to accumulate as much as possible *Vitis vinifera* genome in the offspring this is a favourable situation. The situation for the 25 seedlings cited in the third column is similar. Again the Rpv1-locus flanking markers indicate the presence of the phylloxera resistance but the crossover and hence the switch to a *Vitis vinifera* sequences occurred between the markers Gf.13-01 and VMC9h4.2.

Tab.1 : Status of SSR-markers linked to phylloxera resistance in a progeny of 'Pinot blanc' crossed with three different phylloxera resistant genotypes out of the cross Gf.V.3125 x 'Boerner'

Marker	location on genetic map (cM)	absence/presence (+/-) of resistance related alleles							
		column No.							
		1	2	3	4	5	6	7	8
VMC9h4.2	47,4	+	+	-	+	-	+	-	-
Gf.13-01	59,0	+	+	+	+	-	-	-	-
Rpv1	61,8								
Gf.13-09	64,7	+	+	+	-	+	-	-	-
Gf.13-11	69,5	+	-	+	-	+	-	+	-
Total No. of seedlings		**116**	**17**	**25**	**9**	**14**	**67**	**16**	**402**

The seedlings indicated in column No 4 and 5 exhibit only one half of the Rpv1-locus. The resistance correlated alleles for both markers are found on one side of the Rpv1-locus while the resistance correlated alleles on the other side of the Rpv1-locus are missing. In these cases crossovers happened between the two directly flanking markers of the Rpv1-locus. Hence for these groups of seedlings a statement about the presence or absence of the phylloxera resistance cannot be made. Nevertheless these seedlings might be interesting for further research. Phenotyping these genotypes for phylloxera resistance could be helpful in identifying further markers even more narrow to the locus. All seedlings with the marker profile summarized in

columns 6 to 8 obviously do not carry the alleles for phylloxera resistance and will be discarded from the breeding programme.
In a second step all seedlings of the pBC1-generation containing the Rpv1- locus were subjected for further marker analysis (background selection). For estimating the portion of *Vitis vinifera* in the genome of these seedlings preferably SSR-markers evenly distributed over the whole genome were analyzed (Frisch and Melchinger 2005). Analyses included Gf.V3125 and 'Boerner', the parents of the resistant F1 donor plants. These analyses permitted the verification of whether or not the individual alleles tested are derived from 'Boerner' or from the *Vitis vinifera* genotype Gf.V3125. Fig. 1 summarizes the results based on 58 tested SSR-markers.

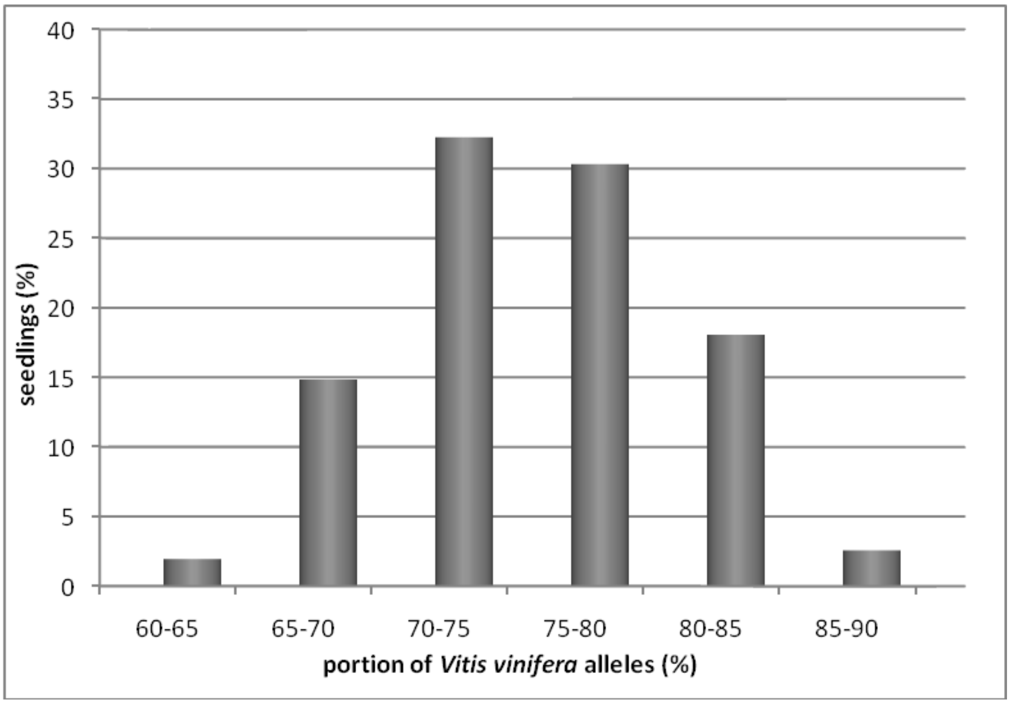

Fig. 1: Distribution of the portion of *Vitis vinifera* alleles in the pBC1-generation [(*Vitis cinera* x *Vitis riparia*) x *Vitis vinifera*] x *Vitis vinifera* based on 58 analysed SSR-markers.

Results show that there is a considerable variation of the *Vitis vinifera* proportion within the pBC1-generation ranging from 60% up to 90%. 75% of all loci which were analysed could be traced back to *Vitis vinifera* confirming the expectation that in a pBC1-generation on average three quarters of alleles should originate from the recurrent parent. Making use of the considerable deviation of the *Vitis vinifera* proportion genotypes with the highest percentage of *Vitis vinifera* genome were selected as parents for the next pseudo backcross generation (pBC2). Selecting for a target locus and performing background selection accelerate process considerably. Using this strategy in each crossing step one can be expect to select breeding lines with the target locus and a *Vitis vinifera* proportion ahead of 98% already in the pBC3-generation. Compared to a pseudo backcross programme with traditional selection techniques the use of MAS saves about two generations. MAS combined with an optimized training system for the seedlings selected for further crossing will lead to a seed to seed cycle of three or even two years. Thus the application of MAS allows the introduction of desired traits from wild species into the *Vitis vinifera* gene pool within a period of around 15 years making marker assisted backcross breeding (MABCB) to a valuable tool for opening up genetic resources.

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New Perspective GeorgianXAmerican Phylloxera Resistant Hybrid Rootstocks of Vine

Otar Kurtiashvili

Senior scientific worker of Telavi Testing Station of the Scientific-Research Institute of Horticulture, Viticulture and Oenology of the Scientific Academy of Agriculture of Georgia
JSC Telavi Wine Celler
The Head of Viticulture Department
V. Kurdgelauri, 2200, Telavi, Georgia
tewincel@tewincel.com

Abstract(*English*)

In Telavi GeorgianXAmerican Phylloxera Resistant Rootstocks were bread, which are in close relation with Georgian races genetically and better adapted with ecologic conditions of Georgia: *RkatsiteliXRiparia Gluar No 14, RkatsiteliX(BerlandieriXRiparia 420^a) No 19 and Shavi KharistvalaX(BerlandieriXRiparia 420^a) No 32.* At the same time clone *BerlandieriXRiparia Teleki 8^b Vazisubani,* and from the introduced rootstocks: *BerlandieriXRiparia Teleki 8^b biotype 9 and BerlandieriXRiparia Krechuneli 2.* All standard rootstocks of Georgia were studied in different ecologic conditions with these rootstocks, namely: *BerlandieriXRiparia Koberi 5^{bb}, 420^a, ShaslaXBerlandieri 41^b, RipariaXRupestris 101-14, 3306, 3309 and Rupestris Dulot.*

New GeorgianXAmerican Phylloxera Resistant Rootstocks with main agrobiologic and economic indicators are surpassed all above mentioned standard rootstocks except of *Koberi 5^{bb}*, and they are even more than *Koberi 5^{bb}* on the carbonated soils. Thus, they develop better than standard rootstocks in all ecologic conditions.

Abstrakt(*German*)

In Versuchsstation Telavi wurden die zu den Georgischen Sorten genetisch nahestehenden und den Ökologieverhältnissen besser angepassten Georgisch×Amerikanischen Phylloxeraresistant Unterlagen der Rebe: Rkatsiteli×Riparia Gluar Nr. 14, Rkatsiteli × (Berlandieri×Riparia 420a) Nr.19 und Shavi Kharistvala×(Berlandieri×Riparia 420a) Nr. 32. Gleichlaufend wurden auch den Klon von Berlandieri×Riparia Teleki 8b Wasisubani, Berlandieri×Riparia Teleki 8b Biotyp 9 und Berlandieri×Riparia Krechuneli 2 gezüchtet.

Neben den obengenannten Unterlagen in den verschiedenen Ökologieverhältnissen wurden alle Standardunterlagen Georgiens: Berlandieri×Riparia Koberi 5bb, 420a, Shasla×Berlandieri 41b, Riparia×Rupestris 101-14, 3306, 3309 und Rupestris Dulo untersucht.

Die neuen Georgisch×Amerikanischen und Phylloxeraresistant Unterlagen der Rebe mit den Hauptagrobiologischen und wirtschaftlichen Hauptkenndaten treffen an allen Standardunterlagen außer Koberi 5bb über und in Karbonatboden treffen sie auch Berlandieri×Riparia Koberi 5bb über. Deshalb entwickeln sie sich in allen Ökologieverhältnissen besser als Standardunterlagen.

Introduction

Phylloxera was found in Georgia in 1881 in the first time and for 1927 it was spread all over Georgia. Trans-Caucasian Phylloxera Committee created nursery garden of American rootstock in West Georgia (town Zestaponi) as far as in 1891, where 63 types of races and rootstock hybrids were brought for future studying. On the base of French experience they have spread: *RipariaXRupestri 101-14, 3306, 3309, BerlandieriXRiparia 420*[a], *ShaslaXBerlandieri 41*[b], *SoloniXRiparia 1616, MurvedriXRupestris 1202 and Rupestris Dulot.*

In 1936 from assortment of above mentioned rootstocks *SoloniXRiparia 1616* and *MurvedriXRupestris 1202* were withdrawn, instead there were brought BerlandieriXRiparia Koberi 5[bb], because of its positive characters. It dominated in a short time and nowadays Georgian viticulture is almost on one rootstock, which in no way corresponds to diverse ecological conditions of Georgia and scale of varieties of engrafted vine.

Nowadays vine rootstocks are required more demands, namely: skills of good adaptation and affinity. Vital capacity, grapes productivity, product quality, chlorosis resistance, drought-resistance, freeze-resistance, salt-resistance, nematode resistance and other are depend on them. That's why, in our opinion, "Phylloxera Resistant Rootstock" is already the narrow sense of the notion and it should be recognized generally as rootstock of vine.

Material and methods

After Georgian viticulturists studied the standard rootstocks in the production conditions, they came to the conclusion that it was necessary to work out new kind of rootstocks being in close relation with Georgian lines genetically and adapted with ecological conditions of Georgia.

With this purpose in the Department of Ampelography and Selection of Scientific-Research Institute of Viticulture and Oenology of Georgia from 1932 special inter races hybridization was conducted, and in result of this since 1950 began studying of received seed rootstock.

Experimental plots were cultivated in different ecologic zones 0.4-0.4 hectare area. Rootstocks were disposed by the method of pair, repeated three times, 25 vines in a variant, area of nutrition 2X2 meters. Form is head. Support – 4 small wire high wall-papers.

Scientific-research work was passing by the general famous ampelographic methods (Lazarevski, 1946, Barskaia, 1967, Ermakov, Apasimovich 1952, Bajanova, Maslova, Popova, 1964).

Observations on leaf phylloxera and chlorosis resistance were carried out by the scheme of 5 scales. Skills of making seeds of shoots of rootstocks are studied, seed distribution according to the horizons in soil. Also indicators of productivity of some Georgian races of vine inoculated on rootstocks.

Results and discussions

Perspective GeorgianXAmerican and detected rootstocks: *RkatsiteliXRiparia Gluar No14,RkatsiteliX(BerlandieriXRiparia420^{a})No19, Shavi KharistvalaX(BerlandieriXRiparia 420^{a}) No 32, BerlandieriXRiparia Teleki 8^{b} Vazisubani, BerlandieriXRiparia Teleki 8^{b} biotype 9, BerlandieriXRiparia Krechuneli 2* are studied in different ecologic conditions. Results are brought in Tab. 1. From Tab. 1 is clear, that new and detected rootstocks taken from *Koberi 5^{bb}* for control are importantly surpassed with many agricultural indicators as on strong carbonated soils, as on soils without carbonate. Also distribution of roots in soil according to the horizons, their architectonic and skills of making roots of shoot rootstocks are given in the Tab. 2, 3 and 4.

Georgian race of vine "cv" *RkatsiteliXRiparia Gluar No 14*. Rootstock is strong growing, sprout is right standing, by what it is distinguished from other rootstocks. Develops a small amount of additional sprouts what makes easy to carry out green operation. Shoots ripen entirely in the first decade of October. It is characterized with practical phylloxera resistance. It damages little by leaf phylloxera in the conditions of overlime (1.83 scales). It has high skills of lignifications of shoots (5 scales). It makes seeds well (84.4%). During inoculation of Georgian races of vine fitting of first-class engrafted rootstocks are in the bounds of 40-50%. In grapes productivity during inoculation of *Rkatsiteli* 284 grams were exceeded on a bottom comparison with *Koberi 5^{bb}*, *Saperavi* with 1100 grams, and *Khikhvi* – with 460 grams.

It grows as on soils without carbonate, as on carbonated soils (20-30% general carbonates). It adapts hard on clay soils and in droughty conditions difficulty. With standard shoot productivity on the soils with overlime was exceeded with 6.925 meters to Koberi 5^{bb} on a hectare.

Georgian race of vine "cv" *RkatsiteliX(BerlandieriXRiparia 420^{a}) No 19*. Rootstock is strong growing, sprout is right standing, develops a small amount of additional sprouts weakly. Shoots ripen entirely in the first decade of October. Lignifications of shoots are estimated with 4 scales. It is characterized with practical phylloxera resistance. It damages little by leaf phylloxera (1.15 scales). Skills of making seeds are 85%. During inoculation of Georgian races of vine fitting of first-class engrafted rootstocks are in the bounds of 47-65% (Goruli green, Chinese, Gorula). By inoculation of *Rkatsiteli* on this rootstock productivity was exceeded with 94 grams on a bottom comparison with *Koberi 5^{bb}*, *Saperavi* with 1170 grams, and *Khikhvi* – with 390 grams.

It grows almost on all type of soils, especially on soils with overlime (50-60% general carbonates). It adapts difficulty in droughty conditions. With standard shoot productivity on the soils with overlime was exceeded with 12.600 meters to Koberi 5^{bb} on a hectare.

Georgian race of vine "cv" *Shavi KharistvalaX(BerlandieriXRiparia 420^{a}) No 32*. Rootstock is strong growing, develops a small amount of additional sprouts weakly. Shoots ripen entirely in the first decade of October. Lignifications of shoots are 5 scales. It is practical phylloxera resistance. It damages little by leaf phylloxera (1.69 scales). Skills of making seeds are well (88.3%). During inoculation of Georgian races of vine fitting of first-class engrafted rootstocks are in the bounds of 57-66%. By inoculation of *Rkatsiteli* on this rootstock productivity was exceeded with 108 grams on a bottom comparison with *Koberi 5^{bb}*, *Saperavi* with 884 grams, and *Khikhvi* – with 1430 grams.

It grows well almost on all type of soils. With standard shoot productivity on the soils with overlime was exceeded with 11.150 meters to Koberi 5^{bb} on a hectare.

BerlandieriXRiparia Teleki 8^{b} Vazisubani. It is detected in 1964. It is different from Teleki 8^{b} with the type of flower (female functionally), with some signs of leaf, color of reed and harvest. Vine is strong growing, develops a small amount of additional sprouts weakly. Shoots ripen entirely at the end of October. Lignifications of shoots are 4 scales. It is practical phylloxera resistance. It damages little by leaf phylloxera with 2.35 scales. Skills of making seeds are 84.1%. During inoculation of Georgian races of vine fitting of first-class engrafted rootstocks are in the bounds of 40-50%. It grows well almost on all type of soils. With standard shoot productivity on the soils with overlime was exceeded with 8.425 meters to *Koberi 5^{bb}* on a hectare.

BerlandieriXRiparia Teleki 8^{b} biotype 9. It is detected from the population of Teleki 8^{b} after the World War II in Rumania. It is brought with other races of 8^{b} biotypes in Georgia in 1964. Rootstock is strong growing, has a weak skill of formation of additional sprouts, what makes easy to carry out green operations. Shoots ripen entirely in the second decade of October. Lignifications of shoots are estimated with 5 scales. Skills of making seeds are 90.6%. It is practical phylloxera resistance. It damages little by leaf phylloxera with 2.78 scales. During inoculation of Georgian races of vine fitting of first-class engrafted rootstocks are 40-50%.

It grows almost on all type of soils, especially on soils which are rich with carbonate (to 50-60% general carbonates). With standard shoot productivity on the soils with overlime was exceeded with 15.700 meters to *Koberi 5^{bb}* on a hectare.

BerlandieriXRiparia Krechuneli 2. It is detected from Koberi 5^{bb} in Rumania. It is brought in Georgia in 1964. Rootstock is strong growing, has a weak skill of formation of additional sprouts, what makes easy to carry out green operations. Shoots ripen entirely at the end of October. Lignifications of shoots are estimated with 5 scales. It is practical phylloxera resistance. It damages little by leaf phylloxera with 2.47 scales. Skills of making seeds are high 91.7%.

It grows almost on all type of soils. With standard shoot productivity on the soils with overlime was exceeded with 15.175 meters to *Koberi 5^{bb}* on a hectare.

Results of experiment of rootstocks in different ecologic conditions

Table #1

#	Name of rootstocks	On strong carbonated soils									On soils without carbonate				
		Intensive of chlorose disease with scales	Intensive of damage of leaf phylloxera with scales	Reed productivity on the bottom with meters	Reed productivity on a hectare	Dry substance in a shoot with %	Quality of shoot lignifications with scales	Easy saccharine in 100 gram dry substance	Difficult saccharine in 100 gram dry substance	Chlorophines in leaves with mg	Intensive of damage of leaf phylloxera with scales	Reed productivity on the bottom with meters	Reed productivity on a hectare	Content of Tree Heart with %	Dry substance in a shoot with %
1	BerlandieriXRiparia 5^{bb} (control)	1.40	2.96	6.28	15.700	55.91	4	1.8	2.1	1.30	1.30	13.90	34.750	15.2	60.20
2	BerlandieriXRiparia 420^{a}	0.52	1.98	5.75	14.375	59.89	4	3.8	8.0	17.30	1.20	10.30	25.750	15.0	59.10
3	ShaslaXBerlandieri 41^{b}	0.90	2.10	7.30	18.250	53.60	4	2.1	2.5	17.2	1.00	6.90	17.250	16.6	54.90
4	RipariaXRupestris 101-14	1.20	2.30	3.70	9.250	56.00	4	1.9	2.3	14.6	2.20	8.00	20.000	21.5	57.70
5	RipariaXRupestris 3306	1.30	2.45	2.00	5.000	55.00	4	2.0	2.6	15.3	1.80	6.50	16.250	24.1	54.5
6	RipariaXRupestris 3309	1.30	2.40	1.50	3.750	56.10	4	2.2	2.8	16.1	1.70	6.60	16.500	13.6	59.70
7	Rupestris Dulot	0.70	2.15	8.10	20.250	57.9	4	1.7	2.0	16.0	0.20	8.10	20.250	17.5	58.10
8	BerlandieriXRiparia Krechuneli 2	1.10	2.47	12.35	30.875	55.97	5	1.8	3.4	13.0	1.10	18.30	45.750	25.6	57.60
9	RkatsiteliXRiparia Gluar #14	1.83	1.83	9.05	22.625	55.88	5	2.4	2.2	12.4	1.40	10.80	27.000	22.8	56.30
10	RkatsiteliX(BerlandieriXRiparia 420^{a}) #19	0.92	1.15	11.32	28.300	53.20	4	2.2	4	18.8	1.60	12.40	31.000	22.4	56.20
11	Shavi kharistvalaX(BerXRip 420^{a}) #32	2.20	1.69	10.74	26.850	61.98	5	2.8	4.2	17.3	1.70	13.80	34.500	21.8	55.50
12	BerlandieriXRiparia Teleki 8^{b} Vazisubani	0.79	2.35	9.65	24.125	46.30	4	2.2	6	15.3	1.40	12.20	30.500	23.2	57.00
13	BerlandieriXRiparia Teleki 8^{b} biotype 9	2.10	2.78	12.80	32.000	57.10	5	2.1	3.5	15.6	1.60	14.10	35.250	22.1	59.10

Distribution of phylloxera resistance rootstocks of vine according to the horizons in soil

Table 2

#	Name of rootstocks	Location	0.20 cm.	20-40 cm.	40-60 cm.	60-80 cm.	80-100 cm	100-120 cm	120-140 cm	140-160 cm.	Number of roots
1	BerlandieriXRiparia Koberi 5[bb](control)	overlime	2.98	31.4	23.70	16.72	11.09	10.21	3.90	0.00	739
		without carbonate	8.67	20.79	17.86	14.18	16.74	9.69	8.25	3.82	600
2	BerlandieriXRiparia 420[a]	overlime	0.00	24.59	22.85	16.66	14.70	11.72	9.48	0.00	581
		without carbonate	9.13	27.07	19.50	18.60	13.94	9.88	1.88	0.00	443
3	BerlandieriXRiparia Krechuneli 2	overlime	0.50	33.35	25.49	9.15	12.69	11.28	7.54	0.00	580
		without carbonate	11.36	23.09	19.63	14.52	16.49	10.71	4.20	0.00	421
4	RkatsiteliXRiparia Gluar #14	overlime	1.50	30.02	24.90	13.07	12.25	10.06	6.24	1.96	777
		without carbonate	11.0	19.23	20.47	13.61	17.30	11.03	5.84	1.46	771
5	RkatsiteliX(Ber.XRip.420[a]) #19	overlime	0.96	31.45	27.34	15.90	12.70	8.67	2.98	0.00	870
		without carbonate	1.98	28.02	32.15	16.50	9.31	10.45	1.59	0.00	352
6	Shavi KharistvalaX(Ber.XRip. 420[a]) #32	overlime	1.28	27.03	23.54	17.18	13.72	12.52	4.73	0.60	293
		without carbonate	3.28	25.56	26.32	20.18	17.27	6.39	0.08	0.00	585
7	BerlandieriXRiparia Teleki 8[b] Vazisubani	overlime	2.14	22.24	18.92	17.41	16.21	13.06	10.02	0.00	654
		without carbonate	13.83	20.89	19.90	20.18	11.31	11.93	1.96	0.00	373
8	BerlandieriXRiparia Teleki 8[b] biotype 9	overlime	0.81	22.77	25.84	15.33	12.28	12.20	3.48	0.00	669
		without carbonate	12.43	25.69	18.47	18.23	17.80	9.81	4.24	0.00	427

Architectonic of root system of rootstocks

Table 3

#	Name of rootstocks	Location	Number of roots	1 mm	1-3 mm	3-5 mm	5-10 mm	10 mm
1	BerlandieriXRiparia 5^{bb} (control)	overlime	739	65.53	25.03	5.33	2.76	1.35
		without carbonate	600	71.20	18.70	7.22	1.39	1.49
2	BerlandieriXRiparia 420^{a}	overlime	581	66.42	26.20	4.99	0.95	1.44
		without carbonate	443	45.30	31.45	16.76	4.19	2.30
3	BerlandieriXRiparia Krechuneli 2	overlime	580	59.46	30.59	8.42	1.22	0.31
		without carbonate	421	59.09	28.87	8.18	1.93	1.93
4	RkatsiteliXRiparia Gluar #14	overlime	777	67.83	24.38	5.17	1.96	0.66
		without carbonate	771	72.88	20.63	4.17	1.49	0.83
5	RkatsiteliX(BerlandieriXRiparia 420^{a}) #19	overlime	870	74.21	20.00	3.85	1.62	0.32
		without carbonate	352	51.22	56.49	8.20	3.89	0.20
6	Shavi kharistvalaX(BerXRip 420^{a}) #32	overlime	293	63.03	26.79	8.00	1.33	0.85
		without carbonate	585	56.88	34.68	5.90	1.68	0.86
7	BerlandieriXRiparia Teleki 8^{b} Vazisubani	overlime	654	71.81	20.25	4.98	1.54	1.42
		without carbonate	373	69.83	18.46	8.68	2.05	0.98
8	BerlandieriXRiparia Teleki 8^{b} biotype 9	overlime	700.3	68.41	28.33	11.25	2.00	0.87
		without carbonate	696.2	70.38	24.63	9.81	1.85	0.91

Skills of making seed of shoots of perspective rootstocks

Table 4

#	Name of rootstocks	Seed making with %	productivity of first-class rootstocks with %	weight of sprout with grams	number of main roots on bottom
1	BerlandieriXRiparia 5^{bb} (control)	91.2	64.6	42.1	9.1
2	BerlandieriXRiparia 420^{a}	31.5	64.5	41.2	9
3	RkatsiteliXRiparia Gluar #14	84.4	70.7	46.6	9.4
4	RkatsiteliX(BerlandieriXRiparia 420^{a}) #19	85	71.5	44.7	9.5
5	Shavi kharistvalaX(BerXRip 420^{a}) #32	88.3	75	46.3	9.5
6	BerlandieriXRiparia Teleki 8^{b} Vazisubani	84.1	72.3	42.7	9.8
7	BerlandieriXRiparia Teleki 8^{b} biotype 9	90.6	73.8	45.1	9.3
8	BerlandieriXRiparia Krechuneli 2	91.7	74.1	48.3	9.5

Conclusion

It is clear from above represented materials that new detected GeorgianXAmerican and detected rootstock hybrids studied by us: *RkatsiteliXRiparia Gluar No 14, RkatsiteliX(BerlandieriXRiparia 420^{a}) No 19 and Shavi KharistvalaX(BerlandieriXRiparia 420^{a}) No 32*, *BerlandieriXRiparia Teleki 8^{b} Vazisubani*, *BerlandieriXRiparia Teleki 8^{b} biotype 9* and *BerlandieriXRiparia Krechuneli 2* are exceeded with many characters to the best standard rootstocks, namely: *BerlandieriXRiparia Koberi 5^{bb}* and *BerlandieriXRiparia 420^{a}*. Above listed rootstocks are carried in standard assortment of vine of Georgia. They gave us opportunity to increase reed productivity in phylloxera resistance rootstock, in vine rootstock – productivity of first class engraft rootstock, in vineyards – productivity and quality of grapes, should be assimilated soils with overlime, what will transform them as a big reserve of rich productivity of their grapes and making wines of high quality.

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QUALITY AND YIELD PERFORMANCE OF POPULAR TURKISH WINE VARITIES (KALECİK KARASI AND NARİNCE) IN LAKE REGION ECOLOGICAL CONDITIONS

Seçkin GARGIN[1], Alim GÖKTAŞ[1]
Prof. Dr. Ahmet ALTINDİŞLİ[2]
[1]Eğirdir Horticultural Research Institute Eğirdir/Isparta, TURKEY
secking32@hotmail.com, alimg32@hotmail.com
[2]Aegean University, Faculty of Agriculture, Department of Horticulture, İzmir, TURKEY
ahmet.altindisli@ege.edu.tr

ABSTRACT

Turkey possesses a rich variety of grape types; around 1250 varieties are grown. Although Turkey has traditionally specialized in the production table grapes and raisins, it also has 34 kinds of wine grapes, 22 of which are valuable native varieties.

Lakes Region has an important vineyard areas and grape production between Middle Anatolia and Mediterranean Region. Vineyards are located in Isparta and Burdur mostly. This study was carried out in Eğirdir Horticultural Research Institute farmlands. Eğirdir is a district of Isparta. Its altitude is 920 m high and it represents passing zone climate and ecological conditions.

Popular Turkish red wine variety Kalecik Karası and white wine variety Narince had been determined for phenology stages, adaptation, yield and quality properties. Main aim of the study was to evaluate these varieties adaptation facilities and increasing development in rural area.

The vineyard was established in 2005 with in row with spacing 2 m X 3 m with irrigation. Three replications by random blocks design was applied in the study.

Observations analyses were done in 2008 and 2009 years. Phenology stages, yield and quality properties data had been obtained in vineyard. Soluble solid, titratable acidity, pH, number of cluster yield and adaptation ability were determined. Buds begin to open in the third week of April and grapes are harvested in the second week of September. Maximum yield for one wine grape is 22 kg for Kalecik Karası and 12 kg for Narince variety. Average weight of one cluster is 172 g for Kalecik Karası and 409 g for Narince. Both of the two wine varieties had been evaluated adaptable to Lakes Region ecological conditions by 2 years data.

These varieties had been evaluated in a good performance for Lakes Region in Turkey. Further studies will be planned about wine production and quality for these varieties.

Key Words: Lakes Region, wine grape varieties, performance, yield, quality

L'ESPOIR INDIGENE DES VARIETES DES VINS LOCAUX (KALECIK KARASI ET NARINCE), LA PERFORMANCE DE RENDEMENT ET DE QUALITE DANS LES CONDITIONS ECOLOGIQUES AU REGION DU LAC.

La Turquie possède des types d'un raisin d'une variété plus riches; Environ 1250 variétés sont développées. Bien que la Turquie se soit spécialisée traditionnellement dans les raisins et les raisins secs de table de production, elle a également 34 genres de raisins de cuve, 22 dont sont les variétés indigènes valables. La plupart des vins turcs sont faits à partir des variétés indigènes de raisin de cuve. Les producteurs turcs de vin emploient également les raisins indigènes en combinaison avec les variétés européennes des vins à la qualité de changement du nouveau goût pour le marché vitivinicole du monde.

La région de lacs appartient des secteurs de raisins important, elle se situe entre l'Anatolie centrale et la région méditerranéenne. Les vignes sont situées à la providence d'Isparta et de Burdur. Cette étude a été effectuée dans les terres cultivables horticoles d'Institue de recherches d'Egirdir. Egirdir est une ville d'Isparta. L'altitude est de 920metres. L'altitude se représente comme le climat de zone et les conditions écologiques de la région. Le vin rouge de Kalecik Karası est un vin traditionnel et national. Le vin blanc, Narince avait été déterminé aux étapes de phénologie, elle se montre la diversité du rendement d'adaptation et de qualité. Le but principal de l'étude était d'évaluer ces équipements d'adaptation de variétés et développement croissant dans le secteur rural.

Kalecik Karası est une variété anatolienne centrale de raisin de la meilleure qualité pour faire le vin rouge. Il peut être développé dans les conditions microclimatiques à la grâce de région de Kızılırmak (fleuve rouge). Les procès indigènes de variété de Kalecik Karası est très douce pour gouter, il se présente aux arômes fruités.

La variété de Narince vient de la province de Tokat développée dans le sud de plateau d'Anatolie, les montagnes près des rivages de la Mer Noire. Narince se produit des vins riches équilibrés qui ont souvent une teinte de jaune verdâtre sensible, arômes fruités. Le vin de Narince convient au vieillissement, acquièrent un bouquet complexe riche avec le temps.

La place d'essaie de la vignoble a été établie en 2005 avec une rangée d'espacement 2m X 3m. Trois répliques par conception de blocs aléatoire ont été appliquées dans l'étude.

Les observations et les analyses sont faites aux années 2008 et 2009. Le rendement et les étapes de phénologie, la particularité de qualité avaient été obtenus. Le solide soluble, l'acidité titrée, le pH, le nombre de rendement de faisceau et la capacité d'adaptation étaient déterminés. Toutes les deux variétés de vin avaient été adaptables et évalués aux états écologiques dans la région de lacs dans une période de deux ans. Les bourgeons commencent à s'ouvrir à la troisième semaine du mois avril et les raisins sont moissonnés à la deuxième semaine du mois septembre. Le rendement maximum pour un raisin de cuve est de 22 kilogrammes pour Kalecik Karası et de 12 kilogrammes pour la variété de Narince. Le poids moyen d'une grappe est 172 g pour Kalecik Karası et 409 g pour Narince.

Ces variétés avaient été évaluées dans une bonne exécution pour la région de lacs en Turquie. D'autres études seront prévues au sujet de la production vinicole et de la qualité pour ces variétés.

Mots clés : Région de lacs, variétés des raisins, exécution, rendement, qualité.

INTRODUCTION

Turkey's climate conditions are most suitable for grape production in the world. According to FAO statistics data in 2008 3.918.440 ton grapes have been produced in 482.789 ha vineyard area in Turkey (Anonymous, 2010). Turkey is in the rank 6^{th} and 4^{th} for the grape production and grape area respectively. 37% of grape production is used for drying, 30% for table grape, 30% for making grape products and 3% for making wine.

Turkey possesses a rich variety of grape types; around 1250 varieties can be found in genetic sources. Although Turkey has traditionally specialized in the production table grapes and raisins, Turkey also has 34 kinds of native and foreign wine grapes which are cultivated widespread, 22 of which are valuable native varieties. Öküzgözü, Boğazkere, Kalecik Karası, Çal Karası, Emir and Narince are local wine varieties and Cabarnet Sauvignon, Syrah, Merlot, Cinsaut, Alicante Bouschet, Carignane and Chardonnay are known foreign wine varieties cultivated in Turkey.

Turkish wine sector has accelerated since 1990. New foreign and native grape varieties and quality entered to production. Kalecik Karası and Narince varieties have been world famous varieties. Foreign and native wine varieties which are grown in Turkey are listed in table 1 (Oraman, 1996),(Gümüş, 2008).

Table 1. Some of the important grape varieties in Turkey for producing wine

	Native wine grape varieties	Foreign wine grape varieties
White wine varieties	Narince Emir Misket Sultaniye Yapıncak	Chardonnay Sauvignon Blanc Muscat Riesling Muscadella Semillon Blanc
Red wine varieties	Kalecik Karası Öküzgözü Adakarası Boğazkere Papazkarası	Cabernet Sauvignon Merlot Gamay Carignane Syrah

Turkey has a very long of vineyard cultivation and wine production. It is well known that Anatolia, the Asian part Turkey, is one of the motherland of vineyards and wine. Evidence indicates that grapes were processed into wine and named "VINO" by the Pre-Hitities who lived in Anatolia between 3000- 4000 B.C.

Turkey has diverse soils and climates allowing wine producers to cultivate several types of grapes for manufacturing different types and tastes of wine. The producers in the sector continue to increase their present wine production capacity and invest utilize modern technologies. As well as the large wine producer companies, there are almost 300 small producers located in Central Anatolia, Marmara- Thrace and the Aegean region. Total wine capacity of the sector is about 120 million liters (Karabayır, 2009).

Turkish Ministry of Agriculture Farmer Registration System reports that enterprise wine area is 1.3 ha and average wine area parcel is 0.5 ha. Insufficient Registration system, deficiency of technical knowledge and small scale vineyards are wine sectors important problems in Turkey (Karabat, 2008). Wine varieties are widely diversified and have improved in quality. The market for wine is expanding in Turkey since more varieties and better quality wines become available. Wine varieties must be evaluated in different locations of Turkey, because Turkey has different suitable climate conditions to produce wines in different tastes.

Grape growing has to adapt somehow to climate change, in order to avoid negative effects. Rising temperatures anticipate phenological phases, extend the vegetative cycle and affect the grape composition resulting in high levels of sugars, rough tannins and oxidizing enzymes and low levels of organic acids, color and some aromatic compounds, affecting the wine quality. The adaptation choices are to shift the growing areas to higher latitudes and elevations, to select suitable varieties and rootstocks, to perform a proper canopy and soil management and to handle irrigation and mineral element supply (Fregoni, 2009).

Lakes Region has an important vineyard areas and grape production between Central Anatolia and Mediterranean region. Vineyards are located in Isparta and Burdur mostly. Table grape drying grape and wine production are one together by small farmer groups (Baydar, 1998). Home made wines are in a good quality for home production. Some local grape varieties are used for wine production in Lakes Region. Foreign and native popular varieties must be evaluated to increase wine quality and quantity. Our study’s main aim was to evaluate adaptation abilities native wine varieties in a different location in Turkey than their own locations and to increase development in rural area. New wine cultivars will be cultivated in the region this means more income for farmers by evaluating native wine varieties in a different location.

Ağaoğlu et.all (1997) said that viticulture cultivation needs vegetation period of days which must be at least 160 days upper than 10°C and it needs at least 1300 hours sunshine time. Ecevit and Baydar (1998) determined 176.2- 222.3 day vegetation period for Isparta in their study, this means Lakes Region has suitable ecology for grape production.

Kalecik Karası variety is a good quality central Anatolian grape for making red wine. It can be grown under the microclimatic conditions of the region (Ankara, Kırıkkale and Cappadocia) thanks to the Kızılırmak (Red River). Its berries are in black-blue in color and in round shape. The indigenous Kalecik Karası variety produces easy to drink, smooth and fruity wines that invoke the aromas and tastes of fig, rose and strawberries. Kalecik Karası grapes are famous for their unique taste, aroma and flavor. This unique quality has been honored with many awards won in international wine competitions.

Picture 1. Kalecik Karası cv.

Picture 2. Narince cv.

Narince variety originally comes from Tokat province and grown on the Anatolia plateau south of the mountains near the Black Sea shores. Narince produces rich and balanced wines which often have a greenish yellow tint and delicate, fruity aromas. Because of their balanced acidity, these wines are suitable for aging and acquire a rich and complex bouquet over time.

Studies about these wine varieties were evaluated in different locations of Turkey. Kalecik karası variety was evaluated in Thrace region by Özen et all, in Adana Çukurova by Tangolar et all, in Konya conditions by Kara. It was aimed to evaluate these varieties in Lakes Region different than the locations were evaluated before in this research.

Özen et. all. (1996) experimented the adaptation of 23 domestic foreign wine grapes in the ecological conditions of Tekirdağ Vineculture Research Institute. In his study; yield growth, must and wine characteristics were investigated. In the result Kalecik Karası, Gamay, Cabarnet Sauvignon was determined to produce quality red vines under ecological conditions of Institute.

Tangolar et all. (2002) evaluated Kalecik Karası variety in Pozantı/Adana conditions. They noticed cluster, berry and must characteristics.

Kalecik Karası variety had been evaluated adaptable and it showed good performance in Konya conditions (Kara, 2005).

Grape varieties local adaptation facilities must be evaluated and according to result new vineyards must be planned with right varieties. Native grape varieties which are in high value for producing wine must be preserved and they must be spreaded to other locations in the result of the adaptation studies.

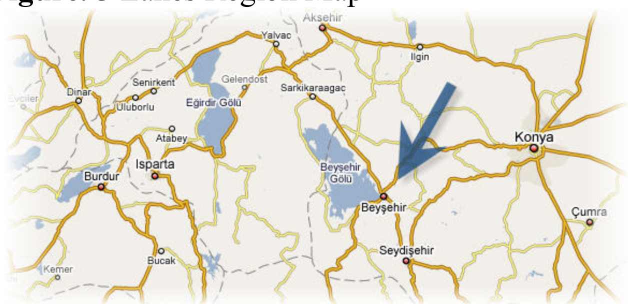
MATERIAL AND METHOD

This study was carried out in Eğirdir Horticultural Research Institute farmlands. Eğirdir is a district of Isparta in Mediterranean Region. Its altitude is 920 m high and it represents passing zone climate and ecological conditions. Geographical coordinates of Eğirdir district; are 37º 50′ 41″, 38º 16′ 55″ N latitude, 30º 57′ 43″, 30º 44′ 39″ E latitude.

Figure. 1 World Map

Figure. 2 Turkey Map

Figure. 3 Lakes Region Map



The vineyard was established in 2005 year with in row with spacing 2 m X 3 m with drip irrigation and on grafted 41B rootstock which was determined according to soil analyses. Soil is in clayish loam texture with 7,8 pH and contains high lime. Vines were trained to a bilateral double cordon and spur pruning system. The spur pruned vines had ten two node spurs per vine and wines were supported by three-wire trellis. There were 3 replications according to experimental figure of randomized blocks and there are 5 grapevines for each plot. The red wine variety Kalecik Karası and the white wine variety Narince were evaluated in the study.

Observations analyses were done in 2008 and 2009 years. Phenology stages, yield and quality properties data had been obtained in vineyard. Soluble solid (%), titratable acidity (g/l), pH, pruning weight (kg/vine), yield (kg/vine) and adaptation ability were determined. Also climate data for 2 years in the study had been recorded.

RESULTS AND DISCUSSION

It was tried to determine native wine grape varieties that were able to adaptable in the Lakes Region and their adaptation values were evaluated. Evaluations, findings and observations were obtained as a result of these analyses and measurements related to two varieties in 2008 and 2009 year with climate conditions are given in table 2. It is seen that climate conditions are suitable for grape growing in the region.

Table 2. Some Climatic data of the experiment area

	Mean temperature (°C)	Minimum temperature (°C)	Mean precipitation (mm)
Long years average	12.2	- 14.9	764
2008 year	11.6	- 10.4	449
2009 year	9.9	- 10.1	1106

Table 3. Monthly Climatic data of the experiment area

Month/year	Mean temperature (°C)	Mean precipitation (mm)
January 2008	-0.2	11.3
February 2008	0.1	35.4
March 2008	8.8	47.2
April 2008	12.2	80.7
May 2008	14.9	23.6
June 2008	21.4	7.1
August 2008	23.9	3.8
July 2008	24.6	12.9
September 2008	19.0	93.0
October 2008	12.3	35.8
November 2008	8.7	74.4
December 2008	3.7	24.7
January 2009	3.4	158.3
February 2009	4.4	173.3
March 2009	5.4	169.4
April 2009	10.7	62.6
May 2009	14.8	63.5
June 2009	20.8	25.1
July 2009	23.2	8.8
August 2009	22.3	10.7
September 2009	17.8	46.8
October 2009	14.8	42.3
November 2009	7.8	77.2
December 2009	6.3	258.6

Grape varieties which were evaluated in the study, all phenological stages were determined. Buds burst time, Full blossom time, verasion period and harvest time were given in 2008 and 2009 years in table 3.

Table 4. Phnelogical stages of the varieties in 2008 and 2009 year

Cultivar /Year	Buds burst time	Full blossom time	Verasion time	Harvest time
Kalecik Karası 2008	16.04.2008	13.06.2008	22.08.2008	14.09.2008
Narince 2008	15.04.2008	14.06.2008	24.08.2008	18.09.2008
Kalecik Karası 2009	21.04.2009	12.06.2009	10.08.2009	04.09.2009
Narince 2009	24.04.2009	16.06.2009	24.08.2009	11.09.2009

We can see by the two years phenologic data, there is enough climate conditions and for these varieties to be grown under Lakes Region ecological conditions.

Among the two variety evaluated in the project yield and quality properties data had been obtained in vineyard. Soluble solid (%), total titratable acidity (g/l), pH, Average bunch weight(g), pruning weight(g), 100 berry weight, yield and adaptation ability were determined. Two years data were given in table 4 and 5.

Table 5. Measurements and analyses performed on 2008 year

Cultivar	Fresh grape yield (kg/vine)	Total soluble solids (%)	Titratable acidity (g/l)	pH	Pruning weight (kg/vine)	100 berry weight (g)	Average bunch weight (g)
Kalecik Karası	14.5±2.1	19.7±2.6	5.5±0.6	2.79±0.3	1.25±0.4	164.06±15.1	207±23.1
Narince	12.2±1.9	18.4±2.7	6.1±0.7	3.01±0.2	1.10±0.3	201.28±24.3	409±22.5

Table 6. Measurements and analyses performed on 2009 year

Cultivar	Fresh grape yield (kg/vine)	Total soluble solids (%)	Titratable acidity (g/l)	pH	Pruning weight (kg/vine)	100 berry weight (g)	Average bunch weight (g)
Kalecik Karası	22.0±1.8	18.8±2.3	5.3±0.7	2.89±0.2	1.45±0.3	269.8±19.3	172±30.8
Narince	11.6±2.5	17.9±2.8	6.8±0.5	2.96±0.2	1.00±0.4	279.4±23.4	286±21.5

CONCLUSIONS

From the experiments conducted in this study these varieties had been evaluated in a good performance for Lakes Region in Turkey. After two years of experiments on these varieties performance results indicate that produce satisfactory yields of good quality grapes. They are suitable for Lakes Region agro-ecological conditions, as well as for the most widespread native grapevine varieties in Turkey. Total soluble solids numbers can be seen low but it was thought from the cropload and extreme climate conditions. In 2009 year total precipitation was higher than long years average and average temperature was lower than the long years average. This caused decrease in total soluble solid and also was affected by the cropload. This can be improved by cropload management and irrigation practices in the next years. Colour verasion had been evaluated significantly important in Lakes region because of day and night temperature differences. It is thought this will let to increase the wine quality. Results presented in this study confirmed the thesis that Kalecik Karasi and Narince are dominant cultivars in Turkish viticulture and wine sector and they can be successfully cultivated in the region. Further studies will be planned about wine production and wine quality for these varieties in the region and some new approaches, growing practices (cropload management and irrigation) will be planned to reach better wine quality for these varieties.

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Strategies for adaptation to climate change, consumption trends and other parameters- an Australian perspective

Paper prepared for the 33rd World Congress of Vine and Wine, Tbilisi - Georgia, 20-27 June 2010

Peter Hayes[1] and Tony Battaglene[2]

Abstract

In a previous paper prepared by the authors[3] we have outlined the importance of climate as a vital component in determining the quality of the grape and ultimately the quality of the wine. Increases in temperature and changed rainfall patterns in both premium cool climate and warm climate regions mean that viticulturists and wine makers are being forced to alter traditional methods to maintain quality in wine. Today's viticultural regions for quality wine production are located in narrow climatic zones that put them at particular risk from both short-term climate variability and long-term climate change.

Because of the strong association between terroir and quality of wine, any change in grape growing attributes due to climate change will have serious economic and social impacts unless producers can adjust. The authors have argued that a very real challenge is how to ensure that the regulatory system is such that will permit the vitivinicultural industry to appropriately adjust to changed conditions and to continue to produce high quality wine.

In this paper we look at the current international developments and what strategies the wine sector can put in place to adapt for climate change. We also argue that consumer expectations will also result in changed environmental behavior from the wine sector and such expectations mean that sustainable practices will not only let the sector adapt, but also become a pre-requisite to selling wine to our consumers.

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1 Vice-President OIV; Email address: grapwine@senet.com.au

2 General Manager, Strategy & International Affairs, Winemakers' Federation of Australia; tel. +61262398304; Mobile +61413014807; Email address: tony@wfa.org.au

3 Hayes, P. and Battaglene, T.; (2006); Regulatory Response to climate change; Le Bulletin de L'OIV; Organisation Internationale de La Vigne et du Vigne, November-December 2006; Vol 79-No 909-910, pp 697-708

Introduction

In a previous paper prepared by the authors[4] we have outlined the importance of climate as a vital component in determining the quality of the grape and ultimately the quality of the wine. Increases in temperature and changed rainfall patterns in both premium cool climate and warm climate regions mean that viticulturists and wine makers are being forced to alter traditional methods to maintain quality in wine. Today's viticultural regions for quality wine production are located in narrow climatic zones that put them at particular risk from both short-term climate variability and long-term climate change.

Because of the strong association between terroir and quality of wine, any change in grape growing attributes due to climate change will have serious economic and social impacts unless producers can adjust. The authors have argued that a very real challenge is how to ensure that the regulatory system is such that will permit the vitivinicultural industry to appropriately adjust to changed conditions and to continue to produce high quality wine.

In this paper we look at the current international developments and what strategies the wine sector can put in place to adapt for climate change. We also argue that consumer expectations will also result in changed environmental behavior from the wine sector and such expectations mean that sustainable practices will not only let the sector adapt, but also become a pre-requisite to selling wine to our consumers.

Background

Over the last century, the mean temperature in Australia has risen by 0.7°C, leading to an increase in the number of very warm days and a decrease in frost and cold days. The prognosis is for an acceleration of climate change during the rest of this century (Pittock *et al.,* 2003).

Within Australia, the work of Webb and others has demonstrated that increase in temperatures in line with climate modelling will result in a projected increase in annual average temperature in viticultural areas by 0.8- 5.2^{0}C in 2070[5]. Climate change is a major threat to existing vineyard areas. The expected increase in temperature, atmospheric CO2, heavy and unseasonal rains, increased humidity, drought and cyclones, are likely to affect crops, quality, pests and diseases and host pathogen interactions, thus impacting on grape production and grape quality within a region. Such increases will potentially make existing production regions problematic and require adaption in either vitivinicultural practices or the planting of new varieties and opening of new regions to maintain production levels of high quality fruit.

To respond to future climates, changes to wine sector practices and government policies may be required. In this paper we look at the current national and international developments and what strategies the Australian wine sector can put in place to adapt for climate change and its impacts on Australian wine regions and biosecurity impacts.

4 Hayes, P. and Battaglene, T.; (2006); Regulatory Response to climate change; Le Bulletin de L'OIV; Organisation Internationale de La Vigne et du Vigne, November-December 2006; Vol 79-No 909-910, pp 697-708

5 **Webb, L.B,** Whetton P.H. and Barlow E.W.R. (2007) **Impact on Australian Viticulture from Greenhouse Induced Temperature Change**

Climate change impacts on quality
Grape production, wine quality and importantly flavour or “style” are very dependent upon climate. There is a major impact of climate on wine style and flavour and this distinguishes wine from other agriculture products.

Key climate challenges affecting grape growing, wine production and quality are:

- Grape quality is more sensitive to temperature change than most other crops
- An increase in average temperature by 2-3oC in a region will significantly change grape quality and wine style
- Increased severity and frequency of heat stress during the growing season
- Forecast increase in unusual weather events such as frost, hail and summer rainfall
- Reduced winter rainfall, increased dependence on scarce irrigation water, decreasing water quality
- Increases in salinity and resultant soil degradation
- Increased water use per hectare
- Increasing levels of atmospheric CO2 will directly impact grape production and water use
- Shorter growing season and earlier ripening causing compression of
- harvest dates and an overload on picking, crushing, transport and winemaking resources

From a marketing perspective this will lead to key challenges as climate change impacts on wine flavours and styles and regional attributes due to the terroir change.

Climate change impacts on biosecurity
The extent to which climate change will affect emergency pests and pathogens and their hosts is not clearly understood. To respond to future climates, changes to industry practices and government policies may be required. Adaptation plans will be developed around these risks to inform industry practice and policy.

A poleward shift in the geographical range of some pests and pathogens has been observed during the last century. Unpublished analyses indicated that if grapevine leaf rust becomes re-established in Darwin, the geographical range was predicted to extend further south to major Australian vine growing regions with a 1-5ºC temperature increase.

Climate change will alter the risks associated with the biosecurity of Australia’s cultivated and natural flora and the access of its agricultural products to international markets. Biosecurity policy will also be strongly influenced by climate change in a number of ways e.g. the re-defining of quarantine zones under future climate change scenarios or the need to modify post-entry quarantine based on new and emerging threats. Understanding these new threats (and opportunities) will enable Australia’s plant industries and quarantine agencies to be better prepared to adapt and respond to any risks.

It is not known how climate change will affect the biology and distribution of known plant hosts in Australia. Nor do we understand the effects of climate change on our currently identified Emergency Plant Pests (EPP). Modelling tools can predict the geographical range of plants and their pests and pathogens but often they do not take into consideration the specific biology of the host, pest or pathogen and their interaction.

Traceability and integrity

A key issue for the wine sector is varietal integrity. Under climate change, the wine sector will be forced to increasingly pursue new varieties. Within Australia, a recent discovery by CSIRO that the Albarino vines in its collection were in fact Savagnin Blanc has brought into focus a range of strategic issues. Not least of these is the use of objective methods such as DNA profiling to confirm identity of grapevine varieties. Under the Australian Wine and Brandy Corporation Label Integrity Program there is a requirement to ensure that if a wine is labeled with a particular varietal description, then it is true to label. The chain of custody required by legislation and subject to criminal penalties in the case of a breach goes back to the grower under the latest amendments to the Act currently before the Australian Parliament. This requires a traceability system for new plantings and an identification system for existing plantings or in the case of a dispute.

There are many more grapevine variety names than there are varieties. For example, there are somewhere between 5000 and 11,000 cultivars of *Vitis vinifera* but over 24,000 names to describe them. The application of DNA profiling for varietal integrity is essential. This will require access to agreed reference DNA profiles for each particular variety. Whilst this may be an issue for the lesser known varieties and clones, the mainstream varieties are more likely to have accessible reference DNA profiles. In order to guarantee varietal integrity there is a need for access to agreed reference DNA profiles to enable confirmation of varietal identity.

This is a global problem with concerns relating to the issue of veracity and authority attached to the foundational plant (s) of named varieties in a number of the global germplasm repositories.

The involvement of the OIV to promote collaborative international engagement directed to the creation and use of an international database and definition of reference varieties is vital. This is needed to underpin future exchange of grapevine material and to provide traceability for claims of variety status in research and development, production, trading and labelling; these may be applicable to the grapevine itself and products thereof; cuttings, plants, fruit, wine, juice etc where varietal attribution is important. The initiative will also add strength to biosecurity initiatives of importance globally and foster complementary actions by other countries.

From the perspective of effective adaptation to climate change, authenticity of variety in terms of characterising particular varieties' adapatability and utilising breeding material of known variety and characteristics will be of fundamental importance.

Australian government and international developments

The Copenhagen Accord was not adopted by delegates to the United Nations climate conference in Copenhagen. Instead, delegates merely 'noted' the agreement's existence, giving it no force whatsoever. The Copenhagen Accord is a sign-on document (i.e. countries that support it will be listed). As it is not a formal agreement within the UNFCCC framework and thus it is not clear how it will be implemented.

Many countries called for the Copenhagen Accord to be acknowledged, as the only agreement that can be reached here at Copenhagen, and that countries/parties should re-convene to ensure that a full agreement on climate change is reached in 2010.

There is reference to two degree temperature limit, committing the parties to reducing global emissions so as to hold the increase in global temperature below two degrees Celsius.

There is also reference to 1.5 degree limits, in ambiguous phrasing. There will be an assessment of the Accord implemented by 2015, including "consideration of strengthening the long-term goal referencing various matters presented by the science, including in relation to temperature rises of 1.5 degrees Celsius."

The agreement includes a goal of Annex 1 parties mobilising jointly US$100 billion a year by 2020 for developing countries and short-term financing "approaching" US$30 billion for 2010-2012.

- It is agreed that a significant proportion of this money would flow through the "Copenhagen Green Climate Fund."
- There is also agreement to establish a Technology Mechanism, without detail of how this would work.

Annex 1 parties (including Australia) will commit to quantified economy-wide emissions targets for 2020 to be submitted to the UN by 31st January. Australia's commitments are a 5% reduction up to -15% or -25%.
'Australia will reduce its greenhouse gas emissions by 25% on 2000 levels by 2020 if the world agrees to an ambitious global deal capable of stabilising levels of greenhouse gases in the atmosphere at 450 ppm CO2-eq or lower. Australia will unconditionally reduce our emissions by 5% below 2000 levels by 2020, and by up to 15% by 2020 if there is a global agreement which falls short of securing atmospheric stabilisation at 450 ppm CO2-eq and under which major developing economies commit to substantially restrain emissions and advanced economies take on commitments comparable to Australia's. '

Mitigation actions by developing countries will be "subject to their domestic measurement, reporting and verification" to be submitted with national communications every two years.

There are also provisions on REDD, adaptation and market mechanisms.

The texts that were generated through the LCA and KP will be included in the reports of the COP and CMP and used as starting inputs for the negotiations in 2010.

A comprehensive climate treaty will happen at some stage, but probably not in Mexico in December 2010. For the Australian wine sector it means that we need to continue to very publically reduce our emissions and lower our carbon footprint.

Domestically, the Australian government has pushed very strongly to introduce a Carbon Emission Trading Scheme (CPRS). The CPRS legislation was introduced into the House of Representatives on 2 February, with debate expected to last around 4 weeks. Currently the government does not have the support within the Senate to introduce such a scheme. While a compromise scheme does not seem possible, it is a possibility and the Green Party has proposed an interim carbon tax proposal. The Australian government has invested too much political capital in the ETS system not to progress unilaterally.

Any such carbon pollution reduction scheme will have cost impacts on the wine sector in Australia. Consequently, the sector needs to focus on reducing the costs on inputs including energy.

Climate change impacts on food production

Climate change will inevitably cause governments to make policy trade-offs as competition between food crops and vines for scarce land and water resources intensify. This means that some mitigation responses may not be conducive to adaptation and may conflict with food security – and vice versa. For instance, viticulture production for wine may lead to competition for land and water resources that are crucial for enhancing system resilience and safeguarding food production. Clear criteria and indicators are necessary to steer decision-making.

Food security is not only an explicit concern under climate change; successful adaptation and mitigation responses in the agricultural sector can only be achieved within the environmental and economic sustainability goals set forth in both the UNFCCC and the Millennium Development Goals.

Consumer demands on the wine sector arising from climate change
Adaptation requires adjusting institutional structures and arrangements. This includes defining adequate national policy and legislative frameworks, and assigning responsibilities within the governance structures of countries and regions. Iterative planning frameworks, participatory approaches and strong stakeholder engagement should be key governance principles for adaptation. In addition, decision-making must remain flexible for dealing with the uncertainties of potential climate change impacts[6].

Livelihood resilience to climate change
Reliable impact scenarios are often not available at the national and sub-national level. When this is the case, adaptation planning should focus on management aimed at increasing livelihood resilience. In this context, FAO strongly argues for priority actions in sustainable land management such as land-use planning and soil and water management, and appropriate management of farming systems, livestock, forests, grasslands, fisheries and aquaculture.

Conservation and sustainable management of biodiversity
The healthy functioning and resilience of ecosystems depends to a great extent on biological diversity. For FAO, the key priorities are to increase understanding of the ecosystem services provided by agricultural and aquatic biodiversity and identify associated impacts of climate change and sustainable responses.

Increased regulation
The wine sector also operates under an increasingly stringent regulatory framework around climate change, industrial emissions and packaging. Many of these policies that

[6] FAO, 2009, Profile for climate change

underpin this regulatory environment support continuous environmental improvement in the sector by considering the interactions at all stages of the food chain.

For example, within Europe the CIAA has identified three major areas of increasing concern about regulation:

- **Climate Change (EU ETS):** The food manufacturing industry accounts for about 1,5% of EU-27 greenhouse gas (GHG) emissions. About 900 food processing installations are covered by the EU Emissions Trading Scheme (ETS) and deliver continuous cuts in CO2 emissions.

- **Industrial Emissions (IPPC):** A possible move towards a rigid implementation of the Best Available Techniques (BAT) reference documents (BREFs) could have wide impacts.

- **Packaging:** Packaging recovery and recycling is highly visible policy response in the European Union and are aimed at balancing food safety and product quality with optimising food and drink products' overall environmental performance

Wine environmental assurance schemes in wine producing countries[7]

Most major wine producing countries have now introduced or are considering introducing environmental assurance schemes. The key objective of these schemes is to minimise the environmental footprint from wine production and ensure high standards of sustainable practices are followed.

The wine sector has been extremely proactive in this field for a number of years. A number of countries have adopted individual schemes, including, *inter alia*, Australia, California, New Zealand and South Africa.

EntWine Australia is the Australian wine industry's voluntary national assurance scheme launched in 2009. The program is administered by the Winemakers' Federation of Australia (WFA), and builds on the Australian Wine Industry Stewardship program (AWIS) launched in 2005. AWIS was a voluntary environmental self-assessment for grapegrowers distributed through the spray diaries of participating wine companies.

The Sustainable Winegrowing Practices (SWP) program was initiated in 2001 with a view to California earning the reputation of world-leader in the adoption of sustainable winegrowing practices. The program is managed by the California Sustainable Winegrowing Alliance, a non-profit organisation. The goals of SWP are based on the proportion of the industry participating in the program. The SWP is launching a voluntary third party certification program on January 13, 2010. The certification program will verify and confirm participation in the program and will become the basis for industry wide improvement targets.

Sustainable Winegrowing New Zealand (SWNZ) was established by volunteer grapegrowers in 1995 as an industry initiative coordinated by Winegrowers of NZ. After a

7 Russell, Amy, (2009), Review of major wine environmental assurance schemes in wine producing countries, Unpublished paper produced for circulation to the World Wine Trade Group.

pilot phase, SWNZ was adopted commercially in 1997 and now has members in all NZ wine regions. With the introduction of a winery program in 2002, SWNZ now applies to both vineyards and wineries. SWNZ aims to provide a best-practice model of environmental practices in the vineyard and winery; guarantee better quality assurance from the vineyard through to the bottle; and address consumer concerns in matters pertaining to the environment and winegrape production.

Participation in South Africa's Integrated Production of Wine (IPW) scheme infers a producer promise that 'South Africa's wines will not contain substances that they should not, and that by adhering to the IPW guidelines, the environment will be keenly protected'. Introduced in 1998, the voluntary membership currently represents 98% of grapes harvested and 95% of South African cellars subscribe to the program. IPW is unique in that it applies to grape growing, winemaking and bottling. Although participation in IPW is voluntary, once participants join the scheme, it is regulatory.

The Scheme for the Integrated Production of Wine was legislated in November 1998 under the Act on Liquor Products and is administered by the South African Wine and Spirit Board. As of 2010, the South African Wine of Origin (WO) and IPW schemes will be linked under a new combined certification seal, to be rebranded as Sustainable Wine South Africa. The IPW requirements will remain voluntary, and therefore producers satisfying WO requirements only will continue to use the existing seal. However, producers satisfying both WO and IPW requirements will be able to use a new combined seal. The system will also enable the certification to apply at the product level – 100% of grapes, the winery and the bottling facility must comply with WO and IPW requirements to wear the combined seal. For products that do not comply, the wine company cannot use the combined seal.

More recently, the Global Wine Sector Environmental Sustainability Principles (the 'Principles') were developed by FIVS in 2006. A key consideration of the Principles is the integration of wine sector environmental programmers being developed or implemented in member countries. FIVS seeks to incorporate these programs whilst ensuring member countries can continue to undertake environmental management most appropriate to their country's regulatory, social, natural resource and biophysical contexts. Accordingly, the global Principles are high level principles, providing direction to member countries whilst enabling members to determine their own environmental and resource allocation priorities.[8] Each of the country programs described previously complies with these principles.

Given the pressure being applied in international markets to provide environmental assurances, a unified international wine industry position on environmental management becomes an important tool for responding to regulatory and market pressures. More importantly, the environmental assurance progams provide the systems required to ensure that the wine sector is able to adapt to climate change and meet the customer requirements for a sustainable low environmental footprint product into the future.

8 RUSSELL Amy (2008) Demonstrating consistency with the Global Wine Sector Environmental Sustainability Principles : An Australian example , Bulletin de l'OIV , vol. 81, no926-928, pp. 269-275

Production responses to climate change

Climate change adaptation by producers is determined by the ability to change behavior in order to respond to a changing climate. This includes devising and implementing solutions to protect livelihoods from negative climate change impacts.

Conclusion

Climate change is a major threat to existing vineyard areas. The expected increase in temperature, atmospheric CO_2, heavy and unseasonal rains, increased humidity, drought and cyclones, are likely to affect crops, quality, pests and diseases and host pathogen interactions.

Research is going to be an integral part of any adaptation program by the global wine sector. Much of this research is pre-competitive and we should actively encourage international collaboration in a number of key areas, in particular relating to vine physiology, winemaking practices (yeasts, enzymes and additives and processing aids) and engineering.

It should also be recognised that national government responses to emission reductions will impose costs on all producers and production efficiencies and sustainability initiatives will be vital to maintain market access.

Environmental programs provide an effective way in which the wine sector can reduce its carbon footprint, and help meet the national and international carbon reduction targets when they appear. The world cares about reducing CO_2 and despite the failure of our governments to reach consensus in Copenhagen, the sector can do much to adapt to climate change and fulfil our sustainability goals.

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METHODS OF CHLOROPHYLL FLUORESCENCE AND GRAPE-VINE RESISTANCE AGAINST ABIOTIC FACTOR

T.Ortoidze, N.Korkotadze, N.Tsertsvadze
Institute of Horticulture, Viticulture and Oenology, Tbilisi, Georgia
6, Marshal Gelovani st., Tbilisi, 0159, Georgia
E-mail: tortoidze@hotmail.com

ABSTRACT

The primary processes of photosynthetic has been used as a natural probe for determining grape-vine resistance against various abiotic factors – temperature, water, and solar irradiation. It was shown that value of variable component of fast fluorescence – Fv, intensity of electron transport between the photosystems – ETR, also intensity of expended electrons in carboxylation ETRn and oxygenation –ETRp, an index of non-photochemical fading – NPQ, allow studying molecular mechanisms of the abiotic factors' impact molecular mechanisms and resulting damage degree.

It is concluded that fast and slow fluorescence characteristics of the grape-vine leaves, provide for investigation of the mechanisms of the abiotic factors' impact on the plant, and for use these in preliminary diagnostics of the comparative resistance of various varieties of the grape-vines.

ZUSAMMENFASSUNG

In der Arbeit sind primäre Prozesse der als natürliche Sonde für die Feststellung der Widerstandsfähigkeit der Weinrebe gegen verschiedene abiotische Faktoren. Es wird gezeigt, dass die Bedeutung der variablen Komponente der schnellen Fluoreszenz – Fv, Intensität des Elektronentransports zwischen den Fotosystemen – ETR, ebenso Intensitäten der für Karboxylisation – ETRn und Oxygenisation – ETRp aufgewandten Elektronen und das mit der Nicht-photochemischen Löschung des Lichtes verbundene Kennzeichen – NPQ uns die Möglichkeit geben, die Molekularmechanismen der Wirkung des abiotischen Faktors und der davon ausgehende Schädigungsgrad zu erlernen.

In der Arbeit ist die Schlussfolgerung gezogen, dass die Kennzeichen der schnellen und verlangsamten Fluoreszenz des Weinblatts uns die Gelegenheit geben, Wirkungsmechanismen der abiotischen Faktoren auf die Pflanze zu erlernen und sie für die vorläufige Diagnostizierung relativer Widerstandsfähigkeit verschiedener Weinrebensorten zu verwenden.

INTRODUCTION

In conditions of global warming in agriculture, and particularly in viticulture, those problems have posed themselves as is a resistance of the grape-vine against high temperature and increased solar irradiation, draught resistance, etc. It is still actual a grape-vines resistance against low temperatures, especially in a case of spring frosts (Duering et al., 1990; Ortoidze, Duering, 2003; Ortoidze, Duering, 2006)).

Considering the above-said, it is obvious that investigation of the grape-vine resistance against the above abiotic factors is highly important; revealing of relatively resistant varieties is important as well.

The present work is dedicated to the study of these problems, namely, determining a resistance of the grape-vine resistance against abiotic factors, with an aid of the chlorophyll fluorescence method.

MATERIAL AND METHODS

Activity of the primary processes of the photosynthesis apparatus in the grape-vine leaves was studied with the method of delayed fluorescence (DF). Recording of the DF was made with the phosphoroscope; the time between an object illumination and fluorescence detection amounted 1 ms (Matorin et al., 1985).

The rapid fluorescence (RF) was evaluated with portable photometric apparatus PAM 2100 (H.Walz, Effeltrich, FRG) (Schreiber, 2005)). This device allows experimenting directly on a plant, without damage to the leaf.

RESULTS AND DISCUSSION

Fig. 1 shows induction straight line of DF for the *Rkatsiteli* leaves; in the induction straight lines of DF the rapid phase O–I-D is linked to the electron transport, slow phase D-P – to energyzing of the photomembrane, while P-S phase – to CO_2 binding (Matorin et al., 1985; Ortoidze et al., 1990). Considering changes of these indices of fluorescence, we can judge on a deterioration degree in the primary processes of photosynthesis (electron transport, photophosphorylation, CO_2 binding). In our works it was shown that K=(P-S)/S coefficient is directly linked to the photomembrane activity – decrease of the photosynthesis activity directly correlates with its decrease.

Fig. 2 shows a variable values of the *Rkatsiteli* leaves' fluorescence - Fv=(Fm-Fo)/Fm, which is connected with the photosynthesis-2 activity. In normal conditions its value equals Fv=0.6-0.7; a stress impact on the leaves results in its decrease, according to which we can judge a reversibility of the stress-induced damages.

According to the reference data, parallel measurements of variable fluorescence and photosynthesis intensity provide for calculation of the electron transport intensity between the photosystems – ETR, as well as at carboxyllation ETR_p and oxygenation – intensities of the electrons spent in ETR_n (Schreiber, 2005; Ortoidze, Duering, 2003):

$$ETR=Y \times 0.85 \times R \times PAR,$$

where *Y* is an intensity of variable fluorescence, *PAR* – active radiation of photosynthesis ($\mu mol.m^{-2}.s^{-1}$), while *R* is a constant and equals R=0.5.

$$ETR_p=1/3\ [ETR+8(A+R)],$$

$$ETR_n=1/3\ [ETR-4(A+R)],$$

Where *ETR* is a total of the electron transport intensity, *A* – intensity of photosynthesis.

A vast part of the light intensity, falling on a leaf, is linked to the so-called non-photochemical extinguishing. Just this process is a mechanism, which guards a leaf from the high intensity of solar irradiation (photic burn). It is with this process that rapid fluorescence NPQ index does connect, which could be calculated with the following formula:

$$NPQ=(Fm-F'm)/F'm,$$

where *Fm* is a maximal fluorescence of the dark-adapted leaf, to the first light impulse, while F'm is a maximal value of fluorescence of a leaf illuminated with constant red light.

Value of the NPQ index is directly connected to the light non-photochemical extinction (Schreiber, 2005).

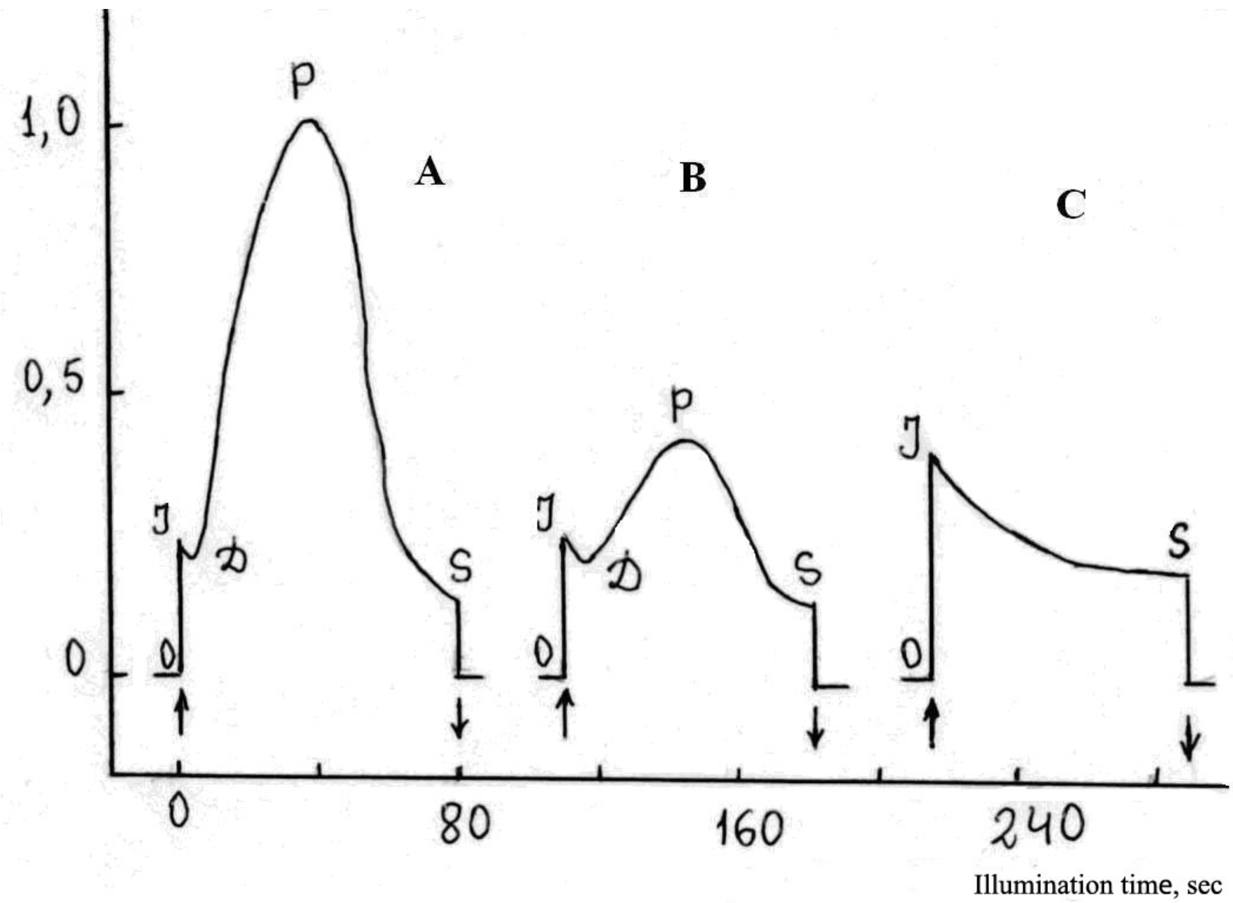

Figure 1. Induced straight lines of the delayed fluorescence in the *Rkatsiteli* leaves: A) control; B) incubated at +45°C; C) incubated at +55°C. (Arrows point at the moments of light on and off).

As it is obvious, any change in the primary photosynthesis processes, could be assessed according to rapid and delayed fluorescence indices. On the other hand, the apparatus of the primary photosynthesis is situated in the chlorophyll thyllacoid, i.e. in the so-called photomembrane.

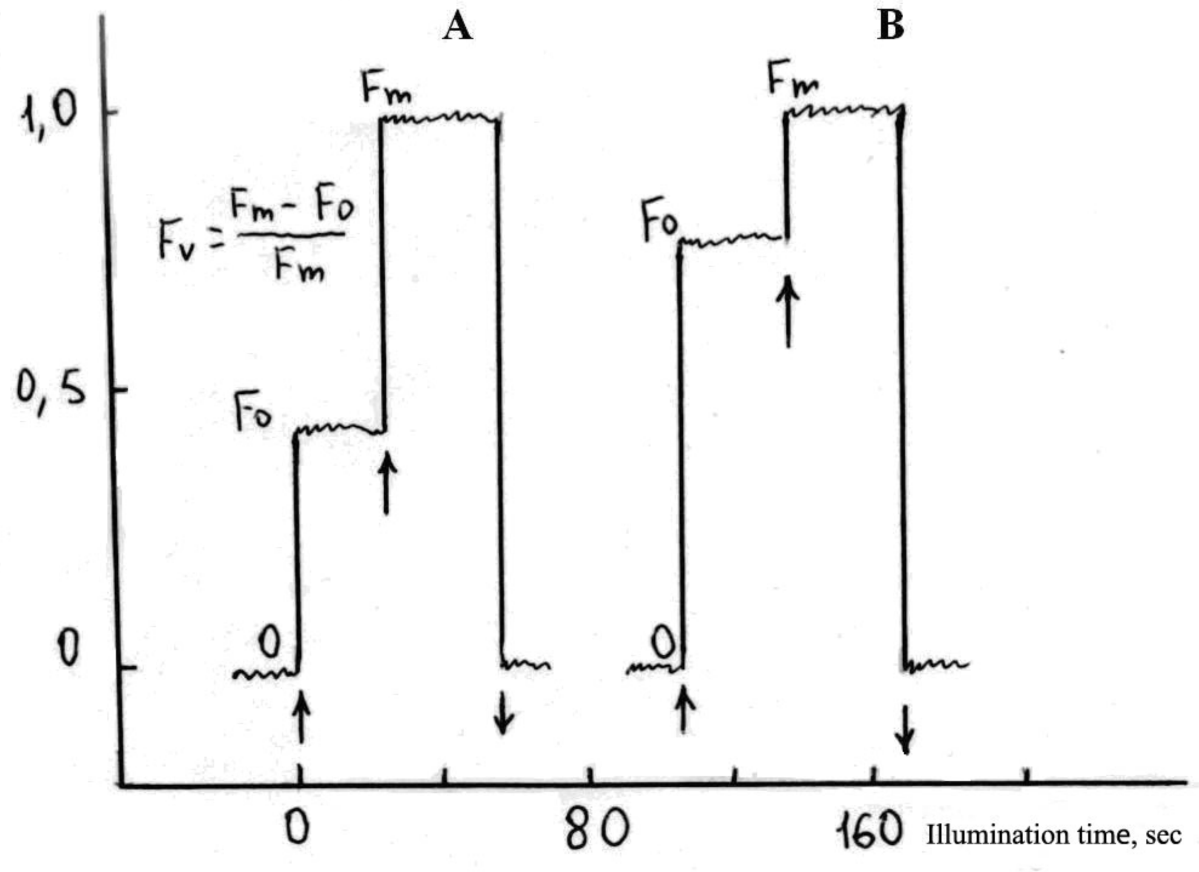

Figure 2. Induction straight lines of the rapid fluorescence in the *Rkatsiteli* leaves: A) control; B) relative volume of water 60% (Arrows point at the light on and off moments).

Now, if we imagine an inverse process: if we act with some stress (e.g. high temperature, water deficit, etc.) on the photomembrane, and induce its damage, it in its turn, will deteriorate a primary processes of photosynthesis, which will be recorded with the fluorescence method. Evaluation of the deterioration degrees of the primary processes of photosynthesis, might provide with the means for judgment on a value of the photomembrane damage (is the damage irreversible, and at which level did it occur). In other words, the primary processes of the photosynthesis we can evaluate as natural probes for determining the stability of photomembranes.

On the other hand, we can consider that all the membranes constituting a cell content are almost of the same resistance (there might be a difference between their resistance. However it will be so negligible that in the applied science it would have no importance). Therefore, we believe that stress, which damages the photomembrane, may also damage other membranes of a cell.

Fig. 1 demonstrates induction straight lines of the *Rkatsiteli* leaves', at 5 min exposure, at high temperature. As it is evident, at 45°C decrease of slow phases D-P and P-S did occur, which depends on decrease of ΔpH values, as well as on decrease of CO_2 binding velocity. However, in this case damage is reversible. At +55°C, the processes of photophosphoryllation and CO_2

binding are completely inhibited; also, intensity of the electron transport between the photosystems, decreases significantly. Irreversible damage to the photomembranes does occur in this case (Matorin et al., 1985).

In a case of water deficiency, value of variable fluorescence decreases (Fig. 2), which points at decreased photosynthesis intensity. During decrease of relative volume of water from 95% to 75%, the ETR_p, which is connected to carbonation, decreases sharply. The result obtained shows that during dehydration the primary processes of the photosynthesis in grape-vine leaves, the most sensitive is a CO_2 binding. The process of photophosphoryllation decreases as well. However, the latter process is reversible.

If we decrease relative volume of water in the grape-vine up to 50%-55%, a sharp decrease of ETR does occur and photosynthesis is inhibited to zero. In such case, a plant undergoes irreversible damage (Ortoidze, Duering, 2006).

In a case of high light irradiation (6000 μmol . $m^{-2}.s^{-1}$, during 10 min) intensity of ETR – electron transport – decreases; however, its oxygenation component ETR_n – increases. Index of NPQ increases, which should be due to non-photochemical shut-down of excessive light illumination. From the biochemical point of view, defense mechanism against a high intensity illumination, should be connected to the chlorophyll transformation: at high intensity illumination violaxanthene transforms into zeaxanthene (Adams et al., 2004).

CONCLUSIONS

Considering all the above-mentioned we can conclude that the primary processes of photosynthesis could be viewed as a natural probe for determining the grape-vine resistance against various abiotic factors; observation of an impact mechanisms of these factors on a plant, is well provided with the fluorescence characteristics of the grape-vine leaves.

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EVOLUTION DU CLIMAT ET VITICULTURE
ANALYSE DE LA PERCEPTION DES PROFESSIONNELS ET DES EXPERTS

ROCHARD J.*, ZABA S.*, CHEVRE C.**

*Institut Français de la Vigne et du Vin
Pôle national développement durable
joel.rochard@vignevin.com

** Vignerons Indépendants de France
christophe.chevre@anfovi.com

RESUME
A partir d'une synthèse bibliographique, un questionnaire sur l'évolution du climat et la viticulture a été établi et mis en ligne sur Internet(www.vignevin.com). Il a pour objectif d'établir un état des lieux concernant l'évolution du climat et ses répercussions sur la filière viticole.

La première partie a pour but de recenser les différentes manifestations de l'évolution du climat sur les exploitations viticoles à partir des observations des professionnels.
Au final, le questionnaire porte sur les répercussions concernant le fonctionnement de l'exploitation et les démarches envisagées pour limiter les causes des modifications climatiques. La communication a pour objectif de présenter de manière synthétique de détailler les réponses liées au questionnaire
L'avancée des dates de vendanges relevée par les professionnels français se situe dans une fourchette de 8 à 12 jours.
Le questionnaire souligne une augmentation significative de la sécheresse une augmentation de l'alcool potentiel des moûts de 0,5 à 1°C et une diminution de l'acidité (en H2S04) d'environ 0,5 grammes par litre.
Concernant les pistes de recherche, les aspects considérés comme les plus importants par les professionnels sont respectivement la maîtrise de la température des raisins, des moûts et des vins, la gestion des sols, la désalcoolisation et le pilotage de l'irrigation.

SUMMARY
A questionnaire based on a review of relevant literature has been drawn up and posted online (www.vignevin.com) to establish an overview of climate change and its repercussions on the wine-growing industry.

The aim of the first section is to take an inventory of the various manifestations of climate change on vineyards, based on observations from wine-growing professionals.
Lastly, the questionnaire concerns the repercussions in relation to vineyard management and the measures envisaged to limit the causes of climate change.
The aim of the communication is to present a summary of the problem of "climate change" and itemise the answers to the questionnaire and the aspects developed during the individual interviews with professionals and experts.

French wine-growers have observed that harvesting dates have been moved forward by 8 to 12 days.
The questionnaire highlights a significant rise in droughts, an increase in potential alcohol musts of 0.5 to 1°C and a drop in acidity (in H2S04) of approximately 0.5 grams per litre have been noted.
As for areas of research, the aspects considered the most important by wine-growing professionals are, respectively, temperature control of grapes, musts and wines, soil management, dealcoholisation and programming irrigation.

INTRODUCTION

Afin de répondre aux attentes de la population et de s'adapter à son milieu, l'Institut Français de la Vigne et du Vin www.vignevin.com en collaboration avec les Vignerons Indépendants de France, s'est penché sur les conséquences de l'évolution du climat et les adaptations envisagées par les professionnels.
Dans le cadre de cette étude, une démarche prospective a été menée auprès des professionnels viticoles français ainsi qu'auprès d'experts et professionnels nationaux et internationaux. Les experts et professionnels de cette filière ont été principalement questionnés lors d'entretiens alors que les professionnels des principales régions françaises, (cf. figure 1), ont majoritairement répondu à un questionnaire sur le site de l'IFV (241 réponses interprétables au total fin décembre 2009). Les personnes les plus représentatives sont celles dont l'âge est compris entre 30 et 50 ans (68%).
Contrairement à un sondage, ce questionnaire ne reflète que partiellement la perception par les professionnels des changements climatiques. Néanmoins, les réponses sont globalement en cohérence avec les observations des experts viticoles.

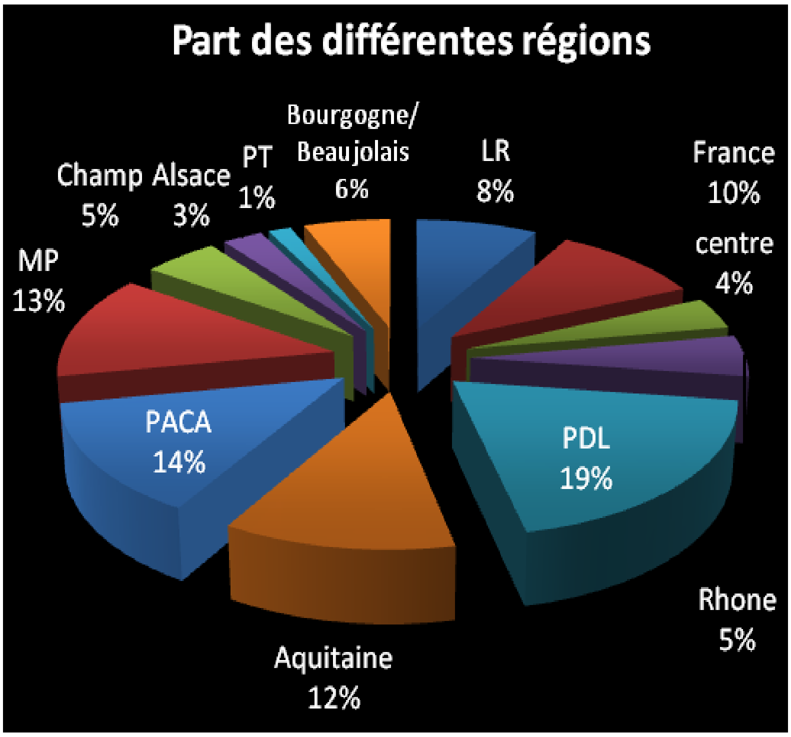


Figure 1 : Pourcentage de réponses dans les différentes régions viticoles

I. Principaux effets perçus

- **Physiologie et phénologie**

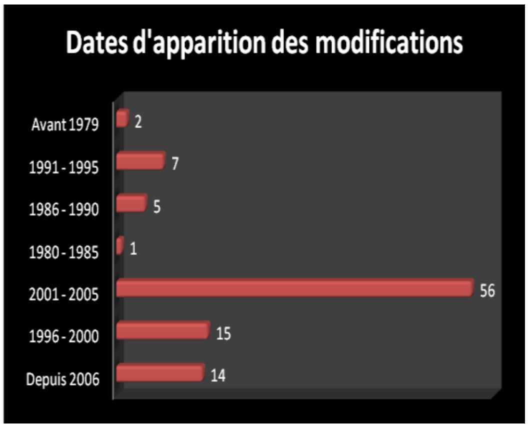


Figure 2 : Dates d'apparition des modifications

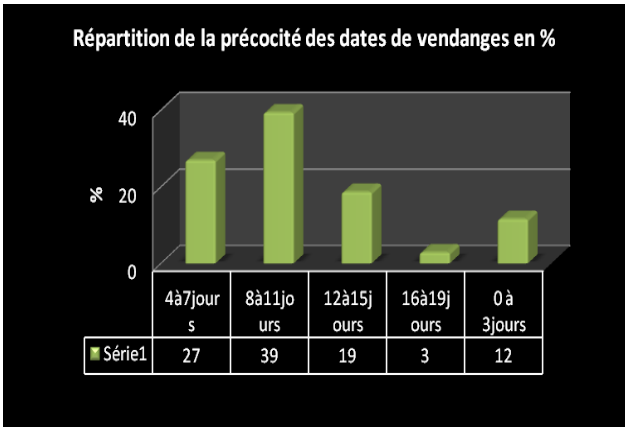


Figure 3 : Répartition de la précocité des dates de vendanges en %

Plus de 50% des réponses (y compris des professionnels les plus âgés) estiment que les changements significatifs sont apparus au cours des quinze dernières années. Les observations réalisées sur le terrain par les vignerons diffèrent des études scientifiques qui mettent en évidence le réchauffement à partir des années 1980 (cf. figure 2). Il est probable que l'année de canicule 2003, perceptible sur l'ensemble du territoire français ait occasionné de nombreuses physiologiques de la vigne, à l'origine de la prise de conscience du réchauffement.
L'étude révèle que quelque soit la région viticole ou la tranche d'âge, les modifications suivantes paraissent les plus importantes.

- L'augmentation du taux de sucre
- La précocité des dates de vendanges
- Les modifications du processus de maturation
- Le raccourcissement du cycle
- La diminution du taux d'acidité

L'avancée des dates de vendanges est majoritairement située entre 8 et 11 jours (cf. figure 3). D'après les études menées par V. Daux et E. Le Roy-Ladurie une avancée de 10 jours sur les dates de vendanges est liée à une augmentation moyenne de 1°C. Ces observations sont cohérentes avec les études menées par Météo France qui situe le réchauffement moyen, au cours des dernières décennies, dans une fourchette comprise entre 0,7°C et 1,1°C.

Le raccourcissement du cycle est lié à une avancée des dates de vendanges, mais également à une précocité de l'ensemble des stades phénologiques. L'étude révèle que pour la floraison et la véraison, l'avancée de 4 à 7 jours est majoritairement observée. Concernant le débourrement, l'avancée est comprise entre 0 et 3 jours (37%). Le raccourcissement du cycle est souligné pour l'ensemble des régions du territoire (cf. figure 4).

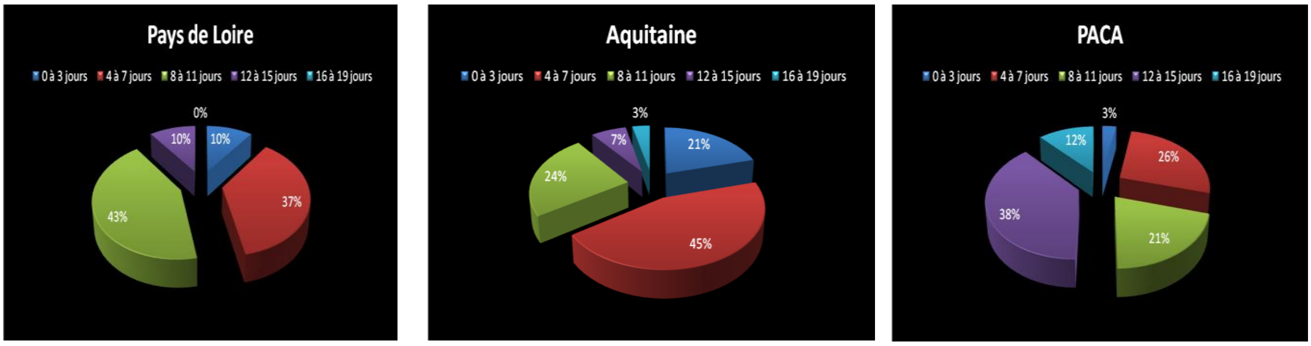


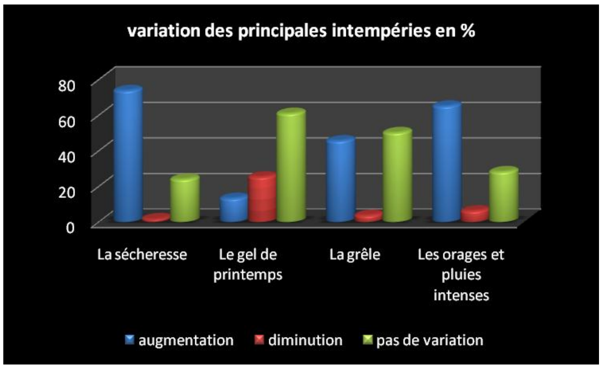
Figure 4 : précocité des dates de vendanges variable en fonction des régions viticoles.

Les autres modifications sont perceptibles au moment de la récolte et affectent les caractéristiques œnologiques des vins. En effet 97% des réponses soulignent que l'augmentation du potentiel alcool est comprise entre 0,5 et 2°. Parallèlement, 81% des personnes interrogées estiment que la diminution de la teneur en acidité est comprise entre 0 et 0,9g/l.

- **Phénomènes extrêmes**

Vis-à-vis des conditions extrêmes et accidents climatiques (cf. figure 5), les aspects suivants ont été mis en évidence :

- Une faible influence sur l'apparition du gel de printemps
- Aucune variation concernant la fréquence de grêle (50%)
- Une variation de fréquence des orages et pluies intenses (65%)
- Une augmentation de la sécheresse à l'origine d'une accentuation des conditions de stress hydrique (71%)



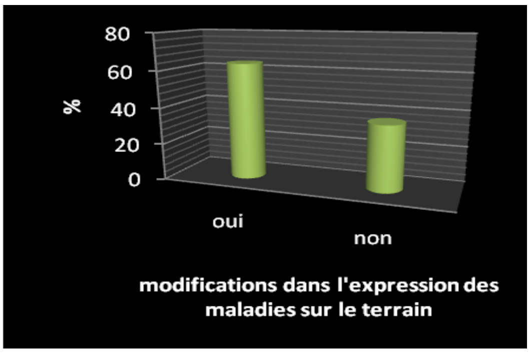


Figure 5 : Variation des principales intempéries en % - Figure 6 : Modifications dans l'expression des maladies sur le terrain

Les résultats de l'enquête font ainsi apparaitre que l'augmentation de sécheresse s'accompagne d'une augmentation des conditions de stress hydrique notamment en Languedoc-Roussillon et en Provence et dans une moindre mesure en Aquitaine.
Ces observations sont en adéquation avec les études climatologiques menées dans les différentes régions. En revanche la grêle et le gel de printemps sont des événements

climatiques difficiles à quantifier qui ne font pas l'objet d'évolutions significatives dans l'interprétation des questions.

- **Maladies et parasites**

Globalement, 64% des réponses soulignent un impact significatif sur les maladies et parasites (cf. figure 6), associées à un changement dans leur expression (56%). Les professionnels (42%) soulignent l'apparition ou la disparition des maladies et 32% estiment que celles-ci se développent à des stades non habituels au cours du cycle végétatif.

II. Piste de recherche

Cette étude permet de mettre en évidence les priorités soulignées par les professionnels concernant les orientations prioritaires de la recherche, en liaison avec les changements climatiques.

Concernant le matériel végétal, la profession viticole souhaite avant tout la mise en place d'observatoires régionaux (49%) et dans une moindre mesure la création de nouvelles variétés (29%) et de nouveaux porte-greffes (16%). Sur le plan viticole, les vignerons, en cohérence avec les chercheurs estiment que la priorité doit se centrer sur l'adaptation de la gestion des sols (68%) et des modes de conduite (48%), l'irrigation (27%) et dans une moindre mesure sur la salinité des sols (10%). Les résultats permettent d'établir un ordre de priorité en fonction du processus à mettre en place, au niveau des pratiques œnologiques :

- Maitrise de la température des raisins, des moûts, des vins (80%)
- Adaptation du transport des raisins, des moûts, des vins (43%)
- Vendange de nuit (26%)
- Maîtrise de l'acidité (20%)
- Réduction de la teneur en sucre (18%)
- Désalcoolisation (8%)

En complément du « questionnaire professionnels » des entretiens individualisés ont été réalisés auprès de chercheurs ou conseillers viticoles

Il apparaît que les priorités diffèrent en fonction des scenarios d'évolution de la température prévue au cours de ce siècle. Ainsi, si l'augmentation reste comprise entre 1,5°C et 3,5°C, la création d'observatoires régionaux est considérée comme la priorité à mettre en œuvre concernant le matériel végétal. Il en est de même de l'adaptation de la gestion des sol. D'un point de vue œnologique, les experts estiment que les éléments prioritaires à mettre en œuvre concernent la maîtrise de la température des raisins, des moûts et des vins ainsi que la désalcoolisation. En revanche, si l'augmentation de la température se poursuit et atteint +5°C, les priorités risquent d'être modifiées. Ainsi, la création de nouvelles variétés ou la modification des cépages semble être incontournable pour s'adapter d'adaptation aux changements climatiques. L'adaptation et le pilotage de l'irrigation risque d'être le volet prioritaire à mettre en œuvre sur les parcelles si le scénario pessimiste se vérifie.

CONCLUSION

Globalement, le questionnaire à permis de cerner la perception par les professionnels des impacts liés aux changements climatiques probables. Les évolutions observées au cours des deux dernières décennies sont cohérentes avec l'avis des experts.

Des similitudes peuvent être mises en évidence en fonction des différents paramètres étudiés. (Languedoc Roussillon et Provence Alpes Côte d'Azur notamment) Pour ces régions, l'augmentation du degré, la baisse d'acidité et une expression plus forte du stress hydrique sont les conséquences les plus marquantes observées à partir de 2003. L'inquiétude semble moins marquée pour les régions septentrionales qui bénéficient depuis quelques années de conditions climatiques favorables à l'épanouissement de millésimes.
Au-delà du questionnaire, il est nécessaire de susciter une dynamique de réflexion stratégique dans les différentes régions pour adapter les pratiques viticoles et œnologiques en fonction du contexte local et de l'évolution observée et des scénarios climatiques régionalisés. La création de réseaux d'échange, d'observatoires parcellaires et variétaux nationaux, voire internationaux, pourrait faciliter cette adaptation par une mutualisation des informations.
L'Organisation Internationale de la Vigne et du Vin doit pouvoir jouer un rôle fédérateur pour cet enjeu majeur de la viticulture.

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RISK OF CLIMATE CHANGE FOR GRAPEVINE PRODUCTION IN MEDITERRANEAN AREAS

P. Resco[1*], S. Quiroga[2], A. Iglesias[3], V. Sotes[4]

[1] Corresponding author, Department of Agriculture, Union of Farmers Associations (COAG): C/ Agustín de Bethancourt, 17 - 5ª, 28003, Madrid, Spain.
presco@coag.org

[2] Department of Statistics, Economic Structure and International Organisation - Universidad de Alcalá, Spain. 28801 Alcala de Henares, Spain
sonia.quiroga@uah.es

[3] Department of Agricultural Economics and Social Sciences, Universidad Politécnica de Madrid (UPM), Avda. Complutense s/n, 28040 Madrid, Spain
ana.iglesias@upm.es

[3] Department of Crop Production, Universidad Politécnica de Madrid (UPM), Avda. Complutense s/n, 28040 Madrid, Spain
vicente.sotes@upm.es

ABSTRACT

Vineyards play an important role in the Mediterranean landscape, thanks to their good adaptation the clime, especially to semi-arid conditions which helps to explain the large extensions throughout Spain. The aim is to evaluate the risk of climate change and the implications for future decisions and policy. The results are useful for exploring solutions for and grapevine management adequate for the future in Mediterranean areas. Historical data of grapevine production in three contrasting locations is used to develop empirical statistical models that are then used to evaluate the impacts of climate change. The uncertainty of future climate conditions is evaluated by using 16 different climate scenarios. We plan to extend this preliminary analysis to include the major grapevine producing regions in Spain and include climate indices and other tools of grapevine adequacy and quality. The results presented here are part of a larger study that includes several other Mediterranean crops and scenarios (Iglesias et al., 2010).

Key words: Climate change, viticulture, management practices, Spain

RESUMEN

El Viñedo desempeña un papel importante en el paisaje mediterráneo, gracias a su buena adaptación al clima, sobre todo a las condiciones semi-áridas, lo que ayuda a explicar las grandes extensiones en toda España. El objetivo es evaluar el riesgo del cambio climático y las implicaciones para las decisiones en el futuro y nuestros resultados pueden servir para explorar soluciones en regiones mediterráneas. Se han utilizado los datos históricos de producción vitícola de tres zonas que representan condiciones muy distintas en la región Mediterránea. En cada zona se ha aplicado un modelo de producción obtenido en estudios anteriores. La incertidumbre de las futuras condiciones climáticas mediante el uso de 16 diferentes escenarios climáticos. Nuestra intención es continuar el estudio para incluir criterios de evaluación y regiones más

relevantes. Los resultados que se presentan aquí son parte de un estudio que incluye otros cultivos Mediterráneos y escenarios de cambio climático adicionales.

Palabras clave: Cambio climático, viticultura, prácticas de manejo, España

1 INTRODUCTION

1.1 Sector

There are in Spain, 1.23 million hectares of extremely diverse vineyards, distributed in over 360000 farms with an average area of 3.34 ha per farm (MARM, 2009): In general, farms are in poor and marginal soils trained as spur-pruned bushes with a very small potential growing and poor yields. The vineyard area associated with a "geographical indication" represents about 62% of the total area of this crop.

In Spain, the vineyard area has experienced in the 1980s and in the first half of the 90s a notable reduction and a significant improvement of the productive potential on the 1990s has happened through rejuvenation of old plantations and changes in production methods, with a tendency towards an increase of the irrigated surface which presently represents by 20 % of total vineyard surface (Sotés, 2007).

1.2 New risks: PAC and environmental, including climate change

It is expected that global temperatures may increase further by 1.8 to 4.0 ºC by 2100. This means that temperature increase since pre-industrial times would exceed 2 °C. intensifying risks in the Mediterranean (IPPC, 2007). Mediterranean may experience substantial drying (precipitation reductions of more than 25%) and warming (temperature increases of 3-5%) by 2080; at the same time, inter-annual variability is projected to increase, especially in the summer (Giorgi et al, 2008).

As farmers manage the majority of land in the EU, the Common Agricultural Policy (CAP) has a role to play in facilitating helping farmers to adapt their production to the Climate change. Climatic variations will have consequences for the availability of water resources, pests and diseases and soils, leading to significant changes in the conditions for agriculture (COM (2009) 147).

Climate change on viticulture is like to be highly variable. More in detail Impacts on the wine sector include a higher risk of frost, a shortening of the ripening period, water stress, which can be highly damaging at the maturity stage, and changes in pest and disease patterns. In the current production areas the variability of fruit production is likely to become higher SEC(2009) 417.

But also trends in energy prices and water framework directive could increase irrigation costs discouraging this practise unless it was economic feasible.

1.3 Measures to mitigate risks

In relation to the new challenges identified by the Commission in the Health Check (including climate change) the council agreed to strengthen the measures in the first and second pillar facing them and increasing up to 5% of the modulation of direct payments in 2012. These measures are focused in soil and crop management. Apart from that also agricultural insurance programmes offer climate risk management alternatives (Dismukes et al., 2004; EC, 2007; Rejda, 2002, Quiroga et al 2009).

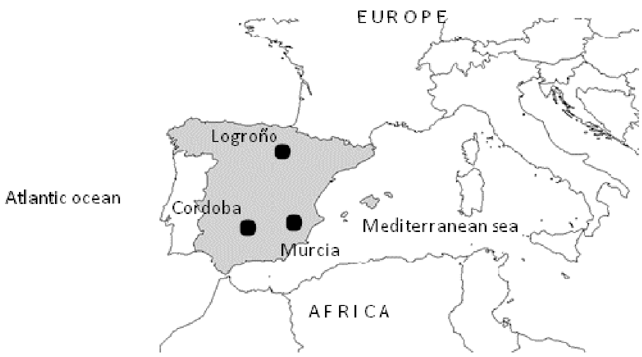
1.4 Objectives and content of the paper

We investigate the response of three grapevine producing regions in Spain, with different precipitation and temperature regimes, to potential climate changes in the Mediterranean area. We based our methodology on the evaluation of production responses to a range of emission scenarios according to different climate change policies. Multiple projections of impacts are generated based on different model of climate change. The results presented here are part of a larger study that includes several other Mediterranean crops and scenarios (Iglesias et al., 2010).

2 METHODS

2.1 Selection of the study areas

Three case studies (Figure 1) were selected as representing different climate risks which determined production in period selected. The data were taken during the 1959-2000, so we have to consider that irrigation was less developed local varieties had higher importance so did traditional management in bushes (more resistant to drought seasons and higher temperatures).



Location (log, lat, att)	T avg annual (deg C)	Precip total annual (mm)	Climatic stress under current conditions and type of agricultural system
Logroño (42.45ºN, -2.33ºW, 353m)	13.4	383	Risk of frost in spring Low risk temperature stress and drought
Cordoba (37.85 ºN, -1.10W, 92 m)	17.9	674	High heat stress during summer
Murcia (38.00 ºN, -4.83W, 0 m)	17.6	305	High heat stress during summer High risk water scarcity and drought

Figure 1. Location and description of the study sites

2.2 Statistical model to yield response

Statistical models of yield response have proven useful to evaluate the effects of extreme events such as droughts, frosts or floods (Dixon et al. 1994; Chavas et al. 2001) and climate change (Lobell et al. 2007; Iglesias et al., 2000). Here we use the model developed by Quiroga and Iglesias (2009) as specified below:

$$\mathrm{Ln}\ Y_t = \text{ɖ}Y_{t-1} + \breve{U}_0 + \breve{U}_{1Mac}Mac_t + \breve{U}_{1Fer}Fert + \breve{U}_{1Irri}Irri_t + \breve{U}_{2i}Tav_{it} + \breve{U}_{3i}Fr_{it} + \breve{U}_{4i}Prec_{it} + \breve{U}_{5i}\ Tmax_{it} + \breve{U}_6Dr_t + \text{ɓ}_{t*} + Impt^*_t + \mathring{U}_t$$

The complete econometric treatment is described in Quiroga and Iglesias 2009; Iglesias and Quiroga 2007. The formulation has been specified to test for lack of heteroscedasticity and autocorrelation of variables. All estimated parameters (α_{1-12}) are significant at the 95 percent level. The mechanisms behind the relationships derived from the statistical analysis may be implied from model coefficient. For example drought explains a significant component of yield variability of grapevines by more than 20%. Contrary to what could be expected no big differences were found in variability during two different periods 1960-1979 and 1980-2000.

2.3 Climate scenarios

Our methodology is based on the evaluation of crop responses using a range of emissions scenarios to provide information of the effects of climate change policy. Apart from taking into account yield differences and locations (Logroño, Cordoba and Murcia), we have also based our projections on 16 climate models from four different sources (CGCM, CSIRO, HadCM, PCM) and using four SRES scenarios (A1, A2, B1, B2) with different impacts in precipitation and temperature change in Spain. (Iglesias et al. 2010).

3 RESULTS

3.1 Impacts of climate change in the different region

Figure 2 show how grapevines production depends on local conditions as different regions could foresee high risks or key opportunities in wine production. In Logroño all future scenarios shows a production increase probably benefiting from a general trend of increase yields thanks to acceleration of vegetative and reproductive growth in a warmer climate (Bindi et al., 1996; Jones and Davis, 2000, Jones et al 2005). Córdoba, with a warmer growing season will experience a clear yield decrease clearly affected by the combination of warmer and dryer conditions. In Murcia impact of global warming is potentially less significant as yields could increase or decrease depending on the scenario.

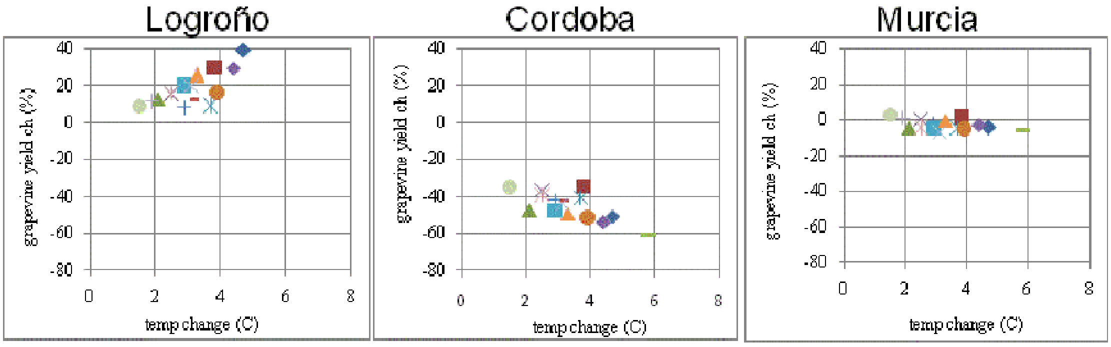


Figure 2. Future climate scenarios in the three representative sites.

3.2 Implications for policy development

Moreover the need of an holistic policy dealing with the consequences of climate change in energy or water that eventually will affect agriculture, also farm level analyses have shown that large reductions in adverse impacts from climate change are possible when adaptation is fully implemented (Mendelsohn et al 1999, FAO 2009). Work will need to be done in the short and in the long term by government agencies and advisory services to assess and identify appropriate varieties and make information on husbandry techniques available to the farming community.

Research institutes and extension services need to identify potential risks and devise appropriate strategies to deal with them (Iglesias *et al.*, 2006) at a regional level. The EC in the white paper ñadapting to climate change: Towards a European framework for actionò advance this possibility proposing specific indicators for agriculture such as an index for adaptive capacity and vulnerability multi-dimensional approach combining climatic, environmental and socio-economic factors at low spatial scale. A first approach to a risk index was developed in the three study sites trying to assess risks. With Monte Carlo simulations using statistical distributions of crop yields and analysing the distribution of probabilities in order to obtain a certain yield (Figure 2). Murcia in our case has the lower variance and Córdoba the highest.

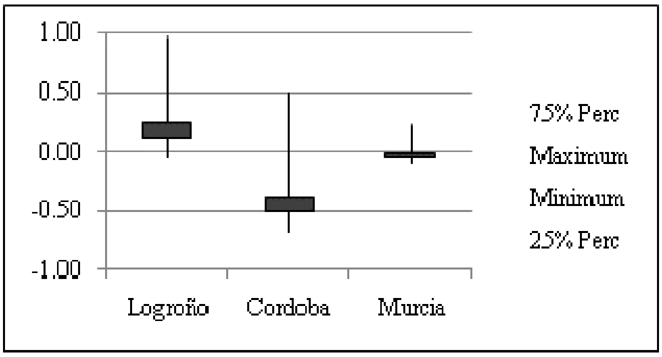


Figure 2. Yield variation derived from Monte Carlo simulations

Mullins et al 1992, Gladstones 1992 and Jones 2005 defined how temperature during the growing season can affect grape quality and viability in at least three ways through prolonged temperatures above 10 deg C, extreme heat in flowering and growth of berries and high diurnal temperature in maturation stage. This information should be used as well as water availability to identify at a regional scale, areas for suitable soil and vineyard management and adapted cultivars; and promoted or disseminated by the corresponding measures to increase resilience and keep or improve quality and yields.

3.3 Limitations of the study

Case studies were limited to three specific areas studied. It is necessary to extend this preliminary analysis to include the major grapevine producing regions in Spain and include climate indices and other tools of grapevine adequacy and quality to get a general assessment.

4 CONCLUSIONS

Using historic climate data, yields of three different traditional winegrape regions (Logroño, Córdoba y Murcia) and projections on 16 climate model from four different sources (CGCM, CSIRO, HadCM,PCM) and using four SRES scenarios (A1, A2, B1, B2) a first approach to climate change consequences was made. The result indicates that hot climate maturity regions could face the most important problems. The Model projection for Cordoba indicates grape harvest reduction up to 60% with no positive scenario even in the more positive cases. High temperatures scenarios may exceed varietally specific optimum temperature threshold. Murcia seems to have higher resilience to new combination foreseen scenarios.

Logroño on the contrary could increase production in the future with the warmer conditions, but higher yields do not guarantee quality or higher yield stability (Bindi et al., 1996; Bindi and Fibbi, 2000, Jones G. 2005). These factors should be taken into consideration in next steps to properly assess risks for climate change in vineyard production in Spain.

ACKNOWLEDGEMENTS

This research has been supported by the project CIRCE of the 6th Framework Research Programme of the European Commission.

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Climate Change and Viticulture – Facing the future

Schultz, H.R.
Forschungsanstalt Geisenheim,
von Lade Str. 1 D-65366 Geisenheim, Germany, h.schultz@fa-gm.de

Introduction

The rapidly increasing world population and the scarcity of suitable land for agricultural food production together with a changing climate will ultimately put pressure on grape producing areas for the use of land and the input of resources. For most grape producing areas the predicted developments in climate will be identical to becoming more marginal for quality production and/or to be forced to improve resource management. This will have a pronounced impact on grapevine physiology, biochemistry and ultimately production methods. Research in the entire area of stress physiology, from the gene to the whole plant and vineyard level (including soils) will need to be expanded to aid in the mitigation of arising problems. Three major challenges can be identified, one related to the lack of knowledge about how plants, micro-organisms and pathogens will respond to a rise in CO_2 concentration, temperature and a possible lack of water simultaneously under field conditions, the second is related to the evaluation of the release of nitrous oxide and methane, two of the most potent greenhouse gases, from viticultural production systems and the development of mitigation strategies, and the third on the broader aspect of resource management in the production chain of wine within the industry and possibilities for its improvement.

CO_2, abiotic and biotic stress factors

For the first challenge, the primary limitation is the establishment of sufficiently large infrastructures to simulate future climate developments such as increased CO_2 concentration and temperature under field conditions. In a recent editorial for the New Phytologist titled “an inconvenient truth” with reference to the Academy award for the best documentary film by former US Vice President Al Gore, Woodward (2007) described and analysed the dilemma between practical experiments with elevated CO_2 concentrations and the need to understand and predict the future responses of plants in the field. Aside from the fact that increasing CO_2 concentrations will impact on global temperature, CO_2 itself is generally beneficial to plant growth, although the response strongly varies between species (Long et al., 2004). However, Woodward (2007) continued that CO_2 enrichment experiments usually don’t mimic the gradual increase in CO_2 plants are experiencing in the field, but rather follow a step-up approach, and possible differences in plant responses to these approaches are unknown. Additionally, CO_2 enrichment is not usually accompanied by warming as would be predicted by climate models because of “the problem of securing long-term funding which is a bothersome limitation to a more general approach” (Woodward, 2007). Recent results from models including the physiological impact of CO_2 on plants (more biomass, reduced g) suggest that rising CO_2 will increase the temperature driven water evaporation from the oceans resulting in an increased absolute water vapour content of the air. However, the decrease in evapotranspiration over land (due to a decrease in stomatal conductance) would still lead to an overall decrease in relative humidity and to an increased evaporative demand according to current knowledge (Boucher et al., 2009). Plant surfaces should then heat up more due to stomatal closure adding to the complexity of expected responses difficult to trace and simulate in conventional experiments.

It is exactly this complexity which necessitates a more global approach to setting-up experimental systems to study the response of grapevines to the combined increase in temperature and CO_2, one of the biggest challenges ahead to understand. Few studies have investigated the response of grapevines to CO_2 either in small FACE (free air carbon dioxide enrichment) systems (Bindi et al., 1995; Bindi et al., 2001a) or in open top chambers (Gonçalves et al., 2009), but these could only describe the impact of increasing CO_2 concentration in the absence of rising air temperature. Nevertheless, the generally predicted increase in biomass was confirmed, yet the effects on water consumption remained unclear (Bindi et al., 1995; Bindi et al., 2001a). These experiments also showed that fruit sugar concentration should increase and acidity levels decrease under elevated CO_2 (Bindi et al., 2001b), but the response of other components contributing to flavour and aroma of grapes were heterogeneous and indicated a significant "chamber effect", with plants grown outside responding differently than plants in open top chambers with or without elevated CO_2 (Gonçalves et al., 2009).

Another area, which needs to receive more attention, is the effect of global warming and increase in ambient CO_2 concentration on plant-pathogen interactions. Recent results have shown that these interactions can be modified and could lead to an increase in insect aggressiveness (DeLucia et al,. 2007), population biology and the sequence of potential epidemics (Garret et al., 2006). The basis for these modifications lies within the potential modification of the genome of micro-organisms and/or insect pathogens or the expression patterns of genes (Travers et al., 2009). Thus, there is a potential threat to agricultural productions systems which goes well beyond the mere spread of diseases into areas where these have not been known previously due to global warming.

Nitrous oxide, methane and the carbon budget of vineyards

An additional challenge will be the missing information about how much viticultural production systems contribute to the release of nitrous oxide and methane, two of the most potent greenhouse gases, or how these systems could be adapted to become less of a source for these gases or even a sink (at least for methane that seems a possibility) (Dalal et al., 2003). Equally largely unknown are strategies to improve the carbon budget of vineyards, so far in most cases not included in carbon budget protocols (Carlisle et al., 2006). These topics require long-term (> 5 years) research strategies but it is important to start gathering information. To elucidate the complex interactions between compounds and management will be a challenging task but results are urgently needed in particular with respect to:

- Factors relating to the production of nitrous oxide, such as nitrogen leaching/volatilization, fertilization amount, timing and method and the interactions with management practices.

- Factors relating to vineyard carbon-sequestration such as vine biomass and cover crop biomass, since this information is absent from carbon budget protocols in the wine sector

- Factors relating to vineyard short-to-medium-term floor management such as cover crops and tillage.

- Factors relating to vineyard long-term management effects on carbon-sequestration and the interactions with other greenhouse gas emissions.

- Factors relating to methane production and uptake.

Carbon and water footprint of the wine industry

The third challenge for the wine industry is more related to the management of natural resources in the production chain for wine and the resulting carbon or water footprints. Whereas the carbon footprint for entire regions has been roughly estimated (examples for the Champagne and Bordeaux regions, (CIVC, 2007; CIVB, 2009) and some strategies devised to reduce it, the water footprint is an upcoming issue which will affect agriculture in general. Water management is no longer an issue restricted to individual countries or river basins. Even a continental approach is not sufficient. The water footprint of Europe – the total volume of water used for producing all commodities consumed by European citizens – for example has been significantly externalised to other parts of the world. Europe is for example a large consumer of sugar and cotton, two of the most thirsty crops (Hoekstra, Chapagain, 2008). Rising food demand and growing water scarcity (IPCC, 2008) will put increasing pressure on agriculture, which is currently using up about 70% of the world's fresh water resources for irrigation. Currently, issues such as the amount of water imported by a country through products (including the *direct* input of water used for its production and the *indirect* water used for services around this product (transport or packaging) are emerging in the context of water neutral production budgets of countries or sustainability strategies of super market chains. Spain, for instance, is exporting 189 Mm^3 water per year to the UK alone captured in products related to grape production (Chapagain, Orr, 2008). Although these calculations and budgets have not yet had impacts on production strategies in the wine industry, the firsts signs are appearing in California and Australia and will ultimately have a feed-back effect on research related to irrigation management and water use efficiency strategies in viticulture. Additionally, the water issue can not be seen strictly independent from other climate related problems, since the release of nitric oxide and CO_2 from agricultural land contributes significantly to the "greenhouse effect", and since this release depends on soil water content, irrigation management and organic matter content (Avrahami, Bohannan, 2009). For grape production, however, we have currently no information on the contribution and/or possible management strategies of these effects, another significant challenge for future research.

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SYSTÈMES DE PRODUCTION INNOVANTS EN VITICULTURE DURABLE, FACE AUX CHANGEMENTS DU CLIMAT ET DE LA CONSOMMATION

Alain CARBONNEAU, Professeur de Viticulture, Président du G*i*ESCO ,
Montpellier SupAgro, IHEV, bât.28, 2 place Viala, F 34060 Montpellier cedex2,
carbonne@supagro.inra.fr
Dr. Hernan OJEDA, Unité Expérimentale INRA, de Pech Rouge,
ojeda@supagro.inra.fr
Ing. Jean-Louis ESCUDIER, Directeur Unité Expérimentale de Pech Rouge,
escudier@supagro.inra.fr

Titre : Systèmes de production innovants en Viticulture durable, face au changement du climat et de la consommation.

Résumé
Suite à une série de recherches sur les systèmes de conduite (Lyre pliable, Taille Minimale ou Taille simplifiée), les variétés résistantes aux parasites, les technologies d'élaboration des vins ou de nouveaux produits, une synthèse des innovations sélectionnées qui tiennent compte des changements liés au climat et aux modes de consommation est proposée. Un dispositif expérimental de divers modèles de systèmes de production qui intègrent les innovations précédente s'impose dans le nouveau contexte d'une Viticulture durable définie pour la zone méditerranéenne de la France.

Mots clés: Viticulture durable, qualité de l'environnement, préservation des ressources, production, rentabilité, qualité de vie, raisin de table, qualité du vin, consommation du vin, terroir, changement climatique, matériel végétal, système de culture, système de conduite, Taille Minimale-non taille, Lyre, Lyre pliable, protection intégrée, date de récolte, technologie œnologique, électrodialyse membrane bipolaire, acicification.

Title: Innovative production systems in sustainable Viticulture, facing the climate and consumption changes.

Summary
Innovative production systems in sustainable Viticulture, facing the climate and the consumption changes.
Following series of researches on training systems (foldable Lyre, Minimal Pruning or simplified Pruning), disease resistant varieties, technologies for wine or new products processing, a synthesis of selected innovations which take in account the changes due to climate or consumption behaviours is proposed. An experimental design of different models of production systems which integrates the previous innovations is necessary in the new context of a sustainable Viticulture defined for the Mediterranean zone of France.

Key words : Sustainable viticulture, quality of environment, resource preservation, production, economic viability, quality of living, table grape, wine quality, wine consumption, terroir, climate change, genetic material, cultivation system, training system, Minimal Pruning-no pruning, Lyre, foldable Lyre, integrated protection, harvest date, wine technology, electrodialysis with bipolar membranes, acidification.

Titulo : Sistemas de producción innovadores en Viticultura sostenible ante los cambios climático y del consumo.
Resumen:
Tras una serie de investigaciones sobre sistemas de conducción (Lyra plegable Poda mínima o Poda simplificada), variedades resistentes a las plagas y enfermedades y tecnologías para elaboración de vinos o de nuevos productos, se propone una síntesis de innovaciones escogidas por tener en cuenta los cambios relacionados con el clima y los patrones de consumo. Un dispositivo experimental de diferentes modelos de sistemas de producción que integren las innovaciones anteriores se necessita en el nuevo contexto de una Viticultura Sostenible definida para el área mediterránea de Francia.
Palabras clave: Viticultura sostenible, calidad ambiental, conservación de recursos, producción, rentabilidad, calidad de vida, uva, vino de calidad, consumo de vino, suelo, clima, material vegetal, sistema de cultivo, sistema de conducción, Poda mínima-no poda, Lira, Lira plegable, protección integrada, cosecha, tecnología enológica, electrodialysia con bipolaras membranas, acidificacion.

INTRODUCTION

La Viticulture durable doit d'abord être définie. La Société Américaine d'Agronomie a proposé la définition suivante pour l'agriculture durable : *« A sustainable agriculture is one that, over the long term, enhances environmental quality and the resource base on which agriculture depends; provides for basic human food and fibres needs; is economically viable and enhances the quality of life for farmers and society as a whole. »*
Par analogie, la définition de la Viticulture durable serait: "La **Viticulture durable** est celle qui, sur le long terme, améliore la qualité de l'environnement et la ressource de base dont elle dépend ; assure les demandes de l'homme en produits issus de la culture de la vigne, notamment en raisins et en vins ; est économiquement viable et améliore la qualité de vie des vignerons et de la société dans son ensemble. »
Les objectifs de la Viticulture durable sont donc :
- qualité de l'environnement
- préservation des ressources
- assurance de production
- viabilité-rentabilité économique
- qualité sociale-de vie (existentielle).

NB : Ces objectifs, notamment le respect de l'environnement et du consommateur, n'imposent nullement un recours exclusif à des substances qualifiées de 'naturelles' ce qui est requis en viticulture 'biologique', ni encore moins à des pratiques ésotériques dont le contenu scientifique n'est pas reconnu ce qui est la base de la viticulture 'biodynamique'.

CONSTRUCTION D'UNE DÉMARCHE DE VITICULTURE DURABLE

Sur la base des principes de la Viticulture durable énoncés ci-avant, il convient de construire une démarche adaptée qui prenne en compte, pas à pas, les divers éléments de durabilité afin de mettre au point un système de production tout entier, en tenant compte des effets attendus du changement climatique (Carbonneau, 2006).

1) Potentialités du site :

La première étape d'une démarche de mise au point d'une viticulture durable est sans doute de connaître les potentialités du site ou de l'entreprise. Le climat étant le facteur dominant de l'environnement naturel, il est nécessaire de connaître les caractéristiques climatiques du vignoble aussi précisément que possible, notamment grâce à la méthode validée à l'échelle internationale 'Classification Climatique Multicritères – CCM' (Tonietto et Carbonneau, 2004). Le calcul des indices IS (Indice de Sécheresse), IH (Indice Héliothermique), IF (Indice

de Fraîcheur des nuits), avec éventuellement quelques indices complémentaires spécifiques du lieu, permet de positionner le vignoble considéré par rapport à des références internationales, ainsi que de simuler son devenir au cours du changement climatique.
Rappelons que la Viticulture peut s'adapter au changement climatique par un choix approprié du site, en fonction de la latitude, de l'altitude, de l'exposition du vignoble.
La connaissance des sols complète celle du climat pour caractériser les **Unités Terroir de Base**. A ce niveau, il convient d'estimer la profondeur d'enracinement et la réserve hydrique et minérale potentielle, en plus de la structure, la texture , la composition chimique, l'activité biologique.
Sur ces bases, il est possible d'établir un premier bilan du potentiel viticole général, et des chances de réussite d'un modèle de Viticulture durable.

2) Contexte socio-économique :

Les choix au sein des potentialités se font ensuite en fonction des conditions socio-économiques, en considérant les marchés des vins (international, national, local), l'évolution des habitudes de consommation, les conditions locales au niveau de la gestion de l'entreprise et du territoire. La rentabilité de la Viticulture durable doit être garantie au mieux, de la parcelle à la bouteille, aussi bien par la maîtrise de l'offre et des coûts de production, que par l'adéquation à la demande des produits sur les marchés. Un véritable **marketing des vins issus de Viticulture durable** doit être entrepris.

3) Adaptation du matériel végétal :

Dans la plupart des cas, le choix du matériel végétal, cépage ou porte-greffe, reste classique, notamment en raison de la notoriété de certains cépages de cuve. Toutefois, les progrès de la sélection variétale, en particulier grâce à Alain Bouquet (2009) de l'INRA Montpellier, permettent au niveau de l'expérimentation de choisir des **variétés poly-résistantes** (figure 1).

Figure 1. Démonstration au Domaine INRA de Pech Rouge de la poly-résistance de 3 génotypes obtenus par Alain Bouquet, issus de 5 rétro-croisements sur *Vitis vinifera* à partir d'un hybride *Muscadinia rotundifolia* x Cabernet-Sauvignon, par rapport à 3 témoins.

Leur qualité œnologique a déjà été bien jugée sur les premiers millésimes. Il convient de poursuivre dans la durée cette évaluation en même temps que le suivi de la production, de la croissance et de la durabilité des résistances.

Une première estimation de leur intérêt économique au niveau de la réduction des coûts de production (figure 2), et de leur intérêt environnemental au niveau du bilan de carbone (figure 3), a été réalisée par Hernan Ojeda sur des références obtenues au domaine INRA de Pech Rouge.

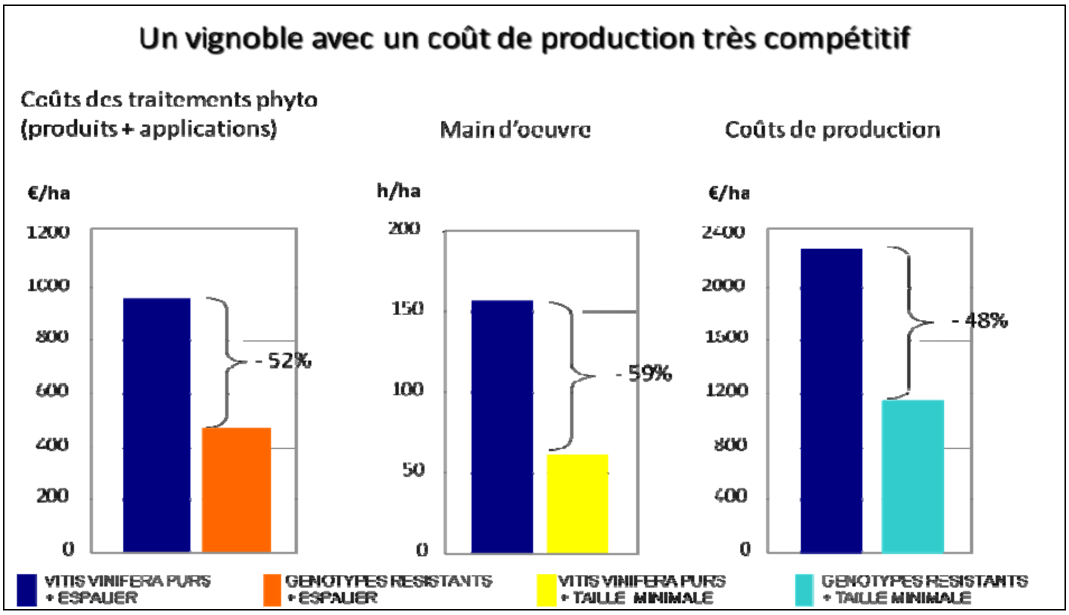


Figure 2. Avantages économiques d'une variété résistante par rapport à une variété classique, conduites soit en Espalier, soit en Taille Minimale-non taille.

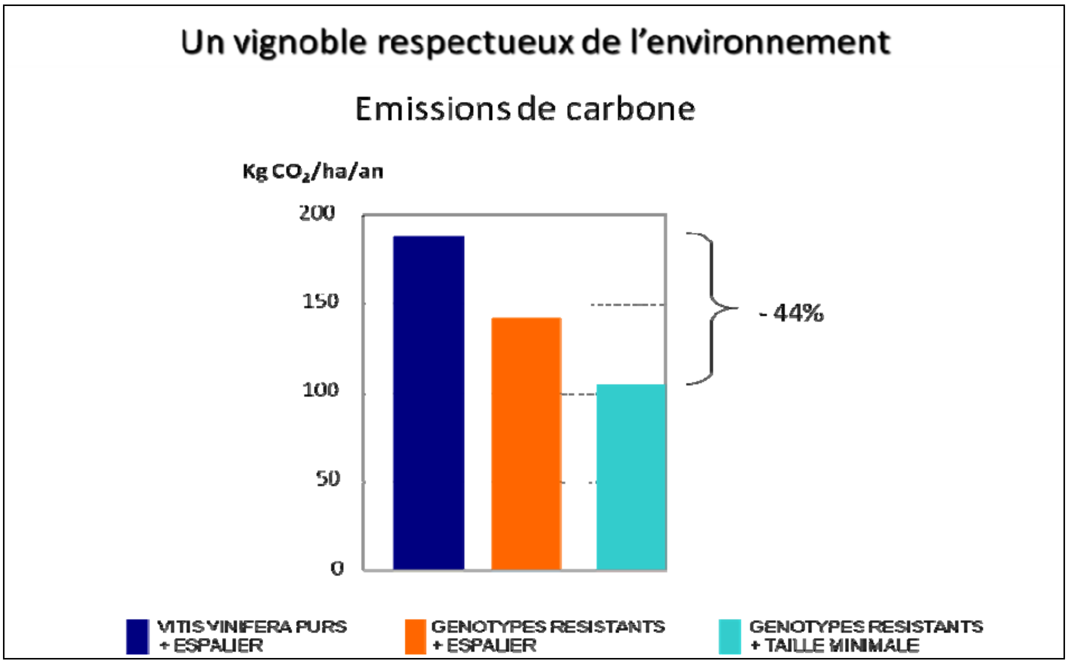


Figure 3. Estimation des émissions de CO_2 pour des cépages classiques ou résistants, conduits en Espalier ou en Taille Minimale-non taille. La réduction des émissions de carbone est liée à la réduction du nombre des interventions culturales, soit les pulvérisations, soit la taille.

4) Choix du système cultural :

Au terme d'une série de recherches sur les systèmes de conduite et de culture du vignoble au cours des 30 dernières années, il est possible de sélectionner 2 nouveaux modèles de culture du vignoble permettant à la fois un progrès selon les critères de durabilité, et une meilleure adaptation aux demandes du marché des vins. (Carbonneau et al., 2007)

Au niveau du système de conduite, la référence de base est la vigne conduite en **Espalier** à feuillage haut et en rangs étroits séparés de 2m environ, qui correspond à l'optimum de l'architecture 'plan relevé'. La vigne en **Lyre** (vendange manuelle) ou en **Lyre pliable** (vendange mécanique), apporte un gain qualitatif général, une possibilité d'augmentation du rendement (20-30%), une meilleure maîtrise de la vigueur et une réduction des coûts en vendange manuelle. Cette technique est recommandée en Viticulture durable pour des productions de vins de haut de gamme voire de cru, ou de raisins de table. La vigne en **Taille Minimale-non taille** permet de réduire au plus bas possible les coûts de production, une mécanisation intégrale, et sous réserve du contrôle de la puissance des ceps et de la maîtrise de leur alimentation hydrique, à une réponse qualitative intéressante pour des niveaux de production d'environ un tiers supérieur. Il est à noter aussi une moindre sensibilité aux maladies de la souche constatée à ce jour, et un espoir de bonne durabilité. La taille Minimale-non taille est conseillées dans de nombreux cas pour les productions de vins d'entrée et de cœur de gamme.

Les systèmes culturaux durables étudiés s'appuient essentiellement sur ces types de conduite, combinés à une **gestion raisonnée de l'eau et de l'azote**, notamment sur l'**enherbement contrôlé** et/ou **l'irrigation qualitative**. Le raisonnement de ces interventions (pilotage de l'irrigation, modalités d'enherbement), avec pour l'aspect hydrique la mesure référence du potentiel hydrique foliaire de base, a pour objet de maîtriser des niveaux modérés de contrainte hydrique afin de garantir la qualité (Carbonneau et al., 2007). Des exemples probants et innovants comme la combinaison 'Lyre-enherbement' dans des coteaux bourguignons chez des vignerons d'Auxey-Duresses, ou des essais de Taille Minimale-non taille dans diverses situations de vigueur à Montpellier SupAgro/INRA, permettent un progrès dans le sens de la viticulture durable.

NB : La Lyre pliable peut être utilisée, par repli du palissage à la fin de la véraison, comme un moyen d'économiser l'eau sous sécheresse en l'absence d'irrigation, et pas seulement pour permettre la récolte mécanique. La Taille Minimale de son côté permet d'obtenir des vins moins alcoolisés (1-2 % TAV) avec une meilleure acidité sous condition chaude et sèche.

5) Protection intégrée :

Les décisions en matière de protection intégrée du vignoble sont particulièrement spécifiques à chacun. Pour cette raison, un seul exemple est pris d'un vignoble commercial situé en *haut Languedoc* sous un macroclimat de type méditerranéen tempéré (Hormazabal, Lyon, Carbonneau, 2002). Dans ces conditions les choix suivants ont été validés depuis 2002.

- Lutte contre les ravageurs : Le choix de la *suppression de tout insecticide* a été fait dans cette zone où la présence de la cicadelle verte vectrice de la Flavescence dorée n'a pas été constatée. L'expérience depuis 2002 a montré que les dégâts d'acariens en tout genre et de vers de la grappe ont été ici absents. Dans le cas où ils apparaîtraient, le recours à un insecticide biodégradable serait envisagé, en particulier à titre expérimental, l'huile de Neem qui a fait ses preuves de polyvalence dans d'autres vignobles du monde.
- Lutte contre les parasites :

  Aucun moyen de lutte directe contre la *pourriture grise* n'a été utilisé, le contrôle se faisant par la maîtrise de la vigueur (enherbement de la partie basse du coteau), un palissage ouvert en Lyre, un effeuillage tardif localisé occasionnel selon les années.

  La lutte contre l' *oïdium* a pu se faire dans de bonnes conditions avec une première intervention systématique avec du soufre mouillable en tout début de croissance (en mélange avec un autre fongicide anti-péronosporales), suivie d'une ou deux (selon les années) applications de lactoferrine (extrait de lait de vache) dont l'efficacité s'est avérée suffisante du moins sous pression d'intensité moyenne avant et au moment de la floraison, et après nouaison selon les risques de l'année une dernière application au soufre mouillable.

La lutte contre l'*excoriose* s'est avérée correcte grâce à une application précoce (en même temps que le premier traitement au soufre) d'un fongicide relativement polyvalent à effet préventif à base de phosétyl-aluminium (inducteur d'éliciteurs, stimulateurs de défenses naturelles).

La lutte contre le *mildiou* et le *black rot* a été fondée sur l'usage de cette même molécule de phosétyl-aluminium, en général en association avec l'anti-oïdium, jusqu'à la nouaison. Après cette date, une dernière application de bouillie bordelaise a permis d'assurer un contrôle total du mildiou et d'autres parasites.

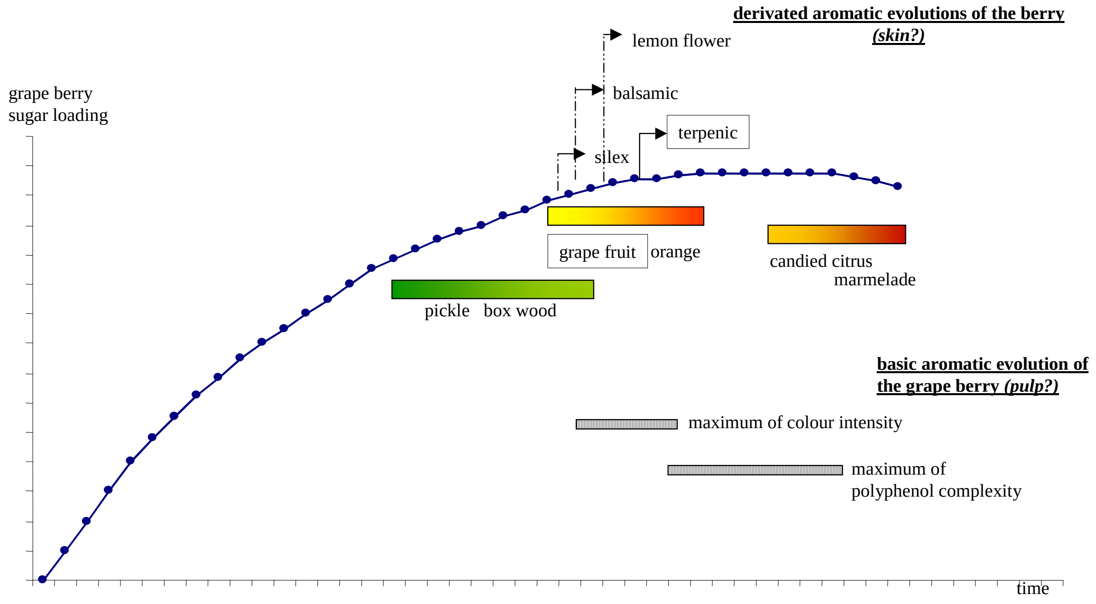
En moyenne, **4 pulvérisations de fongicides par an** (aux doses habituellement recommandées) ont été effectuées et permis d'obtenir un excellent état sanitaire du feuillage et des raisins (à l'exception de quelques attaques occasionnelles et supportables de black rot). *Le changement climatique permet ici de réduire la pression parasitaire estivale*. Il est à noter que la première application est systématique, et que les deux ou trois suivantes sont calées sur les risques réels de pression parasitaire. Le niveau des impacts de ces produits sur l'environnement n'est pas connu ici, mais il est certainement extrêmement faible ou nul ; de même celui des résidus dans les vins sera prochainement évalué, sachant qu'en fonction du nombre réduit de traitements et de leur positionnement essentiellement avant la fin de la nouaison, il devrait être aussi extrêmement faible. Dans la suite de l'expérimentation le recours à une autre molécule élicitrice que le phosétyl-aluminium sera envisagé si elle répond au double critère d'efficacité et de biodégradabilité.

Il est à noter que cet exemple de stratégie de traitement, à l'exception de l'usage du phosétyl-aluminium, répond au cahier des charges de la viticulture biologique. Mais le choix de cette molécule a été préféré à celui systématique du 'cuivre' afin de mieux préserver l'intégrité chimique et biologique du sol, ainsi que d'éventuels excès de résidus dans les vins. Ce cas de figure montre la distinction qui existe entre la *viticulture durable* et la *viticulture biologique*, la viticulture durable pouvant utiliser des produits de synthèse s'ils sont efficaces et pas ou très peu 'polluants', et s'interdire des produits naturels si leurs effets sont contraires aux objectifs, ce qui est le cas de l'usage immodéré du cuivre.

6) Date de récolte :

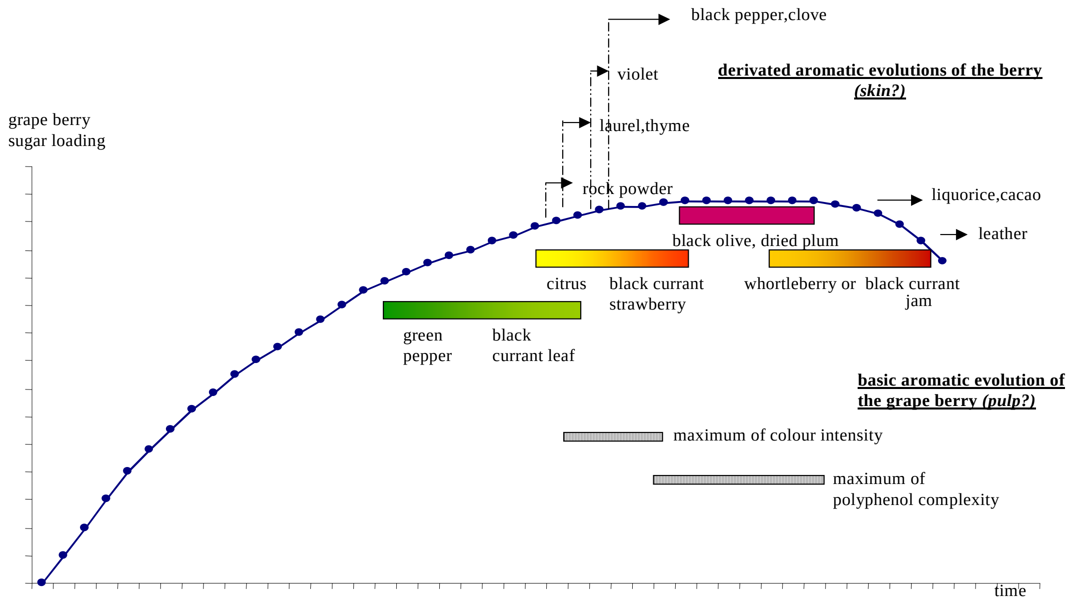
Le choix de la date de récolte, au terme d'un suivi suffisamment précis de la maturation du raisin (rappel de la méthode OIV de prélèvement de 200 baies par fractions de grappes dans la zone médiane du cep) est fonction de la physiologie de la maturation du raisin et du type de vin que l'on cherche à commercialiser. A ce sujet, il a été montré l'intérêt de mesurer l'évolution de l'activité physiologique du raisin pendant sa maturation, par la 'charge en sucres du raisin' ou quantité de sucres par raisin, et non pas par la concentration en sucres dans le raisin (Hunter, 2004 ; Carbonneau et al., 2007). Sur cette base, l'expérience conjointe des suivis de maturation et de l'analyse (sensorielle et chimique) des vins, permet d'anticiper le profil sensoriel des futurs vins par référence au niveau d'évolution de la charge en sucres du raisin (Carbonneau, 2007). Les figures 1 et 2 illustrent ce point pour les cépages Sauvignon et Syrah respectivement.

Cette méthode, même si elle n'est pas spécifique à la viticulture durable, est particulièrement utile dans ce contexte où l'on essaie de concilier l'optimisation de la qualité du produit et son succès économique permettant à l'entreprise d'être rentable.



UNFOLDING OF TYPICITY (links between aromatic impressions)
Typical SAUVIGNON

Figure 4. Relation entre la charge en sucres du raisin (g sucres/baie) en fonction du temps depuis la véraison et le profil aromatique des vins obtenus avec des dates de récolte étalées. A noter la présence d'un 'déroulé fruité' suivant la maturation, et les autres séries aromatiques 'dérivées'. Exemple du Sauvignon en Languedoc.



UNFOLDING OF TYPICITY (links between aromatic impressions)
Complex SYRAH

Figure 5. Relation entre la charge en sucres du raisin (g sucres/baie) en fonction du temps depuis la véraison et le profil aromatique des vins obtenus avec des dates de récolte étalées. A noter la présence d'un 'déroulé fruité' suivant la maturation, et les autres séries aromatiques 'dérivées'. Exemple de la Syrah en Languedoc.

7) Technologies de transformation :

Les technologies de transformation interagissent avec le système cultural pour déterminer les caractéristiques des produits. Elles ont, elles aussi, leur effet au niveau de la durabilité du système de production. Il est intéressant en général d'utiliser des techniques physiques propres, comme dans l'exemple de la régulation du pH par électrodialyse avec membranes bipolaires (Ojeda et al., 2007). L'augmentation du pH des vins en fonction du changement climatique notamment, pose des problèmes d'équilibre et de conservation des vins. La technique d'électrodialyse à membrane bipolaire aboutit dans des conditions définies à abaisser significativement le pH en respectant les autres équilibres du vin (tableau 1). Ces traitements peuvent être réalisés facilement sur les sites de production ou de conditionnement des vins en particulier par des unités mobiles. De même, cette technique permet maintenant de corriger l'excès d'alcool constaté dans de nombreux vins (Bes et al., 2009), et est déjà utilisée à cet effet en Europe, aux USA et en Australie. La combinaison de cette technique avec des systèmes de conduite capables de réduire le pH comme la Taille Minimale-non taille, offre une solution efficace et compatible avec une viticulture durable au problème de la maîtrise du pH, en particulier pour les vignobles méditerranéens.

Tableau 1: Caractéristiques chimiques et comparaison entre différents types de vins, traits ou non traits par électrodialyse avec membranes bipolaires.

Paramètre	Vin rouge 1		Vin rouge 2		Vin rouge 3		Vin Muscat 1		Vin Muscat 2	
	Non traité	Traité	Not treated	Treated	Not treated	Treated	Not treated	Treated	Not treated	Treated
Extrait sec (g/l)	26.1	24.5	27.6	27.1	25.8	24.5				
Sucres résiduels (g/l)	1.3	1.3	1.3	1.3	1.4	1.5				
Alcool (% at 20°C)	11.7	11.7	11.3	11.3	11.8	11.8	15.3	16.3	16.7	15.6
pH	4.03	3.79	3.83	3.68	3.68	3.46	3.68	3.41	3.68	3.6
Acidité totale (g/l H_2SO_4)	2.75	3.25	3.30	3.70	3.20	3.65	3.5	4.25	3.35	4
Acid. volatile (g/l H_2SO_4)	0.57	0.55	0.45	0.46	0.45	0.43	0.29	0.34	0.5	0.53
Acide tartrique (g/l)	0.3	0.3	0.5	0.4	1.6	1.6	1.75	1.75	1.6	1.7
Acide malic (g/l)							0.90	0.87	0.77	0.99
Acide lactique (g/l)	2.5	2.4	3.3	3.5	1.6	1.6				
SO2 libre (mg/l)	32	40	16	17	27	26	7	8	8	9
SO2 total (mg/l)	76	86	44	44	92	90	80	80	58	58
Potassium (mg/l)	1840	1520	1860	1570	1410	1100	1374	1180	1511	1401
Sodium (mg/l)	32	21	47	43	31	28				
Calcium (mg/l)	66	63	128	103	57	49	91	82	110	108
Abs. 420 nm (1cm)	2.57	2.60	2.53	2.34	2.99	2.98				
Abs. 520 nm (1cm)	2.81	3.22	2.37	2.30	3.35	3.65				

Conclusion : Ces premiers essais montrent la faisabilité d'une Viticulture durable dans les régions méditerranéennes françaises

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BUD DORMANCY RELEASE INDUCTION IN ROSE NIAGARA (*Vitis vinifera* x V. *labrusca* L.) GRAPEVINE THROUGH GARLIC EXTRACT IN SUSTAINABLE VITICULTURE.

Erasmo J. Paioli-Pires[1*], João D. G. Maia[2], Mara F. Moura[1], Renato V. Botelho[3], Maurilo M.Terra[1], Marco A. Tecchio[1]
[1]Instituto Agronômico (IAC). Av. Barão de Itapura 1481. CP 28. 13012-970 Campinas. Brasil.
[[*]ejppires@iac.sp.gov.br, mouram@iac.sp.gov.br, mmterra@iac.sp.gov.br, tecchio@iac.sp.gov.br]
[2]Embrapa Uva e Vinho. Córrego da Barra Bonita. CP 241. 15700-000 Jales. Brasil.
[dimas@cnpuv.embrapa.br]
[3]Universidade Estadual do Centro-Oeste. R. Simeão Varella de Sá 3. 85040-080 Guarapuava. Brasil.
[rbotelho@unicentro.br]

ABSTRACT
In temperate climate the grapevines have a dormancy period in the winter and need a chilling exposure necessary to budburst in the next spring. Can growth continuously when cultivated in warm-climate regions. In Brazil, in regions with mild winters, the chemicals treatments to bud breaking dormancy are $CaCN_2$ e H_2CN_2. With the increasing restrictions on use of synthetic substances in sustainable viticulture production, the purpose of this study was to examine the effect of garlic extract 0, 1.5, 3.0, 4.5, 6.0 added to mineral oil 2%, compared with H_2CN_2 2.5% or 3% and mineral oil 2% on budbreak, shoot growth and production of Rose Niagara grapevines in vineyards located in Jales at 20°016'08"S., 50º32'45"W., 478m asl, pruning in March 25 and May 28, 2009; and Jundiaí at 23º11'11'S., 46º53'06'W., 761m asl pruning in August 17, 2009, São Paulo Stat, Brazil. The treatments were effectives for bud dormancy release in Jundiaí and in Jales in the pruning of March 25. In Jales in the pruning of May 28, the effective treatment was H_2CN_2 3%

RESUMEN
En el clima templado la vid presenta un período de dormancia requiriendo un acumulo de horas de frío para brotar en la primavera siguiente. Puede hasta vegetar continuamente cuando cultivada en regiones tropicales. En Brasil, en regiones de invierno ameno, los químicos para romper la dormancia son $CaCN_2$ y H_2CN_2. Con la restricción de sustancias sintéticas para la viticultura sostenible, en este estudio investigaran el efecto del extracto de ajo 0, 1,5, 3,0, 4,5, 6,0 añadido a óleo mineral 2%, comparados con H_2CN_2 2,5% o 3% y óleo mineral 2% en la ruptura de la dormancia de yemas, desarrollo de los brotes y en la producción de la vid Niagara Rosada en viñedos ubicados en Jales _ 20°016'08"S., 50º32'45"O., altitud de 478m, podados en 25 de marzo y 28 de mayo de 2009; y Jundiaí a 23º11'11'S., 46º53'06'W., altitud de 761m podado al 17 de agosto de 2009, Estado de São Paulo, Brasil. Los tratamientos fueram efectivos para romper la dormancia de las yemas de la vid en Jundiaí y en Jales en la poda al 25 de marzo. En Jales en la poda al 28 de mayo, el mejor tratamiento fue H_2CN_2 3%.

INTRODUCTION

The grapevine, typical deciduous species of temperate climate is broadly cultivated in tropical and subtropical areas, like the zones of cultivation of São Paulo State. In such areas the minimum temperatures in the rest period not always fulfill the needs of chilling required for the species, usually propitiating a late bud breaking, reduced and desuniform sprouting and, almost always causing a pronounced decrease in yield, often creates significant economic problems. According to Westwood (1982), Lyon *et al*. (1989) the wide range of accumulated hours of cold demanded for this specie, between 150 and 1200 hours in temperatures ≤7,2ºC, can be explained by the high genetic variability, and the great amount of varieties distributed in a wide range of climates

Previous literature indicates that the exposition of the buds to chilling inhibits the activity of the catalase enzyme (EC 1.11.16) (Nir *et al*., 1986). Catalase is an enzyme present in aerobics cells that decomposes the hydrogen peroxide (H_2O_2) in molecular oxygen and H_2O. Its physiologic function is eliminated the excess of H_2O_2 produced during the cellular metabolism avoiding its accumulation and consequent cellular damage. However, multiple evidences have shown that H_2O_2 is a molecule that acts as a chemical signal generated by plants in response to biotic and abiotic stress (Prasad *et al*., 1994; Bartosz, 1997; Foyer et al., 1997).

One of the principal effects linked to the cyanamide at the budbreaking, is due to the inhibition of the activity of catalase. The increased level of H_20_2 might cause the activation of the pentose-phosphate pathway, and thus lead to dormancy termination, bud burst and rapid growth (Nir *et al.,* 1984). When the activity of the catalase decrease, an oxidative stress will be induced in several systems due to an increment of the hydrogen peroxide content (Or *et al*., 2000; Shulman *et al*., 1986). Researches have demonstrated that the cyanide and cyanamide contain the very reactive –C≡N group which react with to the enzyme-Fe of catalase, thus inhibiting the decomposition of H_2O_2 which is poisonous to plant cell (Hendricks and Taylorson, 1975, cited by Amberger, 1984). The increase of the peroxides content leads to a switch induced a breathing change from the pathway Embden-Meyerhoff-Parnas to the phosphate-pentose pathway, leading to a reduced nucleotides, which are essential for intensified metabolism and synthesis, and consequently to budbreaking (Amberger, 1984). Or *et al*. (2002), demonstrated that concomitantly to the changes produced in the catalase levels after the application of hydrogen cyanamide, changes occur in the expression of some genes, especially those responsible for the regulation of the catalase genes. The same authors identified the transcript _ genetic information _ of budbreaking of Vitis vinifera after the application of hydrogen cyanamide, which were identified as protein kinase (GDBRPK) and SNF protein kinase. These transcriptors would act as sensors of the stress signal.

Therefore, currently both hydrogen cyanamide (H_2CN_2) and calcium cyanamide ($CaCN_2$) are recommended in Brazil for budbreaking induction of grapevines (Pires and Botelho, 2002). For the Jundiaí region, $CaCN_2$ 20% or H_2CN_2 2.5% has promoted bud breaking of the grapevine Niagara Rosada in 100% of the buds (Pires *et al*., 1985).

Among the products allowed in the organic agriculture they are the sulfur and yours derived (OMRI, 2010).

Aiming to find out natural products, that could substitute the cyanamide in the budbreakink of the grapevine, which could assist the sustainable production of the vineyards, Kubota & Myamuki (1992) demonstrated that the application of garlic paste on the pruning cut in vine branches "Moscatel of Alexandria" promoted the budburst in a way efficient as the calcium cyanamide 20%.

Kubota *et al*. (1999) related that the active substances in garlic to which the break dormancy in grapevines are related the sulfide are volatile compounds with two allyl groups, particularly the diallyl disulfide ($C_6H_{10}S_2$); although other mono, tri and tetra allyl sulfides can also be involved in to break off the dormancy of the grapevine buds. However the mode of action of the substances supra mentioned in the vine metabolism it was not yet cleared (Kubota *et al*., 2002). The mechanism by which sulphur compounds can induce bud breaking continues to be unknown. However, progress has been made in elucidating the implied routes in the regulation of sulphur in relation to the vegative growth of plants (Hawksford and De Kok, 2006, cited by Vargas-Arispuro et al., 2008). In the process of assimilation of sulphur by the plant, inorganic sulphur is fixed as cysteine after a process of reduction (Saito, 2000, cited by Vargas-Arispuro et al., 2008). Cysteine is the initial material for the production of reduced glutathione, which is responsible for detoxing cells through the elimination of free radicals and reactive species that accumulate during different types of stress (Saito, 2004; Zang, 2004, cited by Vargas-Arispuro et al., 2008). If the sulphur molecules derived from garlic can be assimilated by the plant in the latent stage, it can favor the detoxification of the plant and promote bud breaking. At the same time, exogenous applications of reduced glutathione induced bud breaking when it was applied on buds of grapevines of cv. Delaware (Tohbe *et al.,* 1998, cited by Vargas-Arispuro et al., 2008), thus being a possible mechanism for increasing output of this tripeptide.

Single bud cuttings were sprayed with 1.5 or 3.0% garlic extract or 1.5% hydrogen cyanamide. The controls were treated with distilled water. Three lots of canes were submitted to 0, 168, 336, or 508 chilling hour ≤6.0°C, respectively, before spraying. All treatments improved and advanced bud sprouting over the control. The most effective treatment for bud dormancy release was 1.5% H_2CN_2, with up to 80% bud sprouting after 35 days, even for unchilled cuttings. Garlic extract also promoted bud sprouting, attaining greater than 70% budbreak after 35 days for cuttings chilled for 168, 336, and 504 hr. (Botelho *et al*., 2007).

The effects of various chemicals and their concentrations on budbreak of Pione grapevine (*Vitis labrusca* L. x *V. vinifera* L.) were studied. Both 5% and 2% of H_2CN_2 accelerated budbreak significantly and resulted in uniform budbreak, especially at 5 %. $C_6H_{10}S_2$, only a 10 % solution showed any effectiveness in budbreak. As for $C_6H_{10}S_2$, further investigation is needed to establish suitable concentrations and methods for its applications (Potjanapimon *et al*., 2007).

This study was carried out to examine the effect of the garlic extract with mineral oil, compared with the hydrogen cyanamide or mineral oil on budbreaking, on the development of the branches and on the production of the "Rose Niagara" grapevine under a sustainable viticulture crop.

MATERIALS AND METHODS

The experiment was carried out in 2009, in a commercial "Rose Niagara" grapevine vineyards, under the microclimate of Jales at 20°016'08"S., 50º32'45"W., 478m asl with climate classified as Aw; and of Jundiai at 23º11'11'S., 46º53'06'W., 761m asl and climate classified as Cfa, São Paulo State, Brazil.

In Jales, the grapevines were about eleven years old, spaced 3x2m. The plants were grafted on the rootstock IAC 766 (106-8Mgt x *Vitis caribœa*). The training system was trellis with unilateral cordon-training to submit at cane pruning with 6 nodes per cane. The vineyards were pruned in 03/25/2009 and 05/28/2009.

In Jundiai, the grapevines were about nine years old, spaced 2x1m. The plants were grafted on the rootstock IAC 766 (106-8Mgt x Vitis *caribœa*). The training system was cordon with spur-pruning. Each cordon had six branches that were pruned with one bud. The vineyard was pruned in 08/17/2009.

The treatments included five doses of garlic extract (Bioalho®) at 0, 1.5, 3.0, 4.5, 6.0 added to mineral oil 2%, mineral oil 2% and doses of H_2CN_2 2.5% or 3%. The chemicals were applied, with a painting roll (Figure 1), immediately after the pruning of the branches with the bud in dormant stage (Eichhorn and Lorenz, 1977). The garlic extract Bioalho®, soluble in water, is a natural product obtained from cold extraction by pressing the garlic. In the H_2CN_2 solutions a non ionic adjuvant was added to 2%.

The experimental design was completely randomized, with eight treatments, six replicates and parcels constituted by one plant. The analyzed variables were time to the budbreaking, sprouting percentage, cycle and number of bunches for plant.

The effect of the garlic extract was evaluated by a polynomial regression. The performance of H_2CN_2, mineral oil and the garlic extract were evaluated by the Tukey's test at 5% probability.

Both experiments were harvested when the fruits presented a soluble solids average content of 17ºBrix

RESULTS AND DISCUSSION

The experiment accomplished in Jales considered two situations: a) pruning on March 25, under minimum temperatures in the general, above 20ºC (Table 1), and therefore buds in paralatency, that is, in rest due to the apical bud dominancy , but with potential capacity to sprout (Pinto et al., 2008), therefore there was no effect of the chemicals, applied after pruning, in the buds dormancy breaking (Table 2); b) soon after pruning, air temperature fell down to 5ºC (Table1), inducing the plant to enter in endolatency, that is, buds will only bloom after the accumulation of a certain number of hours of chilling or buds had been treated with chemicals to replace the chilling (Table 3). The application of garlic extracts has not propitiate satisfactory results, which were in disagreement with those by Vargas-Arispuro et al. (2008) whose grapevines showed 100% bud breaking by using the active principles of garlic extracts.

Table 1. Climate conditions. Jales. 2009.

Month	Maximum Absolute Temperature	Minximum Absolute Temperature	Maximum Mensal Temperature Monthly (average)	Minimum Temperature Monthly (average)	Temperatura Média	Pp (mm)
January	36.8	15.0	31.0	20.1	25.6	287.5
February	35.0	18.0	31.8	22.0	26.9	206.6
March	36.8	19.8	32.1	22.4	27.3	159.8
April	34.4	18.0	31.4	20.9	26.1	18.6
May	34.1	10.6	29.8	19.1	24.4	52.8
June	31.6	5.0	26.9	15.0	20.9	18.1
July	33.4	9.2	28.8	16.0	22.4	16.4
August	35.0	6.8	29.8	16.9	23.4	48.8
September	35.4	14	31.2	19.9	25.6	191.8
October	34.2	16.4	31.7	20.1	25.9	218.6
November	35.6	18.0	32.4	20.4	26.4	126.5
December	33.6	16.2	31.3	19.5	25.4	319.8

Table 2. Average number of bunches per plant, sprout percent, days for budbreaking and the cycle of Rose Niagara grapevine under treatments with garlic extracts, mineral oil and H_2CN_2. Jales, Experiment 1, 2009.

Treatments	number of unches	sprout (%)	days for budbreaking	Cycle
Control	31.4 A	80.9 A	10.0	118.0
Mineral oil 2%	32.7 A	85.7 A	10.0	118.0
Garlic extract 1.5 % +M. oil 2%	27.5 A	71.4 A	10.0	118.0
Garlic extract 3.0 % +M. oil 2%	34.8 A	90.5 A	10.0	118.0
Garlic extract 4.5 % + M. oil 2%	33.4 A	85.7 A	10.0	118.0
Garlic extract 6.0 % + M. oil 2%	30.7 A	81.0 A	10.0	118.0
H_2CN_2 2.5%	34.8 A	90.4 A	10.0	118.0
Average	32.2	83.7	10	118
L.S.D. (%)	26.4	24.5	0	0
DMS	14.1	25.4	0	0

Table3. Average number of bunches per plant, sprout percent, days for budbreaking and the cycle of Rose Niagara grapevine under treatments with garlic extracts, mineral oil and H_2CN_2. Jales, Experiment 2, 2009. Data transformed in $(0,5 + x)^{1/2}$

Treatament	Número de Cachos	Brotação (%)	Dias brotação	Ciclo
Control	0 C	0 C	0 B	0 B
Mineral oil 2%	0 C	0 C	0 B	0 B
Garlic extract 1.5 % + M. oil 2%	0 C	0 C	0 B	0 B
Garlic extract 3.0 % + M. oil 2%	0 C	0 C	0 B	0 B
Garlic extract 4.5 % + M. oil 2%	0 C	0 C	0 B	0 B
Garlic extract 6.0 % + M. oil 2%	10.9 B	30.4 B	20 A	125 A
H_2CN_2 3%	28.9 A	75.7 A	20 A	125 A
L.S.D. (%)	14.4	14.1	0	0
DMS	4.6	10.7	0	0

Means followed by different letters in the same column, differ at 5% by Tukey test.

In the experiment accomplished in Jundiaí, the pruning was performed on August 17, when plants had already accumulated enough hours of chilling from May to August (Table 4). In that way, there were no significant statistical effects of the treatments on the studied variables once the buds endolatency was broken by the hours of chilling accumulation and not by the effect of the chemicals (Table 5).

There are strong evidences that one of the principal mechanisms involved in the dormancy bud breaking of temperate climate fruit plants is related to the induction of an oxidative stress (Pinto et al., 2007). In agreement with Pinto et al. (2007), H_2O_2 would function as a chemical sign activating the expression of genes directly or, indirectly, triggering metabolic alterations that can be detected by other molecules, as for instance, a kinase, that would activate or repress the expression of genes responsible for the dormancy breaking. According to Pérez & Lira (2005), either the hydrogen cyanamide application or the exposition to chilling inhibited the activity of the enzyme catalase, the main enzyme responsible for the degradation of of hydrogen peroxide (H_2O_2) in the bud vines. Besides, a momentary increase in H2O2 levels preceds the overcome of the endodormency of Sultana grapevines buds

Table 4. Climatic data of Jundiaí. 2009.

Month	Maximum Absolute Temperature	Minximum Absolute Temperature	Maximum Mensal Temperature Monthly (average)	Minimum Temperature Monthly (average)	Temperatura Média	Pp (mm)
January	32.7	14.7	27.6	17.7	22.6	287.5
February	32.2	16.6	29.5	18.3	23.9	206.6
March	33.6	15.1	29.7	17.7	23.7	159.8
April	30.0	11.0	26.9	14.8	20.8	18,6
May	28.8	9.6	25.8	12.7	19.2	65.5
June	26.6	2.8	22.0	8.9	15.4	58.9
July	28.5	5.3	22.5	11.4	17.0	131.6
August	29.7	7.6	24.9	11.7	18.3	52.0
September	31.7	10.9	25.9	15.0	20.5	162.2
October	31.0	11.8	26.7	15.5	21.1	101.6
November	33.0	14.5	30.1	18.3	24.2	274.9
December	32.7	15.5	28.2	18.1	23.1	279.2

Table5. Average number of bunches per plant, sprout percent, days for budbreaking and the cycle of Rose Niagara grapevine under treatments with garlic extracts, mineral oil and H_2CN_2. Jundiai 2009.12.5

Treatments	number of bunches	sprout (%)	days for budbreaking	Cycle
control	12.5A	100 A	15.0 B	125.0 A
Mineral oil 2%	7.4 A	100 A	15.8 B	126.6 A
Garlic extract 1.5 % + M. oil 2%	13.6 A	100 A	15.0 B	126.6 A
Garlic extract 3.0 % + M. oil 2%	9.4 A	100 A	16.6 AB	125.0 A
Garlic extract 4.5 % + M. oil 2%	7.0 A	100 A	15.0 B	127.1 A
Garlic extract 6.0 % + M. oil 2%	7.8 A	100 A	18.7 A	127.0 A
H_2CN_2 3%	8.4 A	100 A	15.0 B	125.0 A
Mean	9.4	100	15.9	126.0
L.S.D. (%)	39.4	-	8.7	1.9
F Test	2.5NS	-	5.1**	0.5NS

Means followed by different letters in the same column, differ at 5% by Tukey test.

According to Kubota *et al*. (1999) the garlic active compounds responsible for the dormancy breaking in vines are mainly the diallyl disulfide, although the diallyl mono, tri and tetra sulfide can also be involved. It could be that the active compounds would actuate by the same mechanism proposed by Pinto *et al*. (2008) for the dormancy breaking of temperate climate fruit plants, that is, by oxidative stress through accumulation of H_2O_2. Lemar *et al*. (2005) observed that the application of garlic extracts caused an oxidative stress in cells of *Candida albicans*, but in this case this would lead to the inhibition of fungus colonies growth and to the destruction of its cellular components.

Although the promising results showed by the treatment with Bioalho®, they are still preliminary, needing further researches, mainly about the economical viability and the reduction of doses, as for instance by using different adjuvants. According to Dokoozlian *et al*. (1998), the scales of the buds of vines, that protect them against dehydration and injury by the extreme climatic conditions, are a barrier against the products used for the dormancy breaking. In this case, the adjuvants could increase the penetration of the products in the bud, increasing its efficiency.

CONCLUSIONS

The effective chemical treatment to induce bud dormancy release in Rose Niagara grapevine was the H_2CN_2 application, in endolatent vines. At this same stadium, the bioalho® 6% showed a discreet effect. Further experiments are necessary to study the application of the garlic extract regarding the dose, time and methods of application. .Therefore so far it is not possible to recommend it neither for organic nor for conventional agriculture. When applying H_2CN_2 with a brush or a painting roll (Figure 1), the operator doesn't contact the chemical, and so it could be thought to be applied also in organic viticulture.

Figure 1. Peasant applying H_2CN_2 with painting roller.

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ROLE OF ARTIFICIAL MYCORRHIZATION ON IRON UPTAKE IN CALCAREOUS SOILS, ON STILBENE ROOT SYSNTHESIS AND IN OTHER PGYSIOLOGICAL PROCESSES IN GRAPEVINE

L. Bavaresco [(1)] , M. Gatti [(1)] , M. Zamboni [(1)] , C. Fogher [(2)] , F. Ferrari [(3)]

[(1)] Istituto di Frutti-Viticoltura, luigi.bavaresco@unicatt.it; matteo.gatti@unicatt.it; maurizio.zamboni@unicatt.it; [(2)] Istituto di Agronomia, Genetica e Coltivazioni Erbacee, corrado.fogher@unicatt.it; [(3)] Istituto di Chimica Agraria e Ambientale, federico.ferrari@unicatt.it; Università Cattolica del Sacro Cuore, Via Emilia Parmense, 84, 29100 Piacenza, Italy.

SUMMARY

Mycorrhization is normally occurring in the grapevine, but the natural root infection can be increased by artificial inoculation of mychorrizal fungi when a vineyard is established, with different beneficial effects, as a way of low-input and sustainable agricultural practices. The paper represents a review of over 15-year- research activity of the Viticulture Institute of UCSC, carried out at both pot and field level, aiming at improving vine suitability to calcareous soils and drought, and at regulating the balance among vigour, grape production and quality. Root infection with AM fungus *Glomus mosseae* is able to alleviate symptoms of lime-induced chlorosis and to improve mineral nutrition, especially Fe uptake. Another effect is the enhancement of root stilbene (especially piceid) concentration in some rootstocks. The artificial mychorrization improves the vegetative growth of the young vines, while the grape yield of adult vines is positively affected without negative effects on quality. The treatment improves the vine suitability to drought stress in terms of dry matter production and grape sugar content.

RIASSUNTO

La micorrizazione è un fenomeno che si osserva normalmente nella vite, ma con trattamenti alle radici al momento dell'impianto del vigneto è possibile aumentare il livello di infezione radicale, con vantaggi di vario tipo. Il lavoro rappresenta una review dei risultati ottenuti dall'Istituto di Frutti-Viticoltura in più di 15 anni di sperimentazioni condotte sia in vaso che in vigneto. L'infezione radicale con *Glomus mosseae* è in grado di attenuare i sintomi di clorosi ferrica da calcare e di migliorare la nutrizione minerale della pianta ed in particolare quella ferrica. Un altro risultato interessante della micorrizazione artificiale in terreni calcarei è la capacità di incrementare la sintesi di stilbeni (in particolare il resveratrolo-glucoside) nelle radici di alcuni portinnesti.. Nelle prime fasi di vita di un vigneto, la micorrizazione artificiale aumenta lo sviluppo vegetativo, mentre nella fase di vigneto adulto aumenta leggermente la produzione, mantenendo inalterati i parametri qualitativi dell'uva. Nei confronti della stress idrico il trattamento induce un migliore adattamento della pianta in termini di produzione di sostanza secca e di grado zuccherino dell'uva.

INTRODUCTION

Vesicular-arbuscular mycorrhyzal (AM) fungi such as *Acaulospora* spp, *Gigaspora* spp, *Glomus* spp, *Sclerocystis* spp improve plant growth by increasing the uptake of nutrients, especially phpsphorus in nutrient-poor soils (Bolan, 1991; Gebbing *et al*. 1977; Kothari *et al*. 1991). *Vitis* spp. are commonly infected by AM fungi (Possingham, Groot Obbink, 1971; Schubert, Cravero, 1985) and evidences have been obtained on the ability of those fungi to produce siderophores (Cress *et al*. 1986), enhancing iron uptake in the plants particularly under calcareous conditions. The formation of soluble organic chelates is important for the iron supply of plants growing on calcareous soils, where the solubility of iron minerals is very low. The organic chelate include decomposition products of organic matter, as humic and fulvic acids, organic acids as citrate and malate, and siderophores produced by nearly all micro-organisms (Reid *et al*. 1986). Siderophores are low molecular weight iron (III) chelating agents produced by different organisms under iron deficicncy conditions to supply iron to the cell (Neilands, 1981; Crowley, 2001).

Lime-induced chlorosis affects a lot of annual crops and perennials (including *Vitis* spp.) growing on calcareous soils. The most useful method in viticulture to overcome this stress is to graft the grape varieties on lime-tolerant rootstocks (Fregoni, 1980; Pouget, 1980; Bavaresco *et al*. 2000) and mechanisms of iron-efficiency have been investigated (Bavaresco *et al.* 1991; Brancadoro *et al*. 1995; Nikolic, Kastori, 2000; Nikolic *et al*. 2000), but the ideal rootstock has not yet been obtained, since the lime-tolerant ones induce, as rule, high vigour. That is why it is interesting to test the response to the infection with AM fungi, of vines grafted on different rootstocks, and grown on calcareous soils.

AM fungi can also promote the dry matter production of the vines growing on neutral soils (Schubert *et al.* 1988; Karagiannidis *et al*. 1995; Lindermann, Davis, 2001).

While the artificial infection is effective in the nurseries, it is still under investigations in the vineyard, because some problems have not yet been solved, such as the difficulty to produce a large amount of commercial pathogen-free product, the scarce knowledge on the host-fungus interactions, the likely competition with the soil borne fungi, the impossibility to sterilize the vineyard soil.

The aim of the paper is to make a review of the researches developed over a 15-year- period by the Insitute of Pomology and Viticulture on the role of artificial infection with *Glomus mosseae* and *Glomus intraradicies* at both pot and field level, with a special emphasis on the response to lime-stress conditions.

POT TRIALS ON CALCAREOUS SOIL

The first experiment was set up in 1992, when ungrafted cuttings of 3 rootstocks (140 Ru, SO4, 101-14) were infected with mycorrhizal inoculum (*Glomus mosseae*) and some leaf parameters (Fe^{++}, chlorophyll, mineral elements) were checked (Bavaresco, Fogher, 1992) . The soil was a natural calcareous soil with pH 8.2 and 70% total carbonates. The interest of the trail was focused on the behaviour of 101-14 rootstock which is lime-susceptible and the results showed a significant increasing of Fe (II) and Chl over the untreated plants, despite a low enhancement of the root AM infection (Tab. 1).

Tab. 1. Role of the root treatment on some leaf parameters of 101-14 (Bavaresco, Fogher, 1992)

Treatment	Fe^{++} (µg/g dw)	Fe^{++} (µg/g fw)	Chl (mg/100g dw)	Chl (mg/g fw)	Root AM infection (%)
Control	73	16.3	571	1.19	33
AM infection	85	17.7	711	1.44	36
LSD 0.05	2.8	0.7	34	0.08	n.s.

The correlations between the root AM infection (of all the rootstocks) and the leaf mineral elements is reported in Tab. 2 where there is the confirmation of the positive role on P uptake.

Tab.2. Correlation coefficients ("r") between the root AM infection and the leaf mineral elements (Bavaresco, Fogher, 1992)

	N %	P %	K %	Ca %	Mg %	Fe^{++} ppm	Fe ppm	Mn ppm	Cu ppm	Zn ppm	B ppm
AM %	0.81 *	0.92**	n.s.	n.s.	n.s.	n.s.	n.s.	0.92**	0.89*	n.s.	n.s.

*: significant for $p \leq 0.05$; **: significant for $p \leq 0.01$; n.s.: not significant

The second experiment was run in 1995 (Bavaresco *et al.* 1995) when *V. vinifera* L. cv. Chardonnay clone R8 was grafted on 3 rootstocks (140 Ru, SO4 and 101-14), infected with the AM fungus *Glomus mosseae*, and some leaf parameters (Fe^{++}, chlorophyll, mineral elements) were checked along the growing season (3 samplings). The soil was the same as the previous experiment and even in this case the interest of the trail was focused on the behaviour of 101-14 rootstock which is lime-susceptible; the results showed a significant increasing of Fe (II) and Chl over the untreated plants (Tab. 3).

Tab. 3 Role of the treatment on some leaf parameters of cv. Chardonnay / 101-14, along the growing cycle (Bavaresco *et al*. 1995)

	1st sampling		2nd sampling		3rd sampling		LSD 0.05
	Control	AM infection	Control	AM infection	Control	AM infection	
Fe^{++}(µg/g dw)	50	57	36	39	43	52	2.1
Chl (mg/100g dw)	214	203	319	357	457	492	45

The third experiment was carried out in 1996 (Bavaresco *et al*. 1996a) on Pinot blanc grafted on 140 Ru and 101-14, and infected with the AM fungus *Glomus mosseae*. The soil was the same as the previous experiments and some leaf parameters were recorded, as follows: Fe^{++}, Chl, ash alkalinity, chlorosis rating. The results showed (tab. 4) that the susceptible graft combination (Pinot b/101-14) benefited from the root mycorrhizal infection in terms of iron uptake and chlorosis recovering.

Tab. 4. Role of the treatment on some leaf parameters of Pinot blanc grafted on 2 rootstocks (Bavaresco, Fogher, 2006a)

		Fe^{++} (µg/g dw)	Chl (mg/100g dw)	Ash alkalinity (cmol/kg)	Chlorosis rating *
Pinot b /140 Ru	Control	36	375	106	1
	AM infection	35	344	90	1
Pinot b /101-14	Control	31	155	86	4
	AM infection	33	282	66	2
LSD 0.05		1.4	14	12	-

* 0: no symptoms 5: severe symptoms

The fourth experiment is similar to the previous one, but run with different rootstocks (Pinot blanc grafted on 41B, SO4 and 3309C). Leaf Chl and mineral elements, grape soluble solids and root AM infection were recorded (Bavaresco, Fogher, 1996b) and the results showed a positive effect of the AM infection on alleviating chlorosis, grape soluble solids, leaf Fe and P uptake, especially in the susceptible graft combination (Pinot Blanc/ 3009C) (Tab. 5).

Tab. 5 Role of the treatment on some leaf, grape and root parameters of Pinot b/3009C (Bavaresco, Fogher, 2006b)

	Leaf										Grape	Roots
	Chl (mg/100g dw)	N %	P %	K %	Ca %	Mg %	Fe ppm	Mn ppm	Zn ppm	B ppm	Sol. Solids °Brix	AM infection %
Control	141	2.41	0.25	1.47	0.49	0.24	87	28	18	9	20.9	12.0
AM inf.	180	3.07	0.34	1.63	0.43	0.24	141	25	21	13	24.1	37.9
LSD 0.05	30	0.35	0.05	n.s.	n.s.	n.s.	33	n.s.	n.s.	n.s.	2.0	17.0

The fifth trial was conducted on Chardonnay clone SMA 130 grafted on the following rootstocks: Kober 5BB, 1103 P, 140 Ru, 41 B, SO4, 420 A. The soil was the same as before, and many parameters were recorded (Bavaresco *et al*. 2003) after root infection with *Glomus mosseae*. Tab. 6 reports some of those, namely the total production of dry matter per vine (at the end of the growing cycle) and the root content of trans-piceid, which is a stilbenic compound (Bavaresco *et al*. 2009).

Tab 6. Dry matter production and root *trans*-piceid of Chardonnay SMA 130, depending on the rootstock (Bavaresco *et al*. 2003)

		K 5BB	1103 P	140 Ru	41 B	SO 4	420 A	LSD 0.05
Dry matter (g/vine)	Control	150	159	145	165	115	125	40
	AM inf.	185	158	150	165	128	170	40
	LSD 0.05	n.s.	n.s.	n.s.	n.s.	n.s.	40	
Root *trans*-piceid (μg/g fw)	Control	32	41	28	27	41	40	12.7
	AM inf.	43	35	46	50	30	52	12.7
	LSD 0.05	n.s	n.s	12.7	12.7	n.s.	n.s.	

FIELD TRIAL ON CALCAREOUS SOIL

A field trial lasting 4 years (1995-1998) was carried out on a commercial vineyard (Verona province) of Chardonnay clone R8, grafted on 161.49 C, Kober 5BB and SO4, and infected at planting by *Glomus mosseae*. The soil had a pH of 8.4 and active lime of 10.6%. Leaf SPAD values and mineral elements were detected at fruit set and veraison, and productive and qualitative parameters were recorded at grape harvest. Tab. 7 reports some results (average values of the 4 years), showing a different effect of the AM infection depending on the rootstock. Chlorosis, when occurring, was not alleviated by the root treatment, except for year 1997, when the AM infection on Chardonnay/161.49 was able to recover from chlorosis, at fruit set (data not presented). Leaf iron was in many cases improved by the root infection, but not in a significant way. Grape yield was depressed by the treatment in the graft combination with K 5BB (Bavaresco *et al*., 2002).

Tab. 7. Role of the AM infection on some Chardonnay leaf and grape parameters, depending on the rootstock (Bavaresco *et al*. 2002)

		SPAD fruit set	SPAD veraison	Fe fruit set (ppm)	Fe veraison (ppm)	Yield (kg/vine)	Cluster wt (g)	Soluble Solids °Brix	pH	Titrat. acidity (g/L)
Ch /K5BB	Control	15.8	28.3	70	69	6.4	187	18.0	3.23	7.3
	AM inf.	16.3	26.3	82	84	4.9	167	18.8	3.29	6.7
Ch /161.49C	Control	13.2	27.4	103	70	3.3	133	19.8	3.36	6.0
	AM inf.	14.1	28.0	76	83	4.1	130	19.2	3.36	6.3
Ch /SO4	Control	17.3	31.0	84	79	4.1	150	18.0	3.30	6.8
	AM inf.	18.0	28.3	80	112	4.6	163	17.8	3.35	6.7
LSD 0.05		n.s	n.s	n.s.	n.s.	0.7	15	0.57	0.04	0.32

FIELD TRIALS ON NEUTRAL SOIL

Two field trials were carried out in commercial vineyards, aiming at controlling the effect of root infection at planting, with *Glomus intraradicies* at 3 inoculum rates (30 cc/vine, 60 cc/vine, 120 cc/vine) on the plant growth, measured by the winter pruning weight per plant. The first vineyard was placed in Ancarano di Rivergaro (Piacenza province), poor soil, where Croatina clone MI Cr 10 grafted on 420 A, plant spacing 2 m x 0.8 m, was investigated over a 3-year-period (2002-2004), just after the vineyard was established. The second vineyard was placed in Pietra de Giorgi (Pavia province), fertile soil, where Barbera clone AT 84 grafted on 5C, plant spacing 2.5 m x 1.6, was investigated over the same period (2002-2004). Tab. 8 reports the effect of the different inoculum doses on the winter pruning weight (average values of the three years), showing in both cases a beneficial effect only at dose 60 cc/vine. The big differences between the two vineyards are related to the different vigour of the graft combinations, the different soils and vine spacings.

Tab. 8. Role of AM *(Glomus intraradicies*) infection rates on winter pruning weight (g/vine) (unpublished data)

		Control	30 cc/vine	60 cc/vine	120 cc/vine
Ancarano	Croatina MI Cr 10 / 420 A	145 a	160 ab	190 b	155 ab
Pietra de Giorgi	Barbera AT 84 / 5C	880 a	885 a	1120 b	905 a

Values followed by the same letter (on the line) are not significantly different at 0.05 level, by Tukey test

The productive and qualitative parameters of the Barbera vineyard at Pietra de Giorgi were controlled at the second year of grape production (2004). The AM root infection improved over the control the crop load, without negative effects on grape quality (Tab. 9).

Tab. 9. Role of AM (*G. intraradicies*) infection rates on production and quality of cv. Barbera (unpublished data)

	Yield (kg/vine)	Cluster wt (g)	Berry wt (g)	Bud fertility	Soluble Solids (°Brix)	Titr. Acidity (g/L)	pH	Antho-cyanins (mg/g fw)	Polyphenols (mg/g fw)
Control	7.7 a	266 a	2.6 a	1.8 a	16.8 a	10.7 a	3.03 a	0.90 a	1.55 ab
30 cc/vine	9.0 ab	306 ab	2.4 a	1.7 a	17.1 a	10.9 a	2.98 ab	0.82 a	1.48 a
60 cc/vine	10.3 b	331 b	2.6 a	1.9 a	16.9 a	10.8 a	2.99 ab	0.92 a	1.65 ab
120 cc/vine	9.1 ab	309 ab	2.5 a	1.7 a	16.2 a	11.2 a	2.90 b	0.78 a	1.70 b

Values followed by the same letter (on the column) are not significantly different at 0.05 level, by Tukey test

POT TRIALS RELATED TO DROUGHT STRESS

The experiment included cv. Barbera clone R4 grafted on 3009 C (rootstock susceptible to drought stress), which has been infected with the AM fungus *Glomus intraradicies*, just before potting. The vines were grown on a neutral soil and six treatments were applied, as follows: 1) drip irrigation in order to keep the soil near field capacity; 2) drip irrigation in order to keep the soil near 50% of field capacity; 3) root infection with 30 cc AM inoculum/vine, under irrigation at field capacity; 4) root infection with 30 cc AM inoculum/vine, under irrigation at 50% field capacity; 5) root infection with 120 cc AM inoculum/vine, under irrigation at field capacity; 6) root infection with 120 cc AM inoculum/vine, under irrigation at 50% field capacity.

The dry matter production (grapes and pruning material) was recorded at the end of the annual growing cycle, as well as the soluble solid concentration of grapes at harvest (Tab. 10), during two years (unpublished data). The results showed that mycorrhization (30 cc/vine) under drought stress conditions (50% of field capacity) was able to improve the dry matter production when the vine was young (2002, 1st year of production), while the highest dose (120 cc/vine) had no significant effect. No significant effect was noticed when the vines were older (2004, 3rd year of production). Grape soluble solids were significantly increased by AM infection under stress conditions, only when the inoculum was applied at the highest rate (120 cc/vine).

Tab. 10. Role of drought stress and AM infection rates on dry matter production and grape sugars of cv. Barbera (unpublished data)

	Dry matter (grapes + pruning canes) g/vine		Grape soluble solids (°Brix)	
	2002	2004	2002	2004
Control 1 (field capacity)	101	535	20.7	23.5
Control 2 (50% field capacity)	75	465	18.8	22.9
30 cc AM/vine at field capacity	114	410	20.4	23.3
30 cc AM/vine at 50% field capacity	111	452	20.4	23.1
120 cc AM/vine at field capacity	125	539	21.6	23.6
120 cc AM/vine at 50% field capacity	88	501	22.8	24.2
LSD 0.05	25	65	1.8	1.2

CONCLUSIONS

The utilization of artificial mycorrhization in the vineyard to alleviate lime-induced chlorosis seems to be a promising technique, but further studies are necessary in order to understand at which extent the natural soil borne organisms compete with the supplied AM fungus, and how to keep effective the artificial inoculum along the plant life cycle. As concerning the utilization in the neutral soils to improve the growth, the first results are still very promising, with a clear effect dose-related, as well as in the case of the drought stress.

ACKNOWLEDGEMENTS

The authors wish to thank Italpollina SpA (Rivoli, VR, Italy) company for supplying *G. intraradicies* inoculum and for the financial support; Fiorini estate (Caldiero, VR, Italy), La Stoppa estate (Ancarano, PC, Italy) and Mazzocchi estate (Pietra de Giorgi, PV, Italy) for hosting some of the field trials.

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SOIL INFLUENCE ON VINE PERFORMANCE: FROM ROOT SYSTEM TO WINE QUALITY

D. Tomasi[1], F. Gaiotti[1], G. Bragato[2], D. Mosetti[2], A. Missio[2], F. Battista[1]

(1) CRA-VIT - Viale XXVIII Aprile, 26, 31015 Conegliano (Italy), diego.tomasi@entecra.it
(2) CRA-RPS - Gruppo di Ricerca di Gorizia, Via Trieste, 23, 34170 Gorizia (Italy), gilberto.bragato@entecra.it

ABSTRACT

Many environmental factors, like climate, soil type, land morphology, elevation and hill face, highly affect the grapevine variety performance. Among these factors, soil depth, layering and texture are important sources of variability in grapevine production, due to their effects on soil water availability, vine root distribution and root-to-leaf physiology.

As part of a zoning project carried out from 2006 to 2008 in the AOC *Colli Orientali del Friuli* district (North-East Italy), soil influence on the performance of a local white wine grape variety – the *Friulano*- was investigated.

Different soil types were delineated after a systematic survey of the area. Afterwards, they were characterized for their physical and chemical properties, along with soil moisture seasonal variations; the climate data were collected too.

As soil effects on vine performance could not be successfully interpreted without investigation on root system development, root density and distribution were analyzed. The observed differences among grape and wines produced in diverse soil types confirmed that the soil environment has a high impact on vine production, effecting grape composition and wine quality.

RIASSUNTO

Le performance produttive della vite sono strettamente dipendenti da molteplici fattori abientali, quali il clima, il tipo di suolo, la morfologia, l'altitudine e l'esposizione dei versanti. Tra questi, la profondità del suolo, la stratificazione e la tessitura sono importanti fonti di variabilità nella produzione vitivinicola, soprattutto per le loro ampie ripercussioni sulla quantità di acqua disponibile nel suolo, sulla distribuzione degli apparati radicali della pianta e sui rapporti fra chioma e radici.

Questo studio, condotto nell'ambito di un progetto di zonazione svolto tra il 2006 e il 2008 nella zona DOC Colli Orientali del Friuli, ha analizzato l'influenza del suolo sulle performances vegeto-produttive di una varietà autoctona – il *Friulano*.

Attraverso un'indagine sistematica dell'intera area sono stati individuati i principali tipi di suoli, i quali sono successivamente stati caratterizzati per composizione chimica e fisica e per contenuti idrici stagionali.

Poichè una piena comprensione delle relazioni tra suolo e vite presuppone una conoscenza delle risposte radicali a diverse situazioni pedologiche, si è ritenuto necessario effettuare un'analisi degli apparati radicali, determinando densità e distribuzione delle radici.

I risultati ottenuti nelle diverse realtà pedologiche individuate confermano un'ampia influenza del suolo sulle performance produttive di questa varietà, con effetti evidenti sia sulla composizione dell'uva sia sui caratteri dei vini.

INTRODUZIONE

Il suolo e le sue proprietà fisiche e chimiche hanno un peso rilevante sulle produzioni vitivinicole, contribuendo alla quantità e alla qualità organolettica delle uve (Dutt *et al*. 1981; Van Leewen, 1991; Jackson, Lombard, *1993;* Tomasi *et al*. 1999; Rittiman, Thorson, 2002; White *et al*. 2007). Capacità di ritenzione idrica, disponibilità di nutrienti, proprietà termiche e di aerazione, profondità del suolo esplorabile, colore, pH e carbonati, sono solo alcuni fattori che influenzano lo stato nutritivo, metabolico e fisiologico della vite. E' facile intuire quindi come i caratteri edafici siano un fattore determinante nella tipicità di un vino in quanto direttamente coinvolti nella tempistica di successione fenologica annuale e nel regime di nutrizione idrica e minerale, tutti fattori che nel loro complesso influenzano l'espressione viticola di un dato sito (Barbeau *et al.*,1998; Barbeau, 2003).

Le radici sono l'organo della vite che primariamente viene influenzato dalle proprietà pedologiche di un sito, con effetti evidenti sulla densità e sulla distribuzione orizzontale e verticale (Morlat, Jaquet, 1993; Smart *et al.*, 2006). Se da un lato infatti è accertato che la morfologia e l'architettura degli apparati radicali della vite ha una forte componente genetica, molti studi hanno concordato sul fatto che le condizioni edafiche hanno una larga influenza sulle dinamiche di sviluppo delle radici e sulla loro funzionalità (Puissant, *et al*., 1981; Smart *et al.* 2006); uno stesso portinnesto può quindi presentare una diversa distribuzione radicale in funzione del suolo su cui è posto.

Date le strette relazioni esistenti tra sviluppo radicale ed aereo è facilmente intuibile che la distribuzione e la funzionalità radicale hanno importanti conseguenze sul comportamento complessivo della pianta, con effetti sullo sviluppo vegetativo della chioma, sulla quantità e sulla qualità delle produzioni (Richards, 1983; Morlat e Jaquet, 1993; Hunter e Volschenk, 2001). L'apparato radicale assomma numerose funzioni tra le quali le principali sono l'ancoraggio, l'assorbimento idrico e nutrizionale, la sintesi ormonale legata alla crescita, l'invio alla chioma dei segnali di stress idrico e la funzione di stoccaggio di sostanze di riserva (Davies W. J. *et al*. – 1991; Smart *et al*. 2006). Lo studio degli apparati radicali è quindi in grado di fornire utili indicazioni sullo stato dei suoli e le sue numerose funzioni fisiologiche e biochimiche sono sempre evidenziate dal comportamento della chioma.

Nel triennio 2006-2008 parte dell'area DOC Colli Orientali del Friuli è stata interessata da uno studio di zonazione con l'obiettivo di analizzare le relazioni esistenti tra i fattori ambientali (pedologici e climatici) e la risposta quali-quantitativa di diverse varietà locali ed internazionali.

Le performance vegeto-produttive del vitigno locale *Friulano* sono state indagate in relazione a quattro diverse situazioni pedologiche individuate all'interno dell'area in esame. I risultati emersi sono volti ad un miglior utilizzo dell'area di produzione e allo sviluppo di una viticoltura focalizzata alla qualità e alla tipicità dei vini.

MATERIALI E METODI

La zona oggetto del presente studio comprende un'area complessiva di 2000 ha situati all'interno della DOC "Colli orientali del Friuli" a nord di Manzano (Italia) (46 ° 00'35 "N, 13 ° 25'20" E). L'area, collocata tra i 60 ed i 220 m di quota, è caratterizzata da affioramenti di sedimenti marini e oceanici con alternanza di strati marnosi e arenacei. Nell'ambito di un progetto di zonazione condotto nel triennio 2006-2008, al fine di individuare e delimitare zone omogenee per tipologia di terreno, orografia, morfologia e altimetria è stata effettuata una preliminare caratterizzazione pedologica. Sono state eseguite 236 osservazioni distribuite in modo da coprire omogeneamente l'intera area studiata. Le trivellate sono state eseguite fino ad una profondità di 100 cm, registrando la sequenza verticale degli orizzonti e, per ogni orizzonte, lo spessore, il colore della matrice, la percentuale di figure di ossidoriduzione e la tessitura. Un dataset di 14 attributi è stato elaborato con il programma *simil* (Goodall *et al.*, 1987) e la risultante matrice di dissimilarità è stata processata con pacchetti statistici dedicati di *R* (R La Fondazione per la statistica Computing, 2005) al fine di individuare gruppi omogenei di osservazioni del suolo. Successivamente sono stati descritti 10 profili di suolo rappresentativi riportando la sequenza verticale degli orizzonti. Ogni orizzonte è stato campionato seguendo un metodo di campionamento composito da 4 sub-campioni. I campioni sono stati analizzati in laboratorio per determinare: tessitura, pH, contenuto in sostanza organica, azoto totale, fosforo disponibile, potassio scambiabile, capacità di scambio cationico (CEC) e calcare attivo.

Per lo studio delle relazioni tra il vitigno e il suo ambiente di coltivazione è stata scelta la varietà Friulano (ex Tocai friulano), individuando 4 macrozone di interesse viticolo caratterizzate da differenti tipologie di suolo. In ciascuna di queste sono stati individuati 2 o 3 vigneti campione sui quali valutare i caratteri vegetativi e produttivi. La scelta è stata assegnata scegliendo impianti confrontabili tra loro, con le seguenti caratteristiche: età media: 30 anni; portainnesto Kober 5bb; forma di allevamento: capuccina con 2 capi a frutto per un totale di circa 20 gemme; sesto di impianto: 1,2 x 2,8 m.

Nel triennio, nel corso del periodo vegetativo sono state seguite le dinamiche di maturazione (contenuti in zuccheri, acidi e il pH) delle uve. Alla vendemmia sono state determinate le produzioni/ceppo ed il peso medio del grappolo. I caratteri vegeto-produttivi sono stati valutati anche alla luce degli andamenti climatici stagionali dell'area, definiti utilizzando i dati di 3 centraline meteo presenti nei comuni di Dolegnano, Buttrio e Ipplis.

Stanti le strette relazioni tra suolo, vite e apparati radicali e i molteplici riflessi sul comportamento vegeto-produttivo della, nell'annata 2008 in ciascuna area è stato scelto un vigneto rappresentativo sul quale, con il metodo della trincea (Box, 1996) è stata studiata la densità e la distribuzione degli apparati radicali. Le radici, suddivise in tre classi di dimensioni (<1 mm, 1-3 mm, > 3 mm), sono state conteggiate lungo un profilo verticale aperto a 60 cm dal ceppo e fino alla profondità di 1 metro o fino alla profondità della roccia madre.

In ciascuna annata nei vigneti campione di ogni singola zona è stato raccolto e microvinificato 1 quintale di uva. I vini ottenuti sono stati sottoposti al giudizio di un panel di degustatori esperti al fine di cogliere i caratteri e le differenze riconducibili al suolo e all'ambiente di coltivazione.

RISULTATI E DISCUSSIONI

Caratterizzazione pedologica dell'area

Nella Tab.1 sono riportate le caratteristiche pedologiche delle 4 tipologie di suoli ritenuti più rappresentativi nell'area indagata per la coltivazione della cv *Friulano*.

Tab.1 Caratteristiche pedologiche delle 4 diverse realtà indagate nell'area della DOC Colli Orientali del Friuli

Buttrio – Zona A		**Manzano – Zona B**	
Morfologia: culmine **Pendenza**: subpianeggiante (<3%) **Quota**: 100-125 m s.l.m. **Substrato**: Flysch, sedimenti marini torbiditici con alternanze di marne e arenarie **Uso del suolo**: vigneto		**Morfologia**: culmine **Pendenza**: subpianeggiante (<3%) **Quota**: 100-125 m s.l.m. **Substrato**: Flysch, sedimenti marini torbiditici con alternanze di marne e arenarie **Uso del suolo**: vigneto	
BREVE DESCRIZIONE Suolo sottile con tessitura franco argillosa, neutro, pietrosità superficiale frequente (5-15%) e scheletro abbondante nell'orizzonte sottosuperficiale.	**QUALITA' DEL SUOLO** **Permeabilità**: medio-alta **Drenaggio:** ben drenato–l'acqua è rimossa dal suolo prontamente **AWC:** elevata **Profondità utile alle radici:** limitata dalla presenza di substrato roccioso a 40cm	**BREVE DESCRIZIONE** Suolo sottile con tessitura franco argillosa, debolmente alcalino, pietrosità superficiale frequente (5-15%) e scheletro abbondante nell'orizzonte sottosuperficiale.	**QUALITA' DEL SUOLO** **Permeabilità**: medio- alta **Drenaggio:** ben drenato–l'acqua è rimossa dal suolo prontamente **AWC:** elevata **Profondità utile alle radici:** limitata dalla presenza di substrato roccioso
Manzano – Zona C		**Spessa – Zona D**	
Pendenza: subpianeggiante (<3%) **Quota**: 125-150 m s.l.m. **Substrato**: Flysch, sedimenti marini torbiditici con alternanze di marne e arenarie **Uso del suolo**: vigneto		**Morfologia**: parte alta di versante, curvatura convessa **Pendenza**: pendenza moderata (10-15%) **Quota**: 100-125 m s.l.m. **Substrato**: Flysch, sedimenti marini torbiditici con alternanze di marne e arenarie **Uso del suolo**: vigneto	
BREVE DESCRIZIONE Suolo profondo con tessitura franco argillosa, più sabbioso in profondità, neutro	**QUALITA' DEL SUOLO** **Permeabilità**: medio-bassa **Drenaggio:** ben drenato – l'acqua è rimossa dal suolo prontamente **AWC:** molto elevata **Profondità utile alle radici:** senza limitazione	**BREVE DESCRIZIONE** Suolo profondo con tessitura franco argillosa, debolmente alcalino, screziature grigio-arancioni evidenti e noduli di FeMn abbondanti sotto i 70 cm;	**QUALITA' DEL SUOLO** **Permeabilità**: medio-bassa **Drenaggio:** ben drenato – l'acqua è rimossa dal suolo prontamente **AWC:** molto elevata **Profondità utile alle radici:** limite orizzonte sottosuperficiale argilloso

Analisi degli apparati radicali

Partendo dalla considerazione che la distribuzione delle radici attesta un equilibrio che si è instaurato nel corso del tempo in funzione delle disponibilità idriche e della tessitura del suolo, la sua analisi si è dimostrata di estrema utilità nell'interpretare le relazioni tra suolo e vite. A diverse realtà pedologiche sono infatti corrisposte differenti distribuzioni radicali, i cui effetti sono risultati evidenti anche sullo sviluppo vegeto-produttivo della parte aerea della pianta.

Buttrio - Zona A. Come già descritto si tratta di un suolo molto superficiale con un sottile strato esplorabile dall'apparato radicale. E' quindi evidente la difficoltà delle radice ad approfondirsi e a superare la profondità di 40 cm trovando nello strato marnoso un impedimento nel suo approfondimento. La densità radicale è di 53 radici m^2 localizzate prevalentemente nei primi 20/40 cm e con una certa distribuzione orizzontale confermata dalla presenza di alcune grosse radici. L'umidità dei suoli (fig.5) è su bassi valori, a confermare il prioritario ostacolo opposto dalla tenace matrice del suolo allo sviluppo radicale. L'impedimento nella diffusione radicale comporta una maggior suscettibilità della pianta agli andamenti stagionali (vedi brevi periodi con assenza di precipitazioni) e una ridotta vigoria confermata dal peso del legno di potatura (vedi tab2).

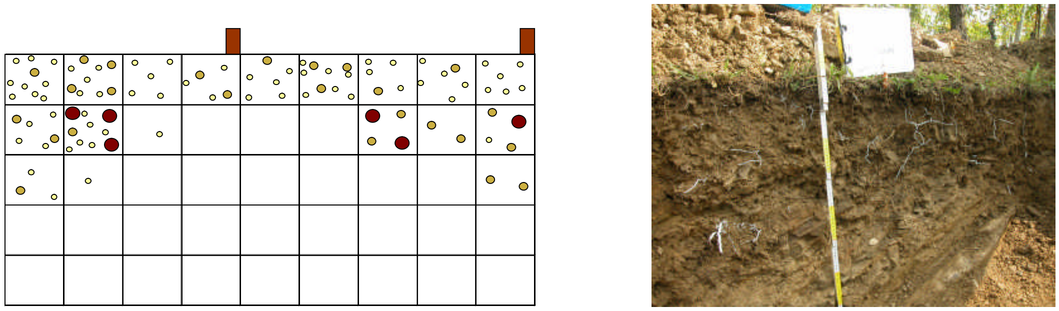

Fig. 1 Densità e distribuzione degli apparati radicali nel vigneto campione della zona A-Buttrio

Manzano – Zona B. La verifica radicale ha riscontrato un folto e consistente apparato radicale che omogeneamente esplora i primi 60 cm di suolo. Un netto impedimento meccanico, dovuto ad uno strato roccioso sottosuperficiale, crea una netta separazione senza possibilità per le radici di oltrepassare questo limite (fig.2). La natura franco-argillosa del primo strato consente una buona diffusione radicale con una alta densità (97 radici m^2) che permea omogeneamente il suolo alla ricerca delle scarse disponibilità idriche risultate in assoluto le più basse (fig.5). In questo caso si combina lo stimolo per la ricerca dell'acqua con una tessitura facilmente esplorabile. La vigoria della pianta si porta in questo caso su valori superiori ai casi precedenti (tab.2) a conferma del sostegno offerto da un più abbondante apparato radicale allo sviluppo aereo della pianta. Si vuole quindi far notare che anche basse disponibilità idriche, se completamente utilizzabili, sostengono efficacemente l'attività della vite.

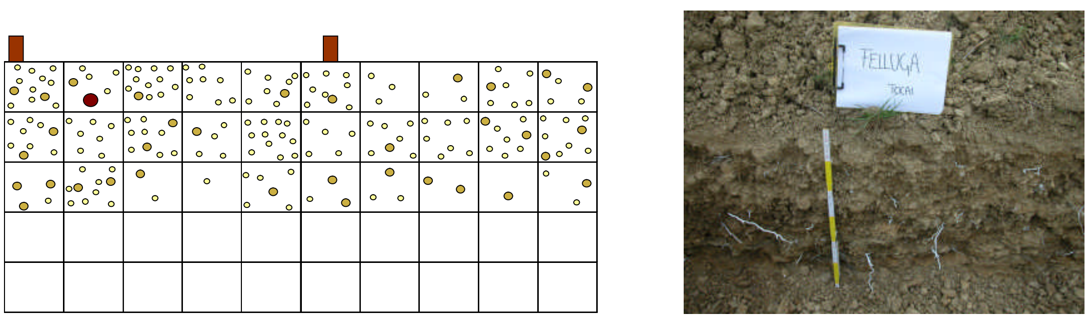

Fig. 2 Densità e distribuzione degli apparati radicali nel vigneto campione della zona B – Manzano

Manzano – Zona C. In questo caso la natura franco-argillosa del suolo (con una maggior quota di sabbia negli orizzonti più profondi), ha permesso una distribuzione radicale che si è portata sino al metro di profondità. La conta radicale ha assegnato una bassa densità pari a 45 radici m^2 con una distribuzione molto rada e non omogenea. Al ridotto numero di radici ha fatto però riscontro l'apparato aereo più vigoroso (tab.2) a confermare le buone disponibilità idriche riscontrate alle diverse misurazioni fig.5. In questo caso la discreta disponibilità di acqua, ha indotto la pianta ad un minor investimento nel suo apparato ipogeo.

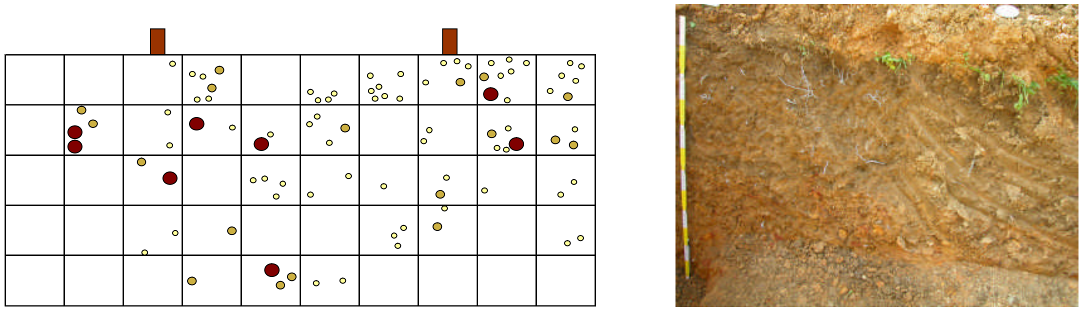

Fig. 3 Densità e distribuzione degli apparati radicali nel vigneto campione della zona C – Manzano

Spessa – Zona D. L'apparato radicale si presenta mediamente distribuito, ma con una bassa densità (42 radici m^2), localizzata principalmente tra 20 e 60/70 cm, in corrispondenza dello scasso operato al momento dell'impianto del vigneto e limitata nel suo approfondimento dal cambio di tessitura. Il ridotto numero di radici si riflette su una bassa vigoria delle viti (vedi fig. 4) dovuta anche alle incostanti e mediamente basse disponibilità idriche rilevate nel corso del ciclo vegetativo (fig.5).

Molto probabilmente la distribuzione radicale risente dei lavori di preparazione del suolo al momento dell'impianto del vigneto, che ha visto la formazione di una suola che separa nettamente e in modo invalicabile i primi due orizzonti, la pianta ha trovato un certo adattamento con la formazione di radici orizzontali.

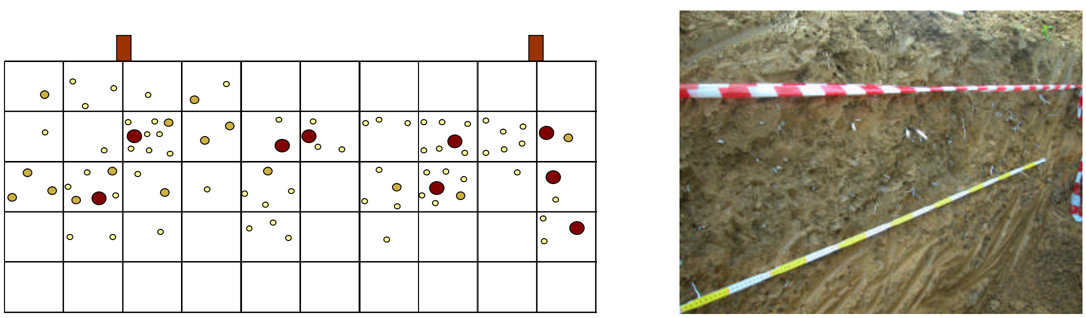

Fig. 4 Densità e distribuzione degli apparati radicali nel vigneto campione della zona D - Spessa

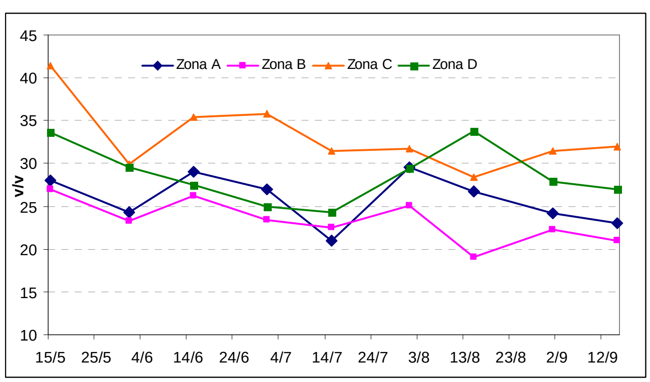

Fig.5 Umidità relativa rilevata nel corso del periodo vegetativo nei suoli indagati (media dati 2006-2008)

Risultati produttivi e qualitativi

I risultati riportati in tab.2 evidenziano come sia le rese produttive sia la composizione delle uve siano parametri fortemente influenzati dai fattori edafici.

I suoli estremamente sottili e scarsamente esplorati dagli apparati radicali nella zona A hanno favorito un naturale contenimento della vigoria della vite (0,6 Kg di legno di potatura/ceppo) risultata tra le più basse rilevate nell'area. Le disponibilità idriche non sempre adeguate associate a queste condizioni edafiche hanno determinato rese produttive inferiori rispetto ad altre zone, associate però a medi contenuti zuccherini e acidici.

Le indagini effettuate nella zona B hanno evidenziato che, anche in questa tipologia di suolo come nel caso precedente, la profondità esplorabile e le disponibilità idriche sono i due fattori che maggiormente influenzano le produzioni. La pianta investe infatti elevate energie nella sintesi di un apparato radicale estremamente denso, necessario ad utilizzare al meglio le limitate riserve d'acqua del terreno e a sostenere il buon apparato aereo. Le rese produttive sono però risultate significativamente inferiori rispetto alle altre aree di indagine (solo 2,2 Kg/ceppo), ma con un ritorno positivo sull'accumulo di zuccheri, che ha riportato i valori più elevati in assoluto (22,6°Brix).

I suoli della zona C sono risultati i terreni più strutturati e con la più elevata profondità esplorabile. Le buone disponibilità idriche che li caratterizzano hanno favorito uno sviluppo vegetativo superiore rispetto a quello rilevato in altre zone (1 Kg di legno di potatura/ceppo). La buona vigoria ha determinato rese produttive elevate (3,9 Kg/ceppo), le più alte riscontrate nella zona di indagine. A queste sono corrisposti però i più bassi contenuti zuccherini (20,7 °Brix) e contenuti acidici significativamente più alti rispetto a quelli tipici delle uve di altre zone, risultato spesso osservato in situazioni in cui le disponibilità idriche permangono abbondanti durante tutto il ciclo vegetativo (Bravdo *et al*. 1985; Esteban *et al*. 2001).

I suoli della zona D sono risultati caratterizzati da medio-basse disponibilità idriche durante tutto il ciclo vegetativo. In questi terreni la presenza di uno strato argilloso a 60-70 cm di profondità ha limitato lo sviluppo e l'approfondimento radicale, portando a vigorie decisamente contenute (0,59 Kg di peso del legno/ceppo). Le rese sono risultate mediamente elevate (3,4 Kg/ceppo), con medi valori zuccherini.

La disponibilità idrica, la profondità esplorabile, la tessitura del suolo e la conseguente densità e distribuzione delle radici sono risultati i fattori che nel loro insieme hanno determinato rese e qualità delle produzioni. Ognuno di questi fattori è strettamente relazionato agli altri e gli effetti sono più o meno significativi a seconda della specifica realtà pedologica esaminata. Come si è visto infatti, a titolo di esempio, buone disponibilità idriche comportano generalmente un decadimento qualitativo per questa varietà (che mal tollera eccessi idrici); quando queste però sono associate ad una profondità limitata del suolo, concorrono all'instaurarsi di un ottimale equilibrio vegeto-produttivo e all'ottenimento di standard qualitativi elevati.

Tab.2 Parametri produttivi e qualitativi delle uve rilevati nelle 4 diverse realtà podologiche indagate (dati medi del triennio 2006-2008)

zona	Produzione /ceppo (Kg)	peso medio grappolo (g)	Legno/ceppo (Kg)	Zuccheri (°Brix)	Acidità (mg/l)	Acido malico (mg/l)	Acido tartarico (mg/l)	pH
Zona A	3,2 b	168 ab	0,60 b	21,5 b	5,4 b	1,8 a	6 b	3,20 a
Zona B	2,2 c	197 a	0,81 ab	22,6 a	5,0 b	1,8 a	6 b	3,26 a
Zona C	3,9 a	155 b	1,01 a	20,7 c	7,0 a	1,6 b	7,3 a	3,01 b
Zona D	3,4 ab	171 ab	0,59 b	21,3 b	5,1 b	1,7 a	6,2 b	3,18 a

L'analisi organolettica effettuata sui vini ottenuti dalle microvinificazioni (fig.6) conferma che l'effetto del suolo si esplica sui caratteri qualitativi delle uve e si estende anche ai prodotti enologici da esse ottenuti. Pur presentando tutti degli standard di gradimento elevati, i vini ottenuti nelle quattro zone di indagine hanno presentato alcune differenze. La maggior preferenza è stata data al vino della zona B, il quale ha presentato la maggior intensità olfattiva, con note marcate di mela, pera e frutta tropicale. L'acidità è risultata inferiore ad altre zone, e il retrogusto è risultato deciso e persistente al palato. Giudizi positivi sono stati riportati anche per i vini ottenuti nella zona A, apprezzati anche per l'ottimo corpo ed equilibrio e per la bassa acidità. I vini della zona C e D hanno evidenziato un'ottima eleganza complessiva, con un buon equilibrio tra sentori agrumati, fruttati e floreali. L'acidità più marcata dell'area C e le leggere note vegetali di entrambe le zone, hanno lievemente penalizzato la qualità complessiva, risultata comunque su livelli elevati.

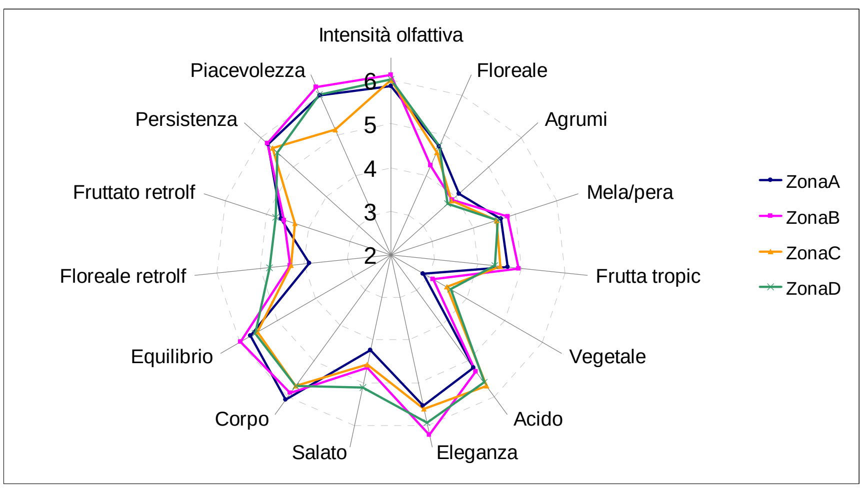

Fig. 6 Profilo sensoriale dei vini ottenuti nelle quattro zone di indagine (media delle annate 2006-2008)

CONCLUSIONI

L'analisi congiunta di tutti i fattori ambientali che partecipano alla qualità complessiva dell'uva e dei vini ha trovato nelle relazioni suolo-apparati radicali una utile chiave di lettura.

Dove minori sono le disponibilità idriche maggiore è la densità radicale atta all'esaurimento delle riserve idriche. In queste condizioni l'apparato aereo viene contenuto nel suo sviluppo con risultati qualitativi sempre premianti. Al contrario, maggiori disponibilità idriche non pienamente sfruttate da apparati radicali poco sviluppati per impedimenti meccanici alla loro diffusione (vedi zona D) conducono ad uve complessivamente meno interessanti a cui corrispondono dei vini non sempre rispondenti agli elevati standard qualitativi attesi. Per contro, se a stesse situazioni di buona disponibilità idrica corrispondono esuberi vegetativi e produttivi (zona C) si compromettono equamente le attese qualitative.

Vogliamo concludere ricordando che oltre al clima, al vitigno, all'uomo e alla sua storia, il suolo, per quanto sopra riportato, dovrebbe prevedere sempre lo studio dell'apparato radicale in esso presente e in così stretta relazione con i suoi caratteri e con la qualità delle produzioni

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PRESENZA DI UOVA DI *SCAPHOIDEUS TITANUS* SU MATERIALE EUROPEO DI PROPAGAZIONE DELLA VITE

V. Forte, F. Rizzini, E. Patriarca, L. Dalla Cia, E. Angelini, M. Borgo
CRA-VIT Centro di Ricerca per la Viticoltura
Viale XXVIII Aprile 26, 31015 Conegliano (Treviso), Italia
elisa.angelini@entecra.it

RIASSUNTO

La flavescenza dorata della vite è una malattia di quarantena diffusa in Europa e trasmessa dalla cicalina *Scaphoideus titanus*. In passato, lo scambio di materiale legnoso di moltiplicazione della vite contenente uova del vettore ha contribuito alla diffusione dell'insetto. Lo scopo di questo lavoro è stato di controllare se tale rischio è ancora presente.

Tralci di *Vitis vinifera*, tralci di portinnesto e barbatelle, provenienti da vivai europei, sono stati posti in condizioni idonee a favorire la schiusura di eventuali uova presenti. I "controlli positivi" erano costituiti da legno raccolto in vigneti infestati dal vettore.

Dai materiali di moltiplicazione in prova non è nato alcun insetto, indipendentemente dall'origine e dal tipo di materiale. Ciò indica che i materiali viticoli certificati ai fini della commercializzazione sono sicuri per quanto riguarda l'assenza del vettore. Nei "controlli positivi", invece, sono nate diverse centinaia di individui di *S. titanus*, sia da tralci di due anni che da tralci di un anno.

SUMMARY

Flavescence dorée (FD) is a European quarantine disease of grapevine, whose vector is the leafhopper *Scaphoideus titanus*. In the past, the trade in grapevine propagation material containing vector eggs contributed to the spreading of the insect. The aim of the present work was to check for the occurrence of this risk.

Commercial grapevine propagation material (scions, rootstocks and rooted grafted plants) from European nurseries were placed in condition to allow emerging of the nymphs. The positive controls were scions and rootstocks from *S. titanus* infested vineyards.

No *S. titanus* individuals were born from the woody material coming from the nurseries, irrespective of the country of origin or the kind of material. This showed that commercial grapevine propagation material used nowadays in trade exchanges is probably reliable as far as the absence of *S. titanus* eggs is concerning. In contrast, several hundred *S. titanus* individuals were born from two and one year old canes collected from an infested vineyard.

INTRODUZIONE

La Flavescenza Dorata (FD) è una malattia da giallumi della vite, di tipo epidemico e di quarantena, causata da fitoplasmi appartenenti al gruppo filogenetico 16SrV (Boudon-Padieu, 2003). Questi fitoplasmi vengono trasmessi da *Scaphoideus titanus* Ball (Auchenorrhyncha Cicadellidae), specie neartica univoltina strettamente associata alla vite, sia a *Vitis vinifera* (Vidano, 1964) sia ad altre specie di vite, in particolare a *Vitis riparia* (Maixner, 1993). Infatti solo su vite l'insetto compie l'intero ciclo biologico e supera il periodo invernale come uovo, che le femmine, a fine estate, depongono sotto il ritidoma o fra gli anfratti corticali, generalmente del legno di vite di due anni (Vidano, 1964; Alma *et al*., 1997).

Entrambi, malattia e vettore, sono presenti in molti Paesi europei, dove persistono e continuano a diffondersi. Numerosi studi sono stati condotti con l'obiettivo di trovare efficaci strategie di lotta nei confronti della malattia e della sua diffusione, orientandosi sia contro il fitoplasma sia contro il suo vettore. La termoterapia, proposta da Caudwell negli anni ’90 e oggetto di molte indagini successive, sembra essere una valida metodologia, che consente il risanamento del materiale viticolo legnoso infetto da FD (Boudon-Padieu, 2003). Contro il vettore l’unico sistema di difesa consiste nei trattamenti insetticidi, che risultano efficaci nei casi in cui vengano effettuati con le modalità adatte, ripetutamente negli anni e su un territorio piuttosto vasto (Mori *et al*., 1999, 2004; Pavan *et al*., 2004). Recenti studi preliminari, che hanno utilizzato il trattamento con acqua calda allo scopo di eliminare le uova di *S. titanus* dal legno di vite, hanno ottenuto risultanti soddisfacenti sul legno di un anno, ma non su quello di due anni (Linder, Schaub, 2009).

Anche in Italia, malgrado i trattamenti siano obbligatori in molte zone viticole, si assiste al perdurare della diffusione della malattia. Spesso ciò viene imputato alla commercializzazione di materiale legnoso di moltiplicazione della vite, che potrebbe contenente le uova del vettore. Questo fatto, infatti, in passato ha comportato la diffusione dell’insetto dall’America all’Europa e successivamente all’interno del territorio europeo (Bertin *et al*., 2007). Inoltre la presenza di uova sul legno potrebbe avere un ruolo importante anche in vigneti abbandonati e siepi di portinnesti, che confinano con vigneti coltivati e che fungono da “serbatoio” per il fitoplasma e per il vettore (Pavan *et al*., 2004; Beanland *et al*., 2006; Lessio, Alma, 2006a; Lessio *et al*., 2007; Forte *et al*., 2009).

Lo scopo di questo lavoro è stato di controllare se nei materiali legnosi di moltiplicazione viticola, che attualmente vengono commercializzati in Europa, siano ancora presenti uova di *S. titanus*. Inoltre si è voluto indagare l’eventuale esistenza di esemplari adulti del vettore all’interno e ai bordi di alcuni vivai viticoli italiani.

MATERIALI E METODI

La ricerca è stata svolta nel 2009 e si è articolata su due aspetti: i) verifica della presenza di uova di *S. titanus* nel legno destinato alla commercializzazione in Europa, ii) monitoraggio dell’insetto all’interno e ai margini dei vivai di moltiplicazione viticola.

Il primo studio è stato condotto in collaborazione con tre Paesi europei, oltre all’Italia: Francia, Slovenia e Svizzera. Sono stati presi in considerazione tralci di varietà di vite da vino, tralci di portinnesto e barbatelle paraffinate e pronte alla vendita, per un totale di 2700 campioni (Tab. 1). Inoltre, come “controllo negativo”, è stata utilizzata la stessa tipologia di materiale (100 marze di cv Merlot clone R12, 100 talee di portinnesto 1103 Paulsen clone CFC 57-34 e 100 barbatelle di Cannonau clone CFC 13 innestate su 779 P clone CFC 53-8), proveniente da un vivaio della Sardegna (Italia), isola in cui non è presente *S. titanus*. Il “controllo positivo”, invece, era costituito da portinnesti di un anno (cv K5BB), provenienti da un vigneto abbandonato in area edificabile della provincia di Treviso (Italia), e da tralci di *V. vinifera* di uno e due anni prelevati da due vigneti infestati da *S. titanus*, rispettivamente della provincia di Frosinone (cv Trebbiano Toscano) e di Venezia (cv Cabernet franc) (Italia). Prima del loro utilizzo per la ricerca, tutti i campioni sono stati conservati in cella refrigerata a 4°C. Date le ridotte dimensioni delle uova di *S. titanus*, che sono difficilmente identificabili sotto il ritidoma del legno di vite, si è ritenuto opportuno utilizzare un metodo indiretto per valutare la presenza del vettore, contando le neanidi nascenti dalle uova eventualmente presenti. Per questo motivo tutto il materiale in studio è stato posto in condizioni idonee alla schiusura delle uova: in camera termostatata a 25°C, 70% Uhr e fotoperiodo 16:8 (Bressan *et al*., 2005). Ogni campione (di 100 pezzi) è stato inoltre diviso in due sub-campioni, di 50 pezzi ciascuno, che sono stati collocati in cella climatica in due momenti diversi, al fine di effettuare l’esperimento in due diverse fasi. Il primo gruppo di sub-campioni è stato posto in camera termostatata dal 20 marzo al 12 giugno (i "controlli positivi" sono stati lasciati in cella fino al 16 luglio), mentre il secondo dal 5 agosto al 10 settembre (i "controlli positivi" sono stati lasciati fino al 3 gennaio). Infatti i "controlli positivi" sono stati lasciati in cella climatica fino alla completa schiusura di tutte le uova, mentre i sub-campioni provenienti dai vivai sono stati tolti dopo che si era verificato il picco di nascite nei "controlli positivi". Ogni sub-campione è stato posto in scatole di allevamento trasparenti di plastica (41x26x30 cm per le barbatelle ed i portinnesti, e 30x20x20 cm per le marze). Per mantenere un adeguato tasso d'umidità, all'interno delle scatole è stata posta agriperlite umida, su cui è stato collocato un tessuto nero, al fine di individuare gli esemplari di *S. titanus* eventualmente caduti sul fondo. Inoltre è stata utilizzata una foglia di vite come esca per le neanidi nascenti. Dalle barbatelle è stata tolta parzialmente la paraffina, per simulare il distacco che si verifica nelle condizioni di campo, evitando però di danneggiare il campione. Gli individui ritrovati nelle scatole sono stati contati ed esaminati allo stereomicroscopio.

Tab. 1: elenco del materiale di propagazione viticola oggetto di indagine.

Paese	Vivaio	Località	Tipo di materiale	Varietà	Quantità (n. pezzi)
Italia	1	Pordenone	Marze	Merlot ISV-F-V2	100
			Portinnesti	K5BB ISV CONEGLIANO 1	100
			Barbatelle	Merlot 181 F / K5BB ISV CONEGLIANO 1	100
	2	Pordenone	Marze	Merlot	100
			Portinnesti	K5BB	100
			Barbatelle	Merlot / K5BB	100
	3	Pordenone	Marze	Merlot ERSA FVG 350	100
			Portinnesti	K5BB VCR 424	100
			Barbatelle	Merlot ERSA FVG 350 / K5BB VCR 102	100
	4	Udine	Marze	Merlot ISV-F-V4	100
			Portinnesti	K5BB MI-K-3	100
			Barbatelle	Merlot ISV-F-V4 / K5BB MI-K-3	100
	5	Verona	Marze	Merlot ISV-F-V4	100
			Portinnesti	K5BB F 114	100
			Barbatelle	Merlot ISV-F-V4 / K5BB F 114	100
	6	Pisa	Marze	Merlot 447 F	100
			Portinnesti	K5BB MI-K-3	100
			Barbatelle	Merlot 447 F / K5BB MI-K-3	100
Francia	7		Marze	Merlot 181 F	100
			Portinnesti	196.17 cl. 99	100
			Barbatelle	Merlot 181 F / 196.17 cl. 99	100
Slovenia	8	Vipava	Marze	Merlot 181 F	100
			Portinnesti	K5BB AU 1	100
			Barbatelle	Merlot 181 F / K5BB AU 1	100
Svizzera	9	Ticino	Marze	Merlot	100
			Portinnesti	K5BB	100
			Barbatelle	Merlot / K5BB	100

La seconda parte dello studio è stata condotta presso due barbatellai presenti in zone viticole tipicamente vivaistiche: in provincia di Verona e di Pordenone (Italia). Ciascun vivaio confinava con un vigneto a conduzione tradizionale trattato secondo le normali pratiche viticole. Il monitoraggio è stato effettuato tramite l'utilizzo di trappole cromotropiche gialle (20 x 24,5 cm, con colla su entrambi i lati) posizionate all'interno sia dei vivai sia dei vigneti, sostituite ogni 20 giorni e osservate con uno stereomicroscopio (Tab. 2). Le trappole sono state collocate a livello della chioma delle barbatelle e delle viti. Inoltre nel mese di settembre è stato effettuato un monitoraggio visivo ai fini di individuare eventuali piante con sintomi da giallumi. I campioni che presentavano sintomi sono stati sottoposti a PCR (Angelini *et al.*, 2007).

Tab. 2: caratteristiche dei vivai monitorati e date di permanenza delle trappole.

Località	Tipologia	Dimensioni (m)	Dimensioni area monitorata con le trappole (m)	Data installazione trappole	Data ritiro trappole	N° postazioni	N° cambi	N°totale trappole	N° trattamenti insetticidi	Principi attivi
Verona	Vivaio	100x300	40x50	16/06/09	1/10/09	12	5	60	6	Spinosad, Chlorpyrifos
	Vigneto	100x300	40x50	16/06/09	1/10/09	12	5	60	1	Chlorpyrifos-methyl
Pordenone	Vivaio	25x250	25x50	30/06/09	22/09/09	12	4	48	3	Deltamethrin, Chlorpyrifos
	Vigneto	40x70	40x50	30/06/09	22/09/09	12	4	48	3	Indoxacarb, Chlorpyrifos

RISULTATI E DISCUSSIONE

Dal materiale legnoso vivaistico proveniente dall'Italia, dalla Francia, dalla Slovenia e dalla Svizzera, in nessuna delle due fasi di sperimentazione sono nate neanidi di *S. titanus* o altri insetti. Malgrado il numero dei campioni possa sembrare limitato (900 marze, 900 portinnesti e 900 barbatelle, per un totale di 2700 pezzi), questo risultato indica che i materiali viticoli commercializzati attualmente in Europa non contengono uova di *S. titanus* e perciò offrono sicurezza per quanto riguarda l'assenza del vettore di FD. Inoltre, tale dato fa presupporre che le pratiche fitosanitarie, adottate in vivaio e nei vigneti di piante madri di marze e di portinnesti, siano efficaci nell'evitare pericolose infestazioni da parte dell'insetto. Nemmeno dai campioni provenienti dalla Sardegna ("controllo negativo") sono stati osservati esemplari del vettore.

All'opposto, nei "controlli positivi" sono nate diverse centinaia di individui di *S. titanus* dai portinnesti di un anno e dai tralci di cv Cabernet franc di due anni, mentre nel materiale di un anno proveniente da Frosinone non sono stati osservati esemplari. Tale risultato accerta che le condizioni adottate nell'esperimento erano idonee alla riuscita della prova. In totale sono nati 924 esemplari di *S. titanus*, di cui 673 individui nel primo gruppo sperimentale e 251 nel secondo (Tab. 3).

Tab. 3: numero di *S. titanus* nati nei "controlli positivi" nei due gruppi sperimentali.

Tempo trascorso in cella climatica (settimane)	Prima fase sperimentale		Seconda fase sperimentale	
	Portinnesti di 1 anno	Tralci di 2 anni	Portinnesti di 1 anno	Tralci di 2 anni
1	0	0	0	0
2	0	0	0	0
3	0	0	0	14
4	0	0	0	27
5	0	0	0	4
6	2	0	2	16
7	1	11	0	7
8	3	12	0	14
9	5	1	0	1
10	1	0	3	36
11	0	37	1	15
12	2	136	0	7
13	5	356	0	6
14	6	76	0	0
15	0	13	0	39
16	0	6	0	16
17	0	0	0	8
18			0	6
19			0	15
20			0	10
21			0	4
22			0	0
23			0	0
Totale	25	648	6	245

Dalle analisi effettuate sul legno raccolto da viti, su incolti e su vigneti con infestazioni di *S. titanus*, è stato possibile acquisire informazioni circa il ruolo dei portinnesti e delle marze. Dai tralci dei portinnesti di un anno le nascite sono iniziate sempre dopo 5-6 settimane dall'introduzione del legno in camera termostatata, mentre dai tralci di *V. vinifera* di due anni sono nati esemplari anche a partire dalla terza settimana (secondo gruppo sperimentale). Nei tralci di K5BB la schiusura è durata da 6 a 9 settimane, mentre nei tralci di cv Cabernet franc tale periodo è variato da 10 a 19 settimane. In media gli esemplari nati dal legno di un anno rappresentano il 3,35% del totale delle nascite ed il 3,47% delle nascite avvenute nel legno di due anni. In totale, il numero di *S. titanus* nati dai portinnesti è stato di quasi 30 volte inferiore rispetto al numero di nati dai tralci di *V. vinifera* di due anni ed il periodo di schiusura delle uova è stato della metà (Fig. 1). Infine nei tralci di due anni si è osservato un alto numero di nascite, che sono risultate distribuite nel tempo in modo diverso nei due gruppi di sperimentazione: nel primo la maggior parte degli esemplari è nata nel corso di 4 settimane, mentre nel secondo le schiusure sono state più omogenee e prolungate nel tempo (Fig. 2). Da questo risultato si potrebbe dedurre che la lunghezza del tempo di conservazione del legno, più elevata nei campioni del secondo gruppo, oltre che la temperatura di mantenimento, abbia influenzato la maturazione delle uova modificandone le modalità di schiusura.

La presenza di neanidi del vettore su tralci di un anno non era mai stata riportata in precedenza, ma è in accordo con dati recenti ottenuti da altri gruppi (Bagnoli, Gargani, 2009; Linder, Schaub, 2009). Tale risultato indica che il legno di un anno è idoneo all'ovideposizione delle uova di *S. titanus* e ribadisce l'importanza da attribuire alla distruzione del legno di potatura. Il ritrovamento di neanidi nei tralci di due anni conferma invece la pericolosità del materiale legnoso proveniente da vigneti infestati dal vettore.

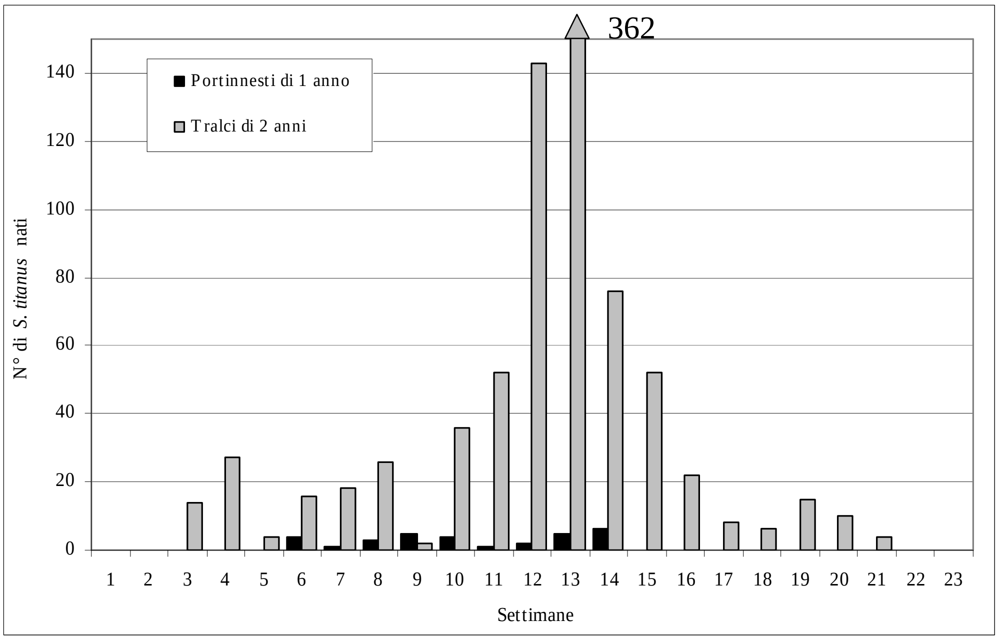

Fig. 1: numero totale di esemplari di *S. titanus* nati dai portinnesti di un anno e dai tralci di *V. vinifera* di due anni.

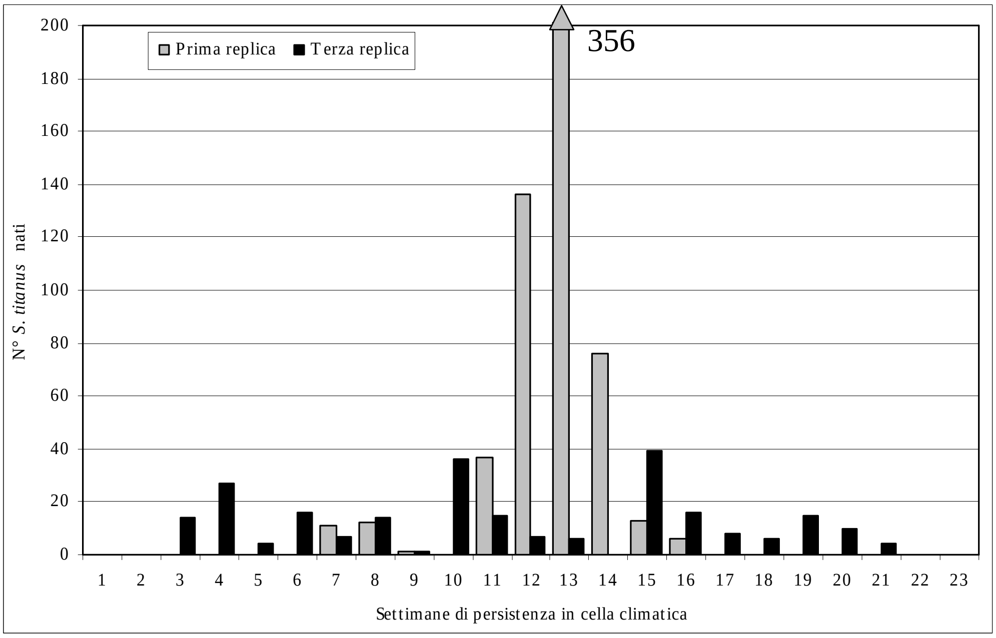

Fig. 2: numero di esemplari di *S. titanus* nati dai tralci di *Vitis vinifera* nella prima e nella seconda fase sperimentale.

Il controllo faunistico della presenza del vettore di FD in vigneto, tramite trappole cromotropiche, ha permesso di catturare 2 esemplari di *S. titanus* nel vigneto in provincia di Pordenone: in particolare un esemplare è stato catturato sul filare direttamente confinante con il barbatellaio e l'altro su un filare distante circa 40 m. Nessun esemplare è stato catturato all'interno del vivaio. In provincia di Verona non sono stati catturati esemplari del vettore né in vigneto né in barbatellaio. Il monitoraggio visivo dei sintomi da giallumi ha consentito di individuare due piante con sintomi sospetti: una vite nel vigneto in provincia di Pordenone ed una barbatella di cv Lambrusco nel vivaio di Verona, che è risultata infetta dal fitoplasma al test molecolare.

CONCLUSIONI

L'indagine faunistica, eseguita su materiale legnoso di vite certificato ai fini della commercializzazione, ha permesso di accertare l'assenza di uova di *S. titanus* sui campioni provenienti dalle zone di maggior produzione di materiale viticolo di Italia, Francia, Slovenia e Svizzera. Questo dato induce a ritenere che il rispetto delle norme comunitarie e nazionali sulla flavescenza dorata, malattia di quarantena, consenta di escludere la presenza di uova di *S. titanus* sui materiali di moltiplicazione Europei. Sarebbe comunque interessante approfondire lo studio, prendendo in considerazione campioni provenienti anche da altri Paesi europei e di tipologie diverse: barbatelle senza paraffina, prima e dopo la forzatura, tralci di *V. vinifera* e portinnesti provenienti da piante madri di diverse età. Sarebbe interessante stabilire un protocollo comune a livello europeo sia per quel che riguarda le pratiche di coltivazione e controllo in vigneto e in vivaio sia per le attività vivaistiche successive.

La schiusura delle uova avvenuta nei "controlli positivi" ha permesso di validare la tecnica sperimentale adottata e confermare alcune conoscenze sull'etologia di *S. titanus* e, in particolare, sulla presenza delle uova anche su legno di un solo anno e su tralci di portinnesti (Bagnoli, Gargani, 2009). Questo risultato pone il problema circa la presenza di siepi e incolti con portinnesti inselvatichiti nonché di vigneti abbandonati, che sono numerosi nelle zone a ridosso dei terreni adibiti a nuovi insediamenti urbani e destinati a rappresentare una situazione di rischio per la diffusione del vettore di FD (Forte *et al*., 2009). Pur sapendo che la capacità di dispersione di *S. titanus* è limitata (Lessio, Alma, 2004, 2006b), future ricerche potrebbero chiarire se effettivamente l'insetto si sposta dalla vite inselvatichita alla vite coltivata e quale sia il rischio reale nelle condizioni di vigneti trattati secondo le attuali normative.

Infine, l'assenza di catture di esemplari di *S. titanus* all'interno dei vivai, seppur siano stati raccolti due individui nelle immediate vicinanze, e il ritrovamento di una barbatella infetta, fa ritenere che le pratiche vivaistiche attualmente adottate, tramite trattamenti insetticidi e controlli fitosanitari, siano efficaci, pur nella necessità di mantenere alta la soglia di controllo. Al pari, i controlli per individuare la presenza di viti affette da giallume aiutano a ridurre i rischi di diffusione della malattia.

RINGRAZIAMENTI

Questo lavoro è stato finanziato tramite il progetto europeo Euphresco PROPSCAPH. Si ringraziano P. Grizon, C. Linder e D. Papura per aver fornito i materiali vivaistici di Slovenia, Svizzera e Francia; B. Bagnoli e G. Pasquini per il "controllo positivo" del Lazio.

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LA REGLEMENTATION DE LA RECOLTE DU RAISIN EN PRENANT EN CONCIDERATION LES PRINCIPES DE PROGRAMMATION

Auteurs : T. Gabisonia, M. Mirvelashvili, M. Vashakidzé, M. Babunashvili
Institution : Institut d'horticulture, de viticulture et de viniculture
6, avenue Maréchal Guélovani, 0159, Tbilisi, Géorgie
E-mail : temurigab@gmail.com ; m_mirvelashvili@hotmail.com

ABSTRACT

Une des conditions principales de la production des produits d'appellation contrôlée est sa réglementation, ce qui est possible avec la programmation de la récolte, dont le critère principal est l'optimisation du complexe de différents facteurs et sur ce fond, la gestion du processus de formation de la récolte biologique.

Dans cet objectif, dans la période des années choisies par nous, nous avons étudié par le biais des essais multi-facteurs la formation de la biomasse, ainsi que les facteurs déterminant l'émission des éléments nutritifs par certains organes de la plante.

A la base des données expérimentales, de différents modèles ont été construits, le meilleur résultat a été obtenu par la fonction logistique. Ce qui nous a permis de déterminer la période de croissance intensive, le début de l'écart de l'optimum et les processus de chute, tout cela ayant une grande importance pratique.

Reglamentation is the main factor for producing Terroir products, which is reached by programming harvest, the most important criteria is optimization of different factors and dealing formation process of biologic harvest (the hole bio mass).
Because of this object, in certain years were examined multi factorial (P^k – factors unity, lead up gradations quantity degree) experiments, on some basic industrial species, as formation of bio mass, as plant's regulatory of taking nutriment elements by separate organs.
Recline of experiment evidences were engineered various models, the best results we got by logistic function: This gave us means of establishing intensive growing period duration, from the beginning of optimum deviation and reduction processes, as of the whole biomass, as of the case study comfortable during the whole vegetation period any time of the plant used food elements amount, which has a huge practical significance.

INTRODUCTION

Le développement de la viticulture et la viniculture en Géorgie a atteint une nouvelle étape, celle de production des vins de l'appellation d'origine contrôlée dont la condition première est la réglementation de la récolte du raisin basée sur la programmation.

La récolte programmée c'est un produit caractérisé de signe spécifique de haute qualité dont la production prend en compte les caractéristiques biologiques du cépage, les conditions écologiques données et le complexe des mesures agrotechnologiques.

La programmation de la récolte comprend le processus de formation de toute la biomasse et les moyens de sa gestion. La description du processus est basée sur la théorie de l'évolution des organismes vivants de Saxe, qui porte la forme de S et est connu en biologie sous le nom de la courbe de Saxe. Pour effectuer une description et la gestion du processus qui suit il est nécessaire d'avoir des données d'expérimentation. [Bondarenko, 1976].

METODOLOGIE

Nous avons conduit une expérimentation d'après un schéma d'essai multi-facteurs sur deux cépages de production dans des environnements écologiques différents.

La matière a été étudiée avec de différentes fonctions. Nous avons reçu le meilleur résultat avec la fonction logistique.

Le modèle reçu décrit la formation des organismes séparés mais aussi de toute la biomasse, ainsi que l'utilisation par eux des éléments nutritifs sur des différentes étapes, ce qui porte une grande importance pratique.

L'expérimentation menée par un essai multi-facteurs nous a permis de constater le rendement optimal des cépages choisis avec l'alliance de divers facteurs dans des régions données. Quand la qualité du produit est supérieure, sa spécificité ainsi qu'une longue période d'exploitation de la plante sont maintenues.

L'expérimentation que nous avons menée pendant trois ans sur les cépages Rkatsitéli et Tchinuri dans de différents environnements écologiques avec un schéma d'essai multi-facteurs, ayant pour but l'observation du rendement optimal dans la région donnée et la création d'un modèle correspondant qui reflèterait son processus de formation [Adler, 1971].

L'étude sur le cépage Rkatsitéli a été conduite dans la région de Kakhéti, dans la partie nord-ouest de la vallée d'Alazani, à 430 mètre d'altitude.

Le sol du terrain d'essai : sol brun du champs, argiles légères, humus 2,0 – 2,24 %.

Le climat : modérément humide, précipitations moyennes annuelles : 770 mm, total des températures actives au dessus de 10° - 3730°.

Le terrain d'essai sur le cépage Tchinuri se trouvait dans la région de Chida Kartli, à 650 mètre d'altitude.

Le sol : argiles lourdes et argiles, humus dans les couches supérieures 4 – 5%.

Le climat : le climat de la zone varie entre sec subtropical et subtropical modérément humide. Le total des températures actives au dessus de 10° - 3500°.

Quatre facteurs ont été étudiés : Irrigation, fertilisation, longueur de tige de fruit, niveau de densité dans chaque cinquième gradation. Afin de mener l'essai sur un schéma complet de facteurs il faut élever le nombre de gradation à la puissance du nombre de facteurs. Dans le cas présent 5^4=625, mais il est pratiquement impossible de mener une expérimentation de variantes aussi nombreuses. C'est pourquoi nous avons choisi une des compositions de schéma à quatre facteurs, nous avons fait un choix équilibré de façon à ce que la valeur informationnelle de l'étude ne soit pas perdue.

RESULTATS

L'observation nous a permis de constater l'influence des facteurs sur les données à étudier ainsi que le degré de leur interaction.

$$Y=a_0+a_1x_1+ a_2x_2+ a_3x_3+ a_4x_4+ a_5x_1 x_2+ a_6x_1 x_3+ a_7x_1 x_4+ a_8x_2 x_3+ a_9x_2 x_4+ a_{10}x_3 x_4+ a_{11}x_1 x_2x_3+ a_{12}x_1 x_2x_4+ a_{13}x_2 x_3x_4+ a_{14}x_1 x_2 x_3x_4+ a_{15}x_1^2+ a_{16} x_2^2+ a_{17} x_3^2+ a_{18} x_4^2$$

De fait nous avons constaté l'interaction entre 18 facteurs au lieu de quatre.

Les coefficients a_1 - a_4 inclus caractérise l'influence de certains facteurs et le coefficient de leur régression est relativement plus haut que $a_5 - a_{10}$, ceux marquant l'alliance de facteurs par paire, le groupe suivant de coefficients $a_{11} - a_{13}$ caractérisent l'interaction de trois facteurs, le coefficient de régression est basse dans ce cas également, il est inférieur aussi dans le cas de quatre facteurs et leurs carrés. Nous pouvons ainsi constater que l'influence de chaque facteur séparé est relativement plus importante que dans le cas de leur alliance quelconque.

En nous appuyant sur les données de recherches de trois ans nous pouvons dire que le rendement optimal du cépage Rkatsitéli dans des conditions de climat et du sol données est 90 – 95 q/ha, avec 20,5% du taux de sucre et 7,5 g/dm^3 du taux d'acidité.

La matière de vin par rapport à d'autres variantes se caractérise d'extrait de haut niveau, des caractéristiques de cépage, un bouquet fin, a reçu une appréciation de dégustation de 8,56 bals.

Le rendement du cépage Tchinuri peut être considéré dans les conditions données et ses similaires 115-120 q/ha, avec 17,8% du taux de sucre et 8,3 g/dm^3 du taux d'acidité. Note d'appréciation – 8,45 bals.

Les paramètres mentionnés peuvent être à la base de la réglementation de la récolte dans le cas des deux cépages, avec les conditions de climat et du sol évidemment similaires. Afin de réussir à obtenir une récolte programmée d'avance, donc réglementée il faut arriver à contrôler et gérer le processus de sa formation.

Dans cet objectif nous avons étudié dans le cas des deux cépages sur cinq variantes différentes les plus contrastées, l'accumulation de la biomasse et le rapport de certains éléments dans la biomasse totale, ainsi que les facteurs déterminant l'utilisation par ces deniers des éléments nutritifs.

Les résultats reçus prouvent que par l'augmentation des normes de facteurs la biomasse totale de la plante augmente également. Pourtant au-dessus d'une certaine limite le pourcentage de la densité de grappes dans la biomasse commence à baisser. Nous avons par ailleurs observé la présence des matières sèches dans certains organes. Dans ce cas aussi son taux était relativement bas dans le cas d'un rendement fort que dans le cas d'un rendement plus modeste. Les résultats obtenus ont été utilisés pour la création d'un modèle.

Comme nous l'avons déjà mentionné, nous avons obtenu le meilleur résultat par la fonction logistique:

$$y = \frac{A}{1+10^{a+bx}} + C$$

D'après les graphiques on voit clairement que dans le cas des normes basses de facteurs agrotechnologiques le point final de la courbe de régression apparaît plus tôt que dans le cas des normes élevées. Ce qui veut dire que les normes élevées déterminent la durée de la croissance.

Il est par ailleurs évident qu'au début de la période de végétation la formation de la biomasse se poursuit plus lentement. Ensuite, c'est la période de la croissance rapide qui poursuit – cette période dure particulièrement longtemps sur le fond de l'agrotechnique élevé.

En nous appuyant sur les données obtenues nous pouvons constater que dans le cas de la densité élevée pour les deux cépages la croissance de la biomasse des organes de végétation est unilatérale. C'est pareil dans le cas de la densité la plus basse, mais ceci est dû à l'insignifiance de la récolte.

GRAPHIQUE N° 3-5

Il est présenté la formation de la biomasse sèche des tiges annuelles.

(Un index de corrélation élevé a été obtenu par la parabole du deuxième degré).

La courbe de régression de la première variante (où le fond agrotechnique était le plus élevé, ainsi la récolte était également plus importante) est inférieure par rapport à d'autres variantes que dans le cas des variantes deux et trois. Ceci est dû au fait que la hausse de la récolte a causé la baisse des matières sèches dans les organes de végétation, ce qui finalement a influencé la productivité et la baisse de rendement la dernière année.

CONCLUSIONS

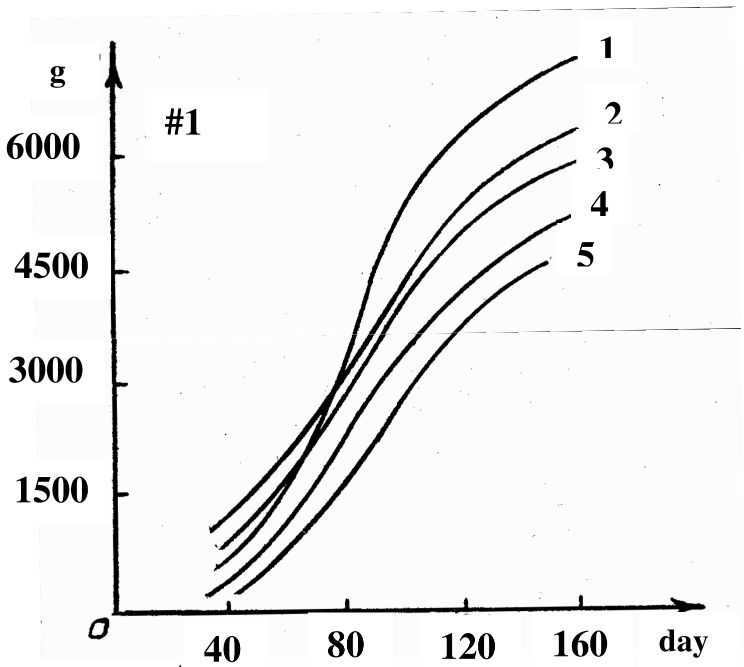
Les données obtenues prouvent que dans le cas du rendement faible l'importance de la biomasse sèche est apparente plus tard que dans le cas du rendement fort. De ce fait, on doit considérer comme rendement optimal, comme nous l'avons déjà noté, 90 – 95 q/ha – le cépage Rkatsitéli et 115 – 120 q/ha – le cépage Tchinuri.

Un des paramètres principaux déterminant la force de la croissance - évolution de la vigne, pouvant être utilisé en tant qu'un certain indicateur – c'est l'intensité de la tige et le degré de son mûrissement. Nous avons constaté sa longueur optimal dans le cas du cépage de Rkatsitéli à 1,5 – 1,6 mètres et celui du cépage Tchunuri – 1,8 – 2,0 mètres. Il a été étudié d'autres paramètres du rendement pour l'obtention d'une récolte réglementée, donc programmée: nombre de grappes, poids moyen de la grappe etc.

Le volume de l'appareil d'assimilation (largeur de la feuille) est un des paramètres pouvant servir à réguler la récolte programmée [Amirjanov, 1980].

Le modèle obtenu par nous permet de déterminer à n'importe quel moment le taux de croissance de la biomasse ainsi que d'autres valeurs déterminant le processus de la formation de la récolte. Dans le cas du besoin ce modèle nous permet d'effectuer des corrections correspondantes.

La programmation de la récolte est étudiée comme un complexe de systèmes dont le modèle conceptuel est le suivant: la plante – les ressources éacologiques – l'agrotechnologie [Tourmanidzé, 2003].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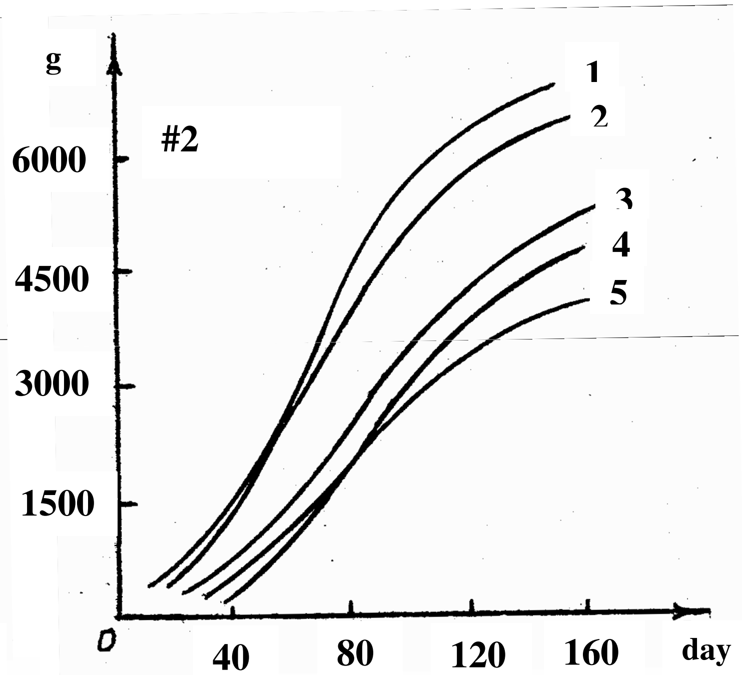
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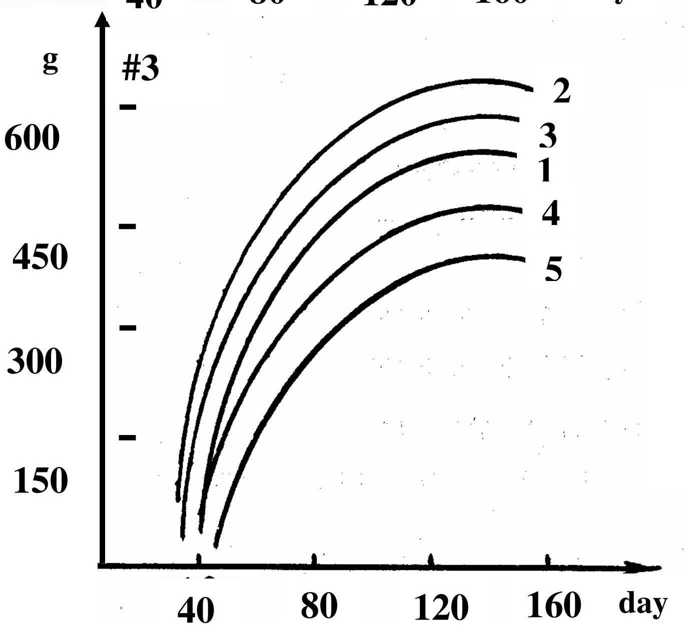
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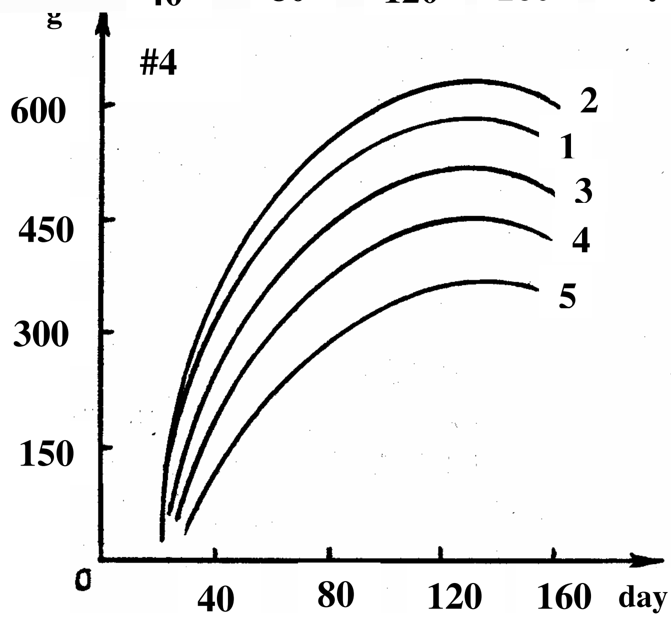
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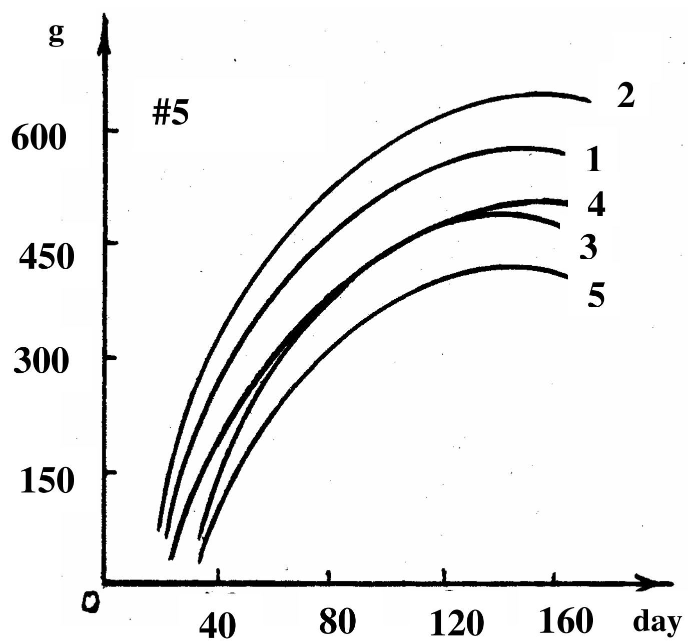
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EFFECTS OF DIFFERENT CULTURE ENVIRONMENTS ON ROOTING

B. ISCI[1] **A. ALTINDISLI**[2]

[1]Ege University, Faculty of Agriculture, Department of Horticulture, Bornova, Izmir, TURKEY, burcak.isci@ege.edu.tr

[2]Ege University, Faculty of Agriculture, Department of Horticulture, Bornova, Izmir, TURKEY, ahmet.altındisli@ege.edu.tr

Abstract. This study was carried out in the hydroponic system available at the Grapevine Production Building of Ege University, Faculty of Agriculture, Department of Horticulture in 2007-2008. As the standard plant material, articles of Alfonse Lavallèe were grafted with 5BB (*V. berlandieri* x *V. Riparia)*, SO4 (*V. berlandieri* x *V. Riparia)*, 1613 Paulsen (*V. berlandieri* x *V. Rupestris)*, cuts of Fercal American grapevine rootstock by using omega grafting. Rootless and grafted Grapevine saplings which have completed callus development were used in the study. Two different aggregate medium; pressed agricultural rock wool and kukupit were used with soil mortar mixture as the control. Each of polystyrene flume type pots in the hydroponic system has a capacity of 20 saplings and the experiment was set to furnish 3 repeats for each medium. Nutrition solution was given to the system at constant intervals. Parameters regarding sapling performance percentages of grafted grapevine saplings, root lengths suggesting the sapling quality, wet shoot weight and wet root weight were analyzed.

In the study it was determined that rock wool (72.333 **a**) and kukupit (66.875 **a**) have produced better results in the aspect of sapling rooting performances when compared to the control (51.429 **b**). Agricultural rock wool gave the best results by 0.899 **a** for wet root weight in grafted grapevine saplings. In the study, effects of different rooting environments on root lengths and wet sucker lengths were found to be insignificant. As structures of both rooting environments allow plating of the grafted root grapevine saplings in the soil without removing them from the tube material and as agricultural rock wool and kukupit mediums are easy and cheap to make and due to their success in rooting performance, they were rendered to be alternative rooting environments for sapling producers.

Key words: grapevine, hydroponic, tube sapling, omega graft, kukupit

EFFETTI DI DIVERSE CULTURE MEDIA ESEGUE IL STEM

Riassunto. Questa ricerca, nel 2007-2008, il Dipartimento di Orticoltura, Facoltà Ege University of Agriculture, appartenente al palazzo situato a vite di produzione di piante nel sistema idroponico sono state effettuate. Vitigni di materiale vegetale, come una penna standard con il Alfons Lavallèe'e, 5BB (*V. berlandieri* x *V. Riparia)*, SO4 (*V. berlandieri* x *V. Riparia)*, 1613 Paulsen (*V. berlandieri* x *V. Rupestris)*, Fercal portinnesti di viti americane appartenenti alla acciai, sono stati immunizzati con vaccino omega. Callo ha completato lo sviluppo di piante di vite senza radici sono stati utilizzati durante l'esperimento. Due diversi mezzi di comunicazione globale; premuto per il controllo dei terreni agricoli con lana di roccia e kukupit mix di mortaio è utilizzato. Sistema idroponico contenuti in ciascun piatto di canali in polistirene ha una capacità di 20 piante e di ogni esperimento ambiente deve essere stabilita con 3 repliche. Soluzione nutritiva è stata data al sistema a intervalli regolari. La percentuale di rendimento vivaio vite innestate, che rivela la qualità delle piantine e lunghezza

della radice, spara peso e l'età del parametro età è correlata alla radice il peso sono stati esaminati.

Studio sistema idroponica nel medio radicazione come lana di roccia agricole (72.333 **a**) kukupit (66.875 **a**), della vite innestate di radicamento efficienza in termini di controllo (51.429 **b**) di un ottimo risultato era stato identificato. Peso rock agricolo di lana delle radici della vite innestate nella 0.899 ha dato i migliori risultati con un. Utilizzato nello studio dei diversi mezzi di comunicazione radicamento della lunghezza della vite innestate radice e l'età non sono stati effetti significativi sulla lunghezza sparare. Entrambi i materiali di radicamento tubo medio rimosso da innesti sospensione radicale del suolo di impianto per permettere la struttura, e che la fattoria con lana di roccia kukupit radicamento ambienti dalla convenienza ed economicità, efficienza radicamento sia successo perché i produttori di piantine una alternativa radicamento mezzo potrebbe essere concluso.

Parole chiave: vite, idroponica, alberelli scuba, il vaccino omega, kukupit

INTRODUCTION

Most of the vine yards in our country are contaminated by phylloxera (*Viteus vitifolii*) and nematods. Thus, American grapevine rootstocks should be used at fields of domestic viticulture and when establishing new vineyard facilities (KISMALI, 1978). There are some insufficiencies in renewal of old vineyards and supplying grafted grapevine saplings required at new vineyard facilities. Grapevine requirement of our country has been determined to be 8-10 millions per year, however our grafted grapevine sapling production can only supply 20-25 % of this demand. In order to meet the sapling deficit in out country both new sapling facilities and new applications enhancing grafted sapling performances are required (ILGIN, AKMAN, KAÇAR, 1990).

From commercial viticulture perspective, grafting on a proper rootstock is a standard vineyard practice in most parts of the world. The majority of commercial plantations presently evade direct planting of selected grape varieties due to susceptibility of cultivated varieties to pests, i.e. microbes, mites, insects, nematodes and more importantly *Phylloxera,* leading to decline. The key for successful commercial cultivation is to focus on accessibility to suitable rootstock planting materials in large numbers (M. ALİZADEH, *et al*., 2010).

2-5 % of production stage losses in grafted grapevine sapling production occurs during grafting and 2-30 % of it occurs during germination. Furthermore, 20-72 % of losses occurs during rooting at the nursery (KOCAMAZ, 1991). The structure of nursery soil and soil and climate conditions during 2-3 weeks period following grafted cuttings greatly affect the success of grafted grapevine sapling performance to be achieved. Harvesting losses are significant besides losses incurring during the vegetation period. In recent years, hydroponic systems are used at open fields and greenhouses in order to minimize nursery losses, to meet the increasing grafted grapevine sapling demand, to produce more saplings per unit area, to increase quality, to reduce cost and labor (ŞENGEL, 2005).

This study of rooting grafted grapevine saplings in different rooting environments was carried out by using a hydroponic system at Grapevine Production Facility of Ege University, Faculty of Agriculture, Department of Horticulture. In the study grafted grapevine saplings of Alphonse Lavallèe grape variety grafted by 5BB, SO4 1613 Paulsen, Fercal American grapevine rootstocks were used as plat plant materials. Two different aggregate mediums, cubic presses agricultural rock wool and kukupit which allow grafted rooted sapling to be planted to the soil without removing it from the tube material after the rooting were used.

Thus, it is aimed to minimize losses which may occur during removal of the tube or nylon material of the sapling. In the study, parameters regarding sapling performances of grafted grapevine saplings in different rooting environments; root lengths suggesting the sapling quality; wet sucker weight and wet root weight were analyzed and effectiveness of the environments were suggested.

MATERIALS AND METHODS

This study was carried out at the Grapevine Production facility of Ege University, Faculty of Agriculture, Department of Horticulture in 2007-2008. As the standard plant material, articles of Alfonse Lavallèe were grafted with 5BB, SO4, 1613 Paulsen, cuts of Fercal American grapevine rootstock by using omega grafting (MAY, 1997; ÇELIK, *et al.*, 2002). Grafted cuts completed their kallus development in germination chambers after grafting. These grafted rootstocks were applied paraffin when exiting the germination chamber. Rootless grafted grapevine saplings which have completed their kallus development were planted in cubic pressed agricultural rock wool and kukupit as two different rooting environments in the hydroponic system. For hydroponic cultivation, saplings in tubes and a mixture of pearlite, soil, sand, sawdust and burnt farm manure in 15 cm x 25 cm black plastic bags representing classical tubed sapling production as the control sample for alternative rooting environments were used.

The hydroponic system used for the research have consisted of long flume type pots made of polystyrene where nutrition solution is passed through and fed to the plants. Dimensions of each polystyrene flume type pots available in this hydroponic system consisting of circulation pumps and materials facilitating the circulation of the nutrition solution are 12 cm x 113 cm with a depth of 20 cm. Each of the pots has a capacity of 20 saplings and the experiment was set to facilitate three repetitions for each environment. The drainage system consist of pipes which collect the nutrition solution drained from pots via flumes and carry it back to the tank and pipes transferring the fresh nutrition solution to saplings. The formulation of nutrition solution fed to grafted grapevines is given in Table 1. Optimum conditions for perennial plants were considered for preparation of the nutrition solution (ADAMOVA, 1978; BONDARENKO, *et al.*,1979).

Table 1. Nutrition solution content fed to tube grapevine saplings

Nutrition element	**Dosage (mg/L)**	**Chemical source**	**Required amounts for 1 ton of water**
N	90	Nitric Acit (% 55)	200 ml HNO_3
P	45	MAP (% 61 P_20_5, % 12 N)	169 gr
K	110	KNO_3 (% 13 N, % 46 K_2O, % 38 K)	288 gr
Ca	-	-	-
Mg	45	$MgSO_4.7H_2O$ (% 10Mg)	450 gr
Fe	2	Sequestrene (% 6 Fe)	33,3 gr
Mn	1	$MnSO_4.H_2O$	3,073 gr
Zn	0,75	$ZnSO_4.7H_2O$	3,312 gr
Cu	0,25	$CuSO_4.5H_2O$	0,982 gr
B	0,3	H_3BO_3	1,691 gr
Mo	0,02	$(NH_4)_6Mo_7O_{24}.4\ H_2O$	0,037 gr

- Stock solutions were prepared by using pure water in final volume of 10 L.
- Solution was applied to the saplings by adding 1 L of solution mixture to 100 L of tap water.

In the study, parameters suggesting sapling performances and sapling quality of grafted American grapevine saplings in different rooting environments within the hydroponic system.

The sapling performance was calculated by dividing the number of saplings obtained after the harvest having healthy root and sucker systems to total number of grafted American grapevine rootstocks plated in each environments and multiplying the result by 100.

The wet root weight (g) and wet sucker weight (g), root lengths (cm) which suggest sapling quality of grafted American grapevine rootstocks were measured.

a. Wet root weight (g): Roots grown on the grafted grapevine sapling in each repetition were cut and weighed. Then total of root weights in each repetition was divided by number of saplings to achieve the average root weight per sapling.
b. Wet shoot weight (g): Wet shoot grown on grafted grapevine rootstocks in each repetition were weighed. Then total of sucker weights in each repetition was divided by number of saplings to achieve the average sucker weight per sapling.
c. Root lengths (cm): Grown root lengths were measured and recorded for each plant individually. The longest 3 roots were measured and their average is given for the roots.

The experiment was conducted as per random parcel experiment pattern in 3 repetitions and 20 grafted grapevine sapling were included in each repetition. Data obtained from the experiment subjected to variance analysis by using SPSS (SPS Inc., USA) software, and separate averages differences for each type were determined by *LSD* test.

RESULTS AND DISCUSSION

In this study, the performances of grape saplings grafted by omega graft on desk by using bars belonging to 5BB, SO4, 1613 Paulsen and Fercal American grape-vine rootstock through belonging to Alfonse Lavallèe are examined. Pressed agricultural rock wool and kukupit used as rooting medium and soil mortar is used for control. When results obtained from the experience at the end of two years, it is determined that 4 different American grape-vine rootstocks created statistically meaningful difference in hidroponic system in terms of sapling performances (Table 2).

Table 2. 4 sapling performance of different American grape-vine rootstock

Rootstocks	**Sapling performance (%)**
SO4	73.529 **a**
Fercal	66.944 **ab**
5BB	60.889 **b**
1613 Paulsen	54.375 **c**
***LSD*$_{rootstocks}$**	***13.313***

In the study, saplings grafted by SO4 grape-vine rootstock has taken place in first statistical class with 73.529 **a** sapling performance. In terms of sapling performance, amongst other American grape-vine rootstocks, Fercal obtained 66.944 **ab**, 5BB; 60.889 **b** and 1613 Paulsen 54.375 **c** values respectively (Table 2).

Ilgın *et al.,* (1990) determined that rootstocks showed important effects on differences of sapling performances, first class sapling performances, and rooting characteristics, in their study they carried out for determining species/rootstock combinations in grafted grape sapling cultivation. Also, they determined that species/rootstock combinations have important effects on differences between total sapling and first class sapling performances. Especially, they indicated that sapling performances in Rupestris du Lot (52.8 %), 1613 (51.5 %) ve 110R (41.2 %) grape-vine rootstocks is more important when compared to others [Ramsey (29.8 %) ve 5BB (29.2 %)].

Çelik *et al.,* (1992) examined sapling performances by grafting Cardinal, Alphonse Lavallée and Semillon species on desk and plantation conditions over 5BB, 1103P ve SO4 rootstocks. It is stated that sapling performance of the bars grafted on desk (in species/rootstock combinations) is 39, 09 % - 23, 51 %.

It is stated that agricultural rock wools and kukupit rooting medium gave better results than controls when examined in terms of effects of two different rooting combinations on sapling performances of grafted grape saplings (Table 3).

Table 3. Effect of different rooting medium on sapling performance

MEDIUM	**Sapling performance (%)**
Agricultural rock wools	72.333 **a**
Kukupit	66.875 **a**
Control	51.429 **b**
LSD **medium**	***15.407***

Parameters presenting sapling quality of different American grape-vine rootstocks on grafted grape-vine rootstocks are evaluated for rootstocks in Table 4.

Table 4. Evaluation of the rootstocks in terms of sapling quality parameters

Rootstocks	**Root Length (cm)**	**Wet Root Weight (g)**	**Wet Shoot Weight (g)**
SO_4	19.397 **b**	0.660 **c**	7.162 **a**
Fercal	20.133 **b**	0.752 **b**	6.757 **a**
5BB	25.326 **a**	0.769 **b**	4.784 **c**
1613 Paulsen	17.236 **bc**	0.852 **a**	5.854 **b**
$\boldsymbol{LSD_{rootstocks}}$	***2.241***	***0.058***	***0.575***

5BB is determined to be taking place in the first statistical group with 25.326 **a** root length parameter in term of sapling quality. SO4 and Fercal are determined to be taking place in the same group in root length and wet shoot weight parameters. It is determined that 1613 Paulsen (0.852 **a**) gave the best result in terms of wet root weight parameters of grafted saplings, and followed by 5BB 0,769 **b** and Fercal 0,752 **b**.

Çelik *et al.,* (1992) examined sapling performances of American grape-vine rootstocks after omega graft. They found that shoot development level changed between 3,11-2,74, root development level between 3,35-2,16, and the number of root thicker than 2 mm between 7,45-5,87 pieces. Also, they determined that simple effect of rootstocks and species created important differences in terms of these criteria. Generally, it is determined that 1103P rootstock gives the best results in terms of sapling performance (38,1 %) and shoot development level (2,99) while 5BB rootstock gave the best results in terms of root development level (3,21) and shoot length, however, SO4 rootstock gave the best averages in terms of root number (6,97 pieces).

In Table 5, parameters presenting sapling quality of different rooting milieus in grafted grape-vine rootstocks in hidroponic system are shown. It is determined that agricultural rock wool and kukupit from different rooting medium used in terms of root length and wet shoot weight gave the same result with soil-mortar mixture used as control. It is determined that medium used didn't give rise to a statistical importance on root length and wet shoot weight parameters and all had the same activity.

In the study, it is determined that grafted tube grape-vine saplings constituted control group consisting of kukupit with 1st class 0,899 **a**, agricultural rock wool with 2nd class 0,786 **b** value, and 3rd class 0,590 **c** for wet root weight parameter. According to study findings, kukupit and agricultural rock wool were determined to be materials that can be used in lieu of soil mortar, classical rooting milieu, for rooting grafted grape-vine saplings.

Table 5. Evaluation of different rooting milieus in terms of sapling quality

Medium	**Root Length (cm)**	**Wet Root Weight (g)**	**Wet Shoot Weight (g)**
Agricultural rock wools	20.492	0.786 **b**	6.305
Kukupit	20.513	0.899 **a**	6.042
Control	20.564	0.590 **c**	6.071
LSD $_{medium}$	***ns***	***0.038***	***ns***

In agricultural rock wool, owing to the fact that water holding capacity and aeration capacity are high and that any situation preventing root development is not encountered, considerably high wet root weight (0,786 **b**) occurred. It can be successfully used as rooting milieu because of its easiness and cheapness in its supply and also its success in wet root weight (Table 5).

Both different rooting materials used in the study are found to be admissible as an alternative allowing planting the grafted sapling directly to the soil in tube sapling production. It is of the opinion that agricultural rock wool and kukupit milieus can be an alternative production method for sapling cultivators because of their cheapness.

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WEINBAU UND ENERGIE

H.-P. Schwarz
Fachgebiet Technik der Forschungsanstalt Geisenheim
Von-Lade-Straße 1, D-65366 Geisenheim
hans-peter.schwarz@fa-gm.de

ABSTRACT

Die zunehmende Knappheit fossiler Brennstoffe und der daraus resultierende Preisanstieg für die daraus gewonnene Energie macht den Einsatz alternativer Energieformen im Weinbau immer interessanter. So besteht z. B: mit der thermischen Verwertung energiehaltiger Reststoffe wie Trester und Rebholz die Möglichkeit, CO2-neutrale Energieträger kosten- und umweltorientiert zu verwerten. Zugleich hat die Rebholzentfernung aus dem Weinberg auch eine wichtige phytosanitäre Bedeutung, denn Krankheiten wie Esca können bisweilen nur durch Entfernen befallenen Holzes bekämpft werden. Weitere Möglichkeiten der Energiegewinnung bestehen durch die Brachflächenbewirtschaftung, die mit Energiepflanzen bestellt werden könnten sowie durch den Einsatz von Photovoltaik und Geothermie.

Den Möglichkeiten der Energiegewinnung steht der Primärenergieverbrauch zur Produktion einer Flasche Wein gegenüber, von der Anlage des Weinbergs bis zur Auslieferung des Produktes. Die Aufschlüsselung des Energiebedarfs der einzelnen Produktionsschritte zeigt auch hier Einsparpotentiale. In der Summe bieten Energieeinsparung und Energiegewinnung die Möglichkeit, die Produktionskosten einer Flasche Wein deutlich zu senken.

ABSTRACT

La hausse des prix de l´énergie résultant du manque croissant de combustibles fossiles rend l'application de formes d'énergie alternatives de plus en plus intéressante pour la viticulture. Ainsi, par exemple, il est possible de mettre en valeur des résidus à haut pouvoir énergétique comme le marc et le bois de vigne par une valorisation thermique neutre en CO2, économique et tournée vers l'environnement. En même temps, la sortie des bois du vignoble après la taille présente aussi un intérêt phytosanitaire important, car des maladies du bois comme l´Esca ne peuvent être combattues jusqu´à présent que par une élimination du bois atteint. D'autres possibilités pour la production d'énergie se présentent par l'exploitation de terres en jachère qui pourraient être plantées avec des plantes énergétiques ainsi que par la mise en place de systèmes photovoltaïques et de géothermie.

Face aux possibilités de production d'énergie, il faut considérer la consommation d'énergie primaire liée à la production d'une bouteille de vin, depuis l'installation du vignoble jusqu'à la livraison du produit. L´étude des besoins énergétiques des différentes étapes de production montre des potentiels d'économie. Sur le bilan, les possibilités d'économie d'énergie et de production d'énergie permettraient d'abaisser clairement les coûts énergétiques liés à la production d'une bouteille de vin.

EINFÜHRUNG

Auf dem Weg zu einer nachhaltigen Energieversorgung kommt der Nutzung erneuerbarer Energie eine zentrale Bedeutung zu. Die Endlichkeit fossiler Rohstoffe gebietet es, wo immer sinnvoll möglich, auf nachwachsende Rohstoffe zurückzugreifen.

„Mit dem starken Wachstum der Weltbevölkerung wird der Energiebedarf bis zum Jahre 2020 voraussichtlich um fast 40% steigen. Während der nächsten 50 Jahre wird sogar mit einer Verdreifachung des Energieverbrauchs gerechnet. (…) Geologen gehen davon aus, dass

Erdöl bei gleich bleibendem Anteil am gesamten Energieverbrauch nur noch für ca. weitere 20 Jahre verfügbar sein wird. Erdgas wird unter derselben Voraussetzung noch ungefähr 40 Jahre zur Verfügung stehen“ (Bioconinvest, 2006).

Die energetische Verwendung des jährlichen Winterschnittholzes stellt für Weinbaubetriebe durchaus eine Strategie zur Befreiung aus der Abhängigkeit von Öl und Gas und deren Kosten dar. Es stellt sich aber die Frage inwieweit mit dem Rebholz ein wichtiger Humus- und Nährstofflieferant verloren geht.

Nach dem Rebschnitt fallen je Hektar Ertragsrebfläche bei 4000 bis 5000 Stock pro Hektar etwa 2000 bis 2600 kg nicht zum Anschnitt benötigtes Rebholz an. Der Wassergehalt von frischem Rebholz liegt bei rund 45 %, weshalb eine Lagerung mit Trocknung notwendig ist. Im lufttrockenen Zustand liegt der Wassergehalt lediglich bei ca. 15 % (1400 bis 1800 kg/ha lufttrockenes Rebholz).

DIE TECHNIK DER ENERGETISCHEN REBHOLZVERWERTUNG

Zur Zeit stehen drei Maschinen von drei verschiedenen Herstellern mit zwei unterschiedlichen Arbeitsweisen zur Rebholzaufbereitung zur Verfügung.

Das Prinzip der Rebholzballenpresse basiert auf der Funktionsweise der im Ackerbau seit Jahrzehnten bewährten Strohrundballenpressen. Die Rebholzrundballenpresse ist zapfwellengetrieben und wird am Schlepperheck angebaut. Die Geräte sind in zwei unterschiedlichen Arbeitsbreiten (0,93 m und 1,23 m) lieferbar, das Gewicht liegt je nach Ausstattung bei 480 bis 550 kg. Die Presse hat eine feste, geschlossene Ballenkammer, d.h. die Ballengröße von 60 cm Länge und 40 cm Durchmesser kann nicht variiert werden. Das Ballengewicht schwankt in Abhängigkeit von der Holzfeuchte und der Pressdichte zwischen 15 und 30 kg (Frischgewicht, ca. 100 bis 120 Ballen pro Hektar). Optional wird eine Aufnahmevorrichtung angeboten, die bis zu sechs Rebholzballen fasst. Die Rebholzballen werden wie Stückholz zur Trocknung im Freien abgedeckt gelagert.

Eine weitere Variante der Rebholzbergung stellen die Rebholzhäcksler mit Sammelvorrichtung dar. Das Rebholz wird durch eine Pickup vom Boden aufgenommen, anschließend wird das Holz wie bei einem Schlegelmulcher zerkleinert und in einem Behältnis gesammelt. Die Geräte sind zapfwellengetrieben und werden ebenso am Schlepperheck angebaut. Die Häcksler sind in vier unterschiedlichen Arbeitsbreiten (1,00 m bis 1,80 m) erhältlich, das Gewicht liegt zwischen 900 und 1400 kg.

Als Sammelvorrichtung werden Bunkerlösungen angeboten, dabei muss das Häckselgut in einem entsprechenden Lagerraum getrocknet werden. Eine Alternative bieten luftdurchlässige Big Bags, in denen das gehäckselte Rebholz im Weinberg gesammelt und später gelagert werden kann.

Bei allen Maschinen zur Rebholzbergung entspricht die Arbeitsgeschwindigkeit etwa der eines konventionellen Rebholzhäckslers. Die Rebholzballen können entweder in einem entsprechenden Feststoffbrennkessel oder nach weiterer Aufbereitung in einer Hackschnitzelanlage verwertet werden. Das Häckselgut der Rebholzhäcksler kann unverändert in einer Hackschnitzelanlage verfeuert werden.

ENERGIEGEHALT UND BEWERTUNG

Um die Wirtschaftlichkeit der Verwertung von Rebholz beurteilen zu können, muss dessen Energiegehalt ermittelt werden, damit ein Vergleich mit den alternativen Brennstoffen möglich ist. Rebholz hat im absolut trockenen Zustand (Wassergehalt 0 %) einen durchschnittlichen Heizwert von ca. 4,95 kWh/kg. Bei luftgetrocknetem Rebholz muss bei der Verbrennung jedoch zuerst das darin enthaltene Wasser verdampft werden. Die dazu benötigte Energie beträgt 0,68 kWh/kg Wasser (Verdampfungswärme). Zieht man die für das Verdampfen des Wassers benötigte Energie von der in der verbleibenden Trockenmasse enthaltenen Energie ab, errechnet sich der tatsächliche Heizwert.

Heizwert der Trockenmasse – Verdampfungswärme des Wasseranteils = Heizwert
Beispiel: Rebholz mit 15 % Wassergehalt
(85 % x 4,95 kWh) – (15 % x 0,68 kWh) = 4,1 kWh/kg

Bei 1400 bis 1800 kg lufttrockenem Rebholz pro Hektar ergibt sich ein Energiewert von ca. 5700 bis 7400 kWh/ha. Verglichen mit Heizöl (Heizwert: 10 kWh/l) ergibt sich ein Heizöläquivalent von rund 570 bis 740 Liter Heizöl pro Hektar. Ein Raummeter Buchen- oder Eichenholz hat bei einem Wassergehalt von 20 % einen Energiegehalt von rund 1900 kWh. Somit entspricht im Vergleich mit Hartholz der Energiegehalt des Rebholzes ca. 3 bis 3,9 Raummeter je Hektar Ertragsrebfläche.

Eine exakte Wirtschaftlichkeitsberechnung ist aufgrund stark schwankender Energiepreise schwierig. Bei einem angenommenen Heizölpreis von 75 ct/l ergeben sich für ein Hektar Ertragsrebfläche äquivalente Heizwertkosten von ungefähr 430 bis 560 €.

Durch die Entfernung des Rebholzes geht dem Boden ein wichtiger Teil an organischer Substanz verloren. Um diesen Verlust zu bestimmen und ersetzen zu können ist eine Humusbilanzierung unerlässlich. Eine solche Bilanz zeigt, dass bei einer konventionellen Bewirtschaftung ohne Rebholzentfernung die Humusbilanz mit einem Saldo von – 1,040 t/ha Humus nicht ausgeglichen ist. Um einen nachhaltigen Weinbau zu betreiben, sollte dieser Verlust ohnehin ausgeglichen werden. Wird zusätzlich das Rebholz aus dem Weinberg entfernt, verschiebt sich das Saldo um weitere 0,260 t/ha Humus ins Negative auf – 1,3 t/ha Humus. Um diesen Verlust auszugleichen wäre z.B. eine jährliche Ausbringung von 6 t Stallmist und 3 t Stroh (jeweils Trockenmasse) möglich. Um lediglich den Humusverlust von 0,260 t/ha durch die Rebholzentfernung auszugleichen, wäre eine jährliche Zufuhr von z.B. 2 t/ha Stallmist oder 2 t/ha Stroh (jeweils Trockenmasse) möglich.

Bei der Verbrennung von Rebholz entsteht durchschnittlich ein Ascheanteil von 3,5 % bezogen auf die absolut trockene Masse. In dieser Asche bleiben bis auf den Stickstoff alle Nährstoffe, die im Rebholz enthalten sind, erhalten. Wird davon ausgegangen, dass die Asche verkompostiert und wieder im Weinberg ausgebracht wird, ist lediglich der Verlust an Stickstoff zu berücksichtigen.

Um den Humus- und Stickstoffverlust zu ersetzen werden inkl. Arbeitsaufwand Kosten von rund 100 €/ha veranschlagt. Geht man von einer jährlichen Festkostenbelastung durch die Anschaffung einer Rebholzsammelmaschine von 1550 € aus (A = 15.000 €, Zins = 5 %, Afa 12 Jahre), entstehen bei einer Flächenleistung von 50 ha je Jahr Kosten von rund 31 €/ha zuzüglich variable (Material-) Kosten von 25 €/ha.

Berücksichtigt man weiterhin pauschale Kosten für Transport und Lagerung in Höhe von 30 €/ha ergeben sich Gesamtkosten von 186 €/ha. Daraus ergibt sich ein Energiepreis von 2,5 bis 3,3 ct/kWh oder ein vergleichbarer Heizölpreis von 25 bis 33 ct/l. Anhand der obigen Ausführungen lässt sich erkennen, dass die energetische Rebholzverwertung für Weinbaubetriebe lohnenswert sein kann.

TRESTER

Eine weitere Möglichkeit ist die thermische Nutzung des bei der Weinproduktion anfallenden Traubentresters. Dieser wird getrocknet und anschließend zu Pellets gepresst. Die Tresterpellets können dann ähnlich den Holzpellets zum Heizen in Feststoffbrennanlagen eingesetzt werden. Der Heizwert von Tresterpellets beträgt 20,8 MJ/kg, der von Holzpellets (DIN plus) beträgt hingegen nur unter 18 MJ/Kg.(Quelle: RLP AgroScience). Tresterpellets können nicht nur in großen Heizkraftwerken verarbeitet werden, sondern es besteht auch die Möglichkeit, diese in kleineren Anlagen zu nutzen. Hierbei werden die Tresterpellets in einem Feststoffbrennraum verbrannte. Die anfallende Wärme kann auf zweierlei Arten genutzt werden. Zum Einen wird eine Gasturbine angetrieben, welche elektrischen Strom produziert, zum Anderen kann anfallende Wärme als Prozesswärmen zum Heizen, oder unter Nutzung einer Absorbtionskälteanlage zum Kühlen genutzt werden.

Unter ökonomischen Gesichtspunkten kann eine Anlage zur Produktion von Tresterpellets und deren Umwandlung in Strom und Wärme durchaus sinnvoll sein. Hierbei ist das Hauptaugenmerk auf die richtige Dimensionierung der Anlage zu richten, so dass zum Einen die Produktionsmittel optimal genutzt werden können, zum Anderen die Produkte Strom und Wärme zu jedem Zeitpunkt einen Abnehmer finden. Hierbei ist meist die nutzbare Wärmemenge, oder den Zeitpunkt zu welchem diese bereitgestellt werden kann der begrenzende Faktor.

GEOTHERMIE

Die Geothermie erfüllt die Kriterien der Nachhaltigkeit und gehört somit zu den regenerativen Energiequellen, ihr Potenzial ist nach menschlichem Ermessen nahezu unerschöpflich.

„Theoretisch würde allein die in den oberen 3 Kilometer der Erdkruste gespeicherte Energie ausreichen, um die Welt für etwa 100.000 Jahre mit Energie zu versorgen“ (Lund, 2005).

Geothermische Energie, auch als Erdwärme bezeichne, ist die in Form von Wärme gespeicherte Energie unterhalb der Erdoberfläche. Im Erdinneren sind immense Wärmemengen gespeichert, deren Ursprung größtenteils in der Zerfallsenergie natürlich radioaktiver Isotope liegt, die zum Teil aber auch in Form von Restwärme aus der Zeit der Erdentstehung vorliegt. Ganz oberflächennah kommen Anteile aus der Sonneneinstrahlung auf die Erdoberfläche und aus dem Wärmekontakt mit der Luft dazu. So sind nach heutigen Kenntnissen im Erdkern Temperaturen von über 6000 °C, im oberen Erdmantel noch ca. 1300 °C anzunehmen.

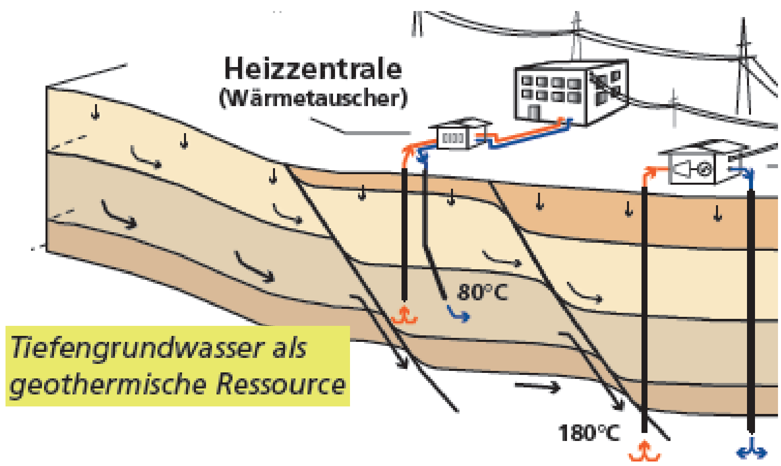

Abbildung 1: Darstellung der Nutzung von Erdwärme (Schweizerische Vereinigung für Geothermie, Geothermie - Info, 2001)

„Der geothermische Wärmestrom durch die Erdoberfläche beträgt über 40 Milliarden kW. Im Schnitt nimmt die Temperatur ab Erdoberfläche pro 100 m Tiefe um etwa 3 °C zu, was einem normalen geothermischen Tiefengradient entspricht. Vielerorts auf der Welt jedoch finden sich sogenannte Wärmeanomalien, das bedeutet, Gebiete mit wesentlich höheren Temperaturgradienten." (Schweizerische Vereinigung für Geothermie, 2007).

Beträgt die Temperatur der geothermischen Wärmequelle weniger als 100 °C, so wird der Wärmeinhalt des geothermischen Fluids mittels eines Wärmetauschers an einen Heizkreislauf abgegeben. Beträgt die Temperatur der geothermischen Wärmequelle über 150 °C, so ist eine Umwandlung der Wärme, mittels Turbine und Generator, in Elektrizität rentabler.

Da die eigentliche Energie, die Geothermie kostenlos ist, wird die Wirtschaftlichkeit einer Geothermienutzung, neben den vorliegenden geologischen Verhältnissen im Untergrund, vor allem durch die Investitions- und Unterhaltskosten der Anlagen bestimmt.

SONNENENERGIE

Als Sonnenenergie oder Solarenergie bezeichnet man die von der Sonne durch Kernfusion erzeugte Energie, die in Teilen als elektromagnetische Strahlung (Strahlungsenergie) zur Erde gelangt.

Diese Energiemenge entspricht etwa dem 10.000 fachen des Weltprimärenergiebedarfs. Die Sonneneinstrahlung stellt somit eine unerschöpfliche Energiequelle dar. Ein großer Teil der eingestrahlten Energie wird direkt reflektiert oder indirekt als Wärmestrahlung wieder abgestrahlt.

Ein Teil der direkt und diffus bis zur Erdoberfläche strahlenden Energie kann durch direkte Umwandlung in Strom (Photovoltaik) oder Wärme (Solarthermie) genutzt werden.

WINDENERGIE

Bei der Windenergie handelt es sich um die kinetische Energie der bewegten Luftmassen der Atmosphäre.

Durch die entstehenden Temperatur- und damit auch Druckunterschiede geraten die Luftmassen zwischen der Zone um den Äquator und den Polen, als auch zwischen der Tag- und der Nachtseite der Erde, in Bewegung. Die Rotation der Erde trägt ebenfalls zur Verwirbelung der Luftmassen bei, ebenso die Schiefstellung der Rotationsachse der Erde zur Ebene, die die Erdbahn durch das Umkreisen der Sonne bildet, führt zu jahreszeitlichen Luftströmungen. Sie ist eine indirekte Form der Sonnenenergie und zählt deshalb zu den erneuerbaren Energien.

Eine sinnvolle Nutzung beginnt, bei dem derzeitigen Stand der Technik, bei mittleren Windgeschwindigkeiten von vier bis fünf Metern pro Sekunde. Bei kleineren Werten ist das Energieangebot zu gering, als dass sich eine Nutzung lohnen würde. Aber nicht nur die durchschnittliche Windgeschwindigkeit ist ausschlaggebend, sonder auch deren jährliche Verteilung.

FAZIT

Die räumlich verteilte, nicht kontinuierlich anfallende Biomasse mit unterschiedlicher Qualität und Quantität, trifft auf eine Energienachfrage, die dezentralisiert ist, genaue Qualitätsanforderungen stellt und in spezifischen Tages- und Nachtrhythmen nachgefragt wird. Besonders wichtig erscheint hier, dass Biomasseanfall und die Energienachfrage nicht synchron sind. Abgeleitet von der Prozesskette der Biomassenutzung ergeben sich Informationsbedürfnisse über die Produktion von Biomasse, zu deren Transporten, Lagerung und Aufbereitung bis zur Energieumwandlung und – bereitstellung. So sind für alle Bereiche der Industrie, Wirtschaft und der Allgemeinheit Energiebedarfszenarien, - bilanzen und – bereitstellungspotentiale zu bilden und zu ermitteln. Zunächst wird der aktuelle Energiebedarf eines Betriebes erfasst und je nach Bereich dargestellt. Anschließend soll untersucht werden, in welchem Umfang eine Umstellung auf erneuerbare Energieträger realisierbar ist. Kommen mehrere Energieträger oder Kombinationen von mehreren in Frage, wird die optimale Variante nach Möglichkeit berechnet. Am Ende soll der Betrieb in der Lage sein, seine eigens benötigte Energie selbst und umweltbewusst bereit zu stellen und gegebenenfalls Energieüberschuss abzugeben.

Literatur: Auf Anfrage

Conservación de las uvas Centennial Seedless y Niagara Rosada con la aplicación, en precosecha, del acido naftalenoacetico (ANA) y del cloruro de calcio ($CaCl_2$)

Maurilo Monteiro Terra[1,7], Rebeca Martins Iricevolto[2], Marcel Mamede de Carvalho Filho[2], Patrícia Cia[3], Eliane Aparecida Benato[4], Marco Antonio Tecchio[1], Erasmo José Paioli Pires[1,7], Mara Fernandes Moura[1], Felipe de Angelis Monteiro Terra[5], Renato Vasconcelos Botelho[6,7], Larissa Villar[2]

[1] Centro de Frutas do Instituto Agronômico – Av. Luiz Pereira dos Santos, 1.500 - CEP 13214-820 – Jundiaí – SP – Brasil. mmterra@iac.sp.gov.br, tecchio@iac.sp.gov.br, ejppires@iac.sp.gov.br, mouram@iac.sp.gov.br
[2] Pós-Graduação do Instituto Agronômico – CP 28, CEP 13012-970 – Campinas – SP – Brasil. reiricevolto@yahoo.com.br, marcelmcf@ig.com.br, larissavillar.agro@gmail.com
[3] Centro de Engenharia e Automação do Instituto Agronômico – CP 26, CEP 13201-970 – Jundiaí – SP – Brasil. pcia@iac.sp.gov.br
[4] Grupo de Engenharia e Pós-Colheita do Instituto de Tecnologia de Alimentos – CP 139, CEP 13070-178 – Campinas – SP – Brasil. benato@ital.sp.gov.br
[5] Pós-Graduação da ESALQ, USP, CP 9, CEP 13418-900 – Piracicaba – SP- Brasil. faterra@esalq.usp.br
[6] Universidade Estadual do Centro-Oeste, UNICENTRO, CEP 85035-120 – Guarapuava – PR – Brasil. rbotelho@unicentro.br
[7]Becário de Productividad en Investigación del CNPq

RESUMEN - Con el objetivo de reducir las pérdidas de uvas de mesa poscosecha por la desgrana y incidencia de podridos de los cvs Centennial Seedless y Niagara Rosada, fueran realizadas, en la campaña de 2007/2008, en viñedos ubicados en los municipios de Jales, Louveira, y São Miguel Arcanjo, Brasil, experimentos empleándose cuatro dosis de ANA 0, 50, 100 y 150 $mg.L^{-1}$, con o sin aplicación de $CaCl_2$ en la dosis de 10 $g.L^{-1}$. Después de la cosecha, fueran evaluadas la porcentaje de desgrana y de podridos, pH, contenido de sólidos solubles (SS) y acidez titulable (AT) y después los racimos fueran almacenados bajo condición ambiente a 25ºC/70%HR, por 3 días, y refrigeración a 1ºC/85%UR, por 21 días, seguido de transferencia para condición ambiente por más 3 días, siendo evaluadas después de eses períodos las mismas variables. El ANA fue eficiente en la reducción de la desgrana y de la incidencia de podridos en los racimos, principalmente después del almacenamiento de los mismos bajo condición ambiente, siendo la dosis de 150 $mg.L^{-1}$ la más efectiva.
Palabras clave: *Vitis sp*, desgrana, podridos de los racimos, ANA, $CaCl_2$

Postharvest conservation of table grapes 'Centennial Seedless' and 'Niagara Rosada' with preharvest use of naphthalene acetic acid (NAA) and calcium chloride ($CaCl_2$)

ABSTRACT - Main problems of table grapes losses for Centennial Seedless and Niagara Rosada varieties have been happening due to postharvest by drop berries and rot. Aiming the reduction of these losses, during the crop 2007/2008, trials were taken place in vineyards localized at the cities of Jales, Louveira and São Miguel Arcanjo, Brazil, using four different doses of NAA 0, 50, 100 e 150 $mg.L^{-1}$, added or not with 10 $g.L^{-1}$ of $CaCl_2$. After harvest, were evaluated berry detachment and decay percentage, pH, soluble solids content (SS) and titratable acidity (TA). After harvest, the clusters were storage at room conditions (25ºC/70% RH) during 3 days and under refrigeration at 1ºC/85% RH for 21 days. After cold storage

clusters were once again storage at room conditions during 3 days, being evaluated on the parameters cited above after this period. NAA was efficient on drop berries and rot cluster reduction, mainly after the storage at room temperature, being the dose of 150 mg.L^{-1} the most effective.
Key words: *Vitis sp*, drop berries, rot cluster, NAA, $CaCl_2$

INTRODUCCIÓN

El Estado de São Paulo, Brasil, es el mayor productor brasileño de uva de mesa, con una superficie de 9.514 hectáreas, producción de 177.934 toneladas y rendimiento promedio de 18.702 kg ha-1 (AGRIANUAL, 2010).

En los últimos años tiene sido observado un descenso de la rentabilidad del cvs Centennial Seedless y Niagara Rosada debido a desgrana y problemas de plagas en poscosecha. La magnitud de estas pérdidas depende del cultivar y de las condiciones climáticas y nutricionales en el que las uvas son producidas (SALUNKHE, DESAI, 1984), sin embargo, estas pérdidas pueden reducirse al mínimo mediante prácticas culturales y el uso de técnicas de manejo pre y poscosecha (CENCI, 1994).

Asociados con la refrigeración, el uso de cloruro de calcio ($CaCl_2$) y la auxina sintética acido naftalenacetico (ANA) en pre o poscosecha, son dos técnicas que pueden retrasar la maduración de los frutos, y ya hay sido estudiados por varios autores, obteniéndose resultados positivos para diversas variedades de uva en diferentes condiciones climáticas y de suelo (CENCI, 1994; LIMA *et al.*, 2002; BRACKMANN *et al.*, 2002; MOURA *et al.*, 2006; BENATO *et al.*, 2006; DANNER *et al.*, 2008; TECCHIO *et al.*, 2009).

CENCI, CHITARRA (1994) estudiaron el potencial de conservación de la uva cv Niagara Rosada cuando sometido a tratamientos de precosecha con ANA y $CaCl_2$ y concluyeran que estos productos promovieran una reducción en el porcentaje de desgrana. Por los resultados obtenidos en este trabajo fue evidenciado la posibilidad del uso de $CaCl_2$ y ANA, a fin de reducir la desgrana, habiendo, sin embargo, la necesidad de ajustar las dosis pues los autores utilizaron solo las dosis de 10 g.L^{-1} de $CaCl_2$ y 100 mg.L^{-1} de ANA. Asociado al ajuste de la dosis se encontró en la literatura variaciones sobre el efecto de los reguladores vegetales dependiendo de la región y de la variedad estudiada.

Este trabajo tuvo como objetivo evaluar los efectos de la aplicación en precosecha del ANA y $CaCl_2$ en la reducción de las pérdidas poscosecha de las uvas cvs Centennial Seedless y Niagara Rosada, derivados de la desgrana y de podridos, bien como estudiar sus efectos sobre la características físicas y químicas de los frutos después de un almacenamiento en condiciones ambiente y de refrigeración.

MATERIAL Y MÉTODOS

Las aplicaciones de $CaCl_2$ y ANA en uvas cvs Centennial Seedless y Niagara Rosada se celebraron en 2007 y 2008 en viñedos ubicados en los municipios de Jales, Louveira y São Miguel Arcanjo, Estado de São Paulo, Brasil. El municipio de Jales esta ubicado a 20° 16'08"S. y 50º32'45"W., con una altitud de 483 m, medias anuales de 1.312 mm de lluvia, la temperatura media de 23,6ºC. Louveira situase a 23°05'11"S. y 46º57'02 "O., con una altitud de 750 m, medias anuales de 1.400 mm de lluvia y la temperatura media de 19,5ºC. São Miguel Arcanjo se encuentra a 23°52'42"S. y 47º59'50 "O., con una altitud de 660 m, precipitaciones medias de 1.396 mm de lluvia y la temperatura media de 20°C.

Para la variedad Centennial Seedless, en el municipio de Jales, el viñedo seleccionado tenía seis años de edad, siendo las plantas sostenidas en el sistema de parral, con un espacio de 5,0 m x 3,0 m, injertadas en el patrón IAC 572. En São Miguel Arcanjo, el viñedo seleccionado tenía cinco años de edad, siendo las plantas también sostenidas en el sistema de parral, espaciados 3,5 m x 3,5 m, injertadas en el patrón 420 A. Para la variedad Niagara Rosada, en Jales, el viñedo tenía cinco años de edad, siendo las plantas sostenidas en el sistema de parral, a una distancia de 2,50 m x 2,00 m, injertados en el patrón IAC 572. En los municipios de Louveira y São Miguel Arcanjo, los viñedos seleccionados tenían seis años de edad, siendo las plantas sostenidas en el sistema de espaldera, espaciadas 2,00 m x 1,00 m, injertadas sobre patrón 106-8 Mgt. Se utilizó en todos los experimentos, el diseño experimental de bloques al azar, dispuestos en factorial 4 x 2, con cinco repeticiones y parcelas experimentales constituidas por 9 racimos seleccionados en cada planta. Así, en cada experimento, los tratamientos fueran representados por cuatro dosis de ANA a 0, 50, 100 y 150 $mg.L^{-1}$, aplicados un día antes de la cosecha, con o sin la aplicación de $CaCl_2$ a 10 $g.L^{-1}$, en el envero. Las aplicaciones del ANA y $CaCl_2$ fueron realizadas por medio de pulverizaciones direccionadas a los racimos, añadiéndose a la solución el adyuvante IHARAGUEN-S ® a 0,3%.

Después de la cosecha, los racimos de cada unidad experimental fueran separados en submuestras de tres racimos, utilizadas para determinación de las variables físicas y químicas. Los análisis se realizaron en el día de la cosecha de los racimos y después del período de almacenamiento, bajo condición ambiente (25±2ºC/70±5%HR) durante tres días, o bajo refrigeración (1±1ºC/85±5%HR), donde permanecieron durante 21 días y luego transferidos a las condiciones ambientales, por más tres días. Después de estos períodos, se determinó el porcentaje de desgrana, la incidencia de podridos, los contenidos de sólidos solubles (SS), el pH y la acidez titulable (AT).

Con los datos obtenidos se llevó a cabo inicialmente en el programa MINITAB la normalidad de todas las variables, con el objetivo de determinar si los datos presentaban distribución normal. Los datos fueron sometidos a análisis de varianza, prueba de Tukey para comparar el efecto del $CaCl_2$ y regresión polinomial para las dosis de ANA. Para los datos expresados en porcentaje (desgrana y podridos), se utilizó la transformación de los datos, por la fórmula arc sen $\sqrt{x/100}$.

RESULTADOS Y DISCUSIÓN

Para la variedad Centennial Seedless, en el experimento realizado en Jales, las dosis de ANA mostraron un efecto de segundo grado con un máximo de desgrana observado con 58 $mg.L^{-1}$ al 24 días de almacenamiento (Fig.1). También hubo un efecto de segundo grado para la incidencia de podridos, siendo la máxima observada con 62 $mg.L^{-1}$ al 24 días de almacenamiento (Fig.1).

En São Miguel Arcanjo, la porcentaje de desgrana y incidencia de podridos fueran influenciadas significativamente por el tratamiento precosecha con $CaCl_2$, siendo la primera variable afectada en todas las evaluaciones y la segunda solamente al 4 días (Tab.1).

La porcentaje de desgrana en los racimos tratados con $CaCl_2$ en precosecha fue mayor en todas las evaluaciones y esto se debe a que la cosecha se lleva a cabo en un mes de temperatura considerada alta. Los valores observados para esta variable hasta a 46% en los racimos de la última evaluación y a 36% en racimos no tratados (Tab.1).

Tab.1. Medias para la porcentaje de desgrana y incidencia de podridos en racimos de uva cv Centennial Seedless en función de la aplicación de $CaCl_2$ (10 g.L^{-1}), São Miguel Arcanjo-SP, Brasil, 2007-2008.

Dosis $CaCl_2$ (g.L^{-1})	**Desgrana (%)**			**Podridos (%)**		
	4	21	26	4	21	26
0	27,0 A	30,0 A	36,0A	25,0 A	29,0 A	42,0 A
10	36,0 B	36,0B	46,0 B	30,0 B	31,0 A	53,0 A

Análisis 3 días después de la cosecha y los racimos mantenidos almacenados bajo condiciones ambiente; 21 días almacenados a 1ºC e HR = 95%; 24 días, siendo 21 almacenados bajo refrigeración y 3 bajo condiciones ambiente. Medias seguidas de la misma letra, en la columna, non presentan diferencia significativa entre si ($P \leq 0,05$).

Para la variedad Niagara Rosada, en el experimento realizado en Jales, se encontró que después de 21 días de almacenamiento en frío seguido de 3 días a temperatura ambiente, la aplicación de ANA un día antes de la cosecha ocasionó una reducción en el porcentaje de desgrana en 56,2% con la dosis de 150 mg.L^{-1} (Fig.1). No hubo influencia significativa de ANA en la incidencia de podridos, tampoco de $CaCl_2$ (10 g.L^{-1}) en la incidencia de podridos y desgrana (Tab.2).

Para las uvas de la región de Louveira, se encontró que después de 21 días de almacenamiento en frío seguidos de 3 días a temperatura ambiente, utilizando 150 mg.L^{-1} de ANA un día antes de la cosecha proporcionó una disminución en el porcentaje de desgrana de 11,7% para 3,4% (71,3%) (Fig.1). Cuanto a la incidencia de podridos, se constató que la aplicación de ANA hasta la dosis de 93 mg.L^{-1} provocó un aumento de 3,67% para 9,60% después de 3 días de almacenamiento bajo condición ambiente (Fig.1).

Similar a los resultados obtenidos para las uvas cv Niagara Rosada provenientes de Jales, el uso de $CaCl_2$ (10 g.L^{-1}) no mostró resultados significativos para desgrana y incidencia de podridos en las uvas provenientes de Louveira y almacenadas en condiciones ambientales y refrigeración (Tab.2).

En relación a la porcentaje de desgrana en uvas del cv Niagara Rosada cosechadas en São Miguel Arcanjo, se encontró que a los 21 días de almacenamiento refrigerado hubo reducción de 5,15% a 3,06% hasta la dosis de 79 mg.L^{-1} de ANA. Ya a los 21 días de almacenamiento en frío seguido de 3 días bajo temperatura ambiente, el uso de ANA hasta a la dosis de 68 mg.L^{-1} proporcionó reducción de 8,74% para 5,26% (Fig.1). La aplicación de ANA hasta la dosis de 73 mg.L^{-1} se redujo de 7,06% para 3,92% la incidencia de podridos a los 21+3 días de almacenamiento (Fig.1). El uso de $CaCl_2$ (10 g.L^{-1}) no influyó significativamente en la incidencia de podridos y en la desgrana en las uvas almacenadas en condiciones ambientales y de refrigeración (Tab.2).

Los resultados obtenidos en el experimento realizado en Jales permitieron concluir que la aplicación del ANA, en precosecha, promovió aumento de la desgrana y de la incidencia de podridos, mientras, en São Miguel Arcanjo, la mayor desgrana y incidencia de podridos fueran resultantes de la aplicación del $CaCl_2$.

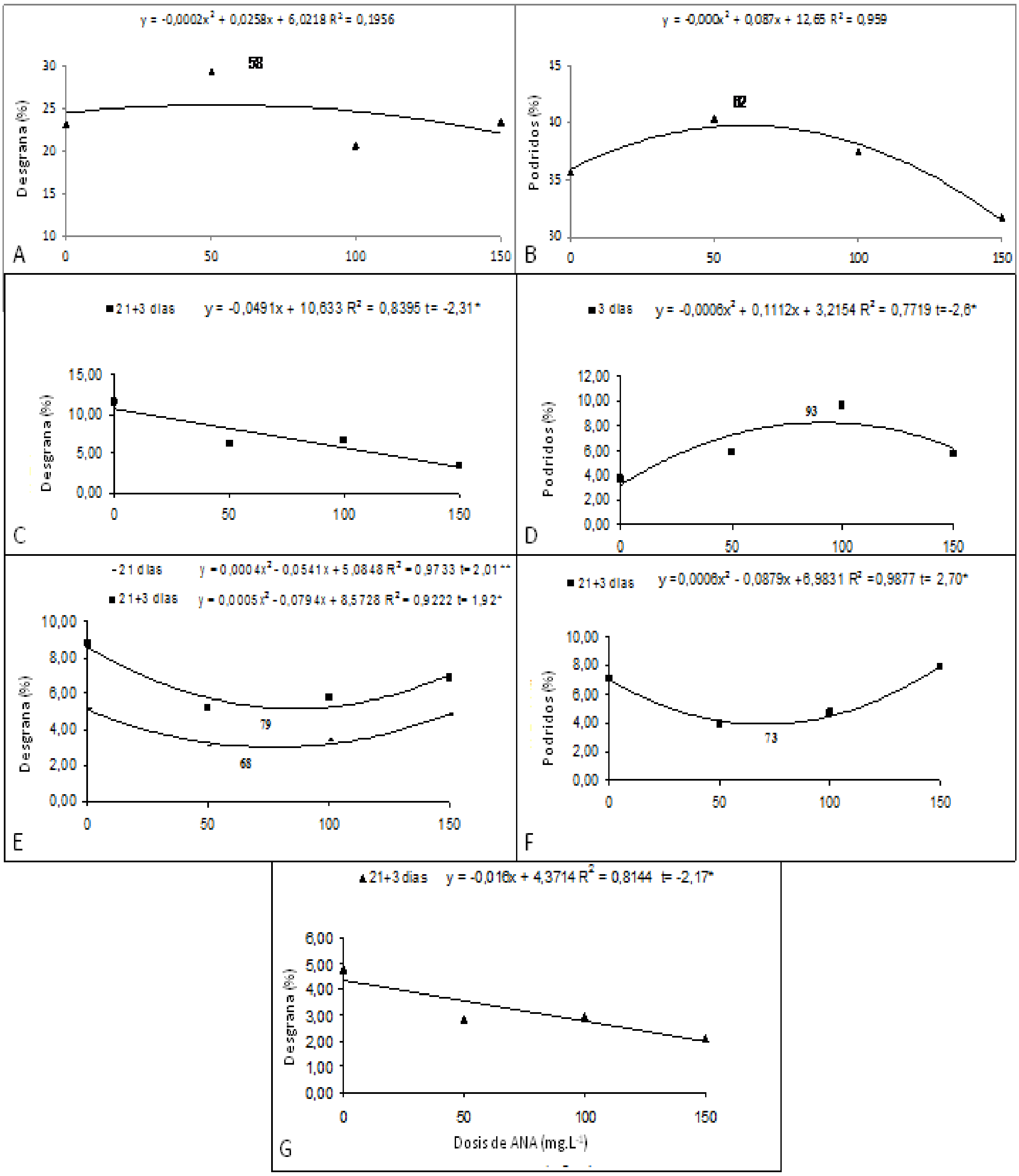

Fig.1. Efectos de la aplicación del ANA en la porcentaje de desgrana (A) y de la interacción entre ANA y $CaCl_2$ en la incidencia de podridos (B) en la uva cv Centennial Seedless evaluada después de 24 días, Jales, SP; porcentaje de desgrana en racimos sometidos a dosis de ANA, después de 21 días de almacenamiento refrigerado seguido de 3 días en condición ambiente (C) y de la incidencia de podridos en racimos sometidos a dosis de ANA, después de 3 días de almacenamiento en condición ambiente (D) en la uva cv Niagara Rosada, Louveira, SP; porcentaje de la desgrana (E) y incidencia de podridos (F) en la uva cv Niagara Rosada sometida a dosis de ANA, después de 21 días de almacenamiento refrigerado seguido de 3 días en condición ambiente, São Miguel Arcanjo, SP; porcentaje de desgrana en los racimos da uva cv Niagara Rosada sometidos a dosis de ANA, después de 21 días de almacenamiento refrigerado seguido de 3 días en condición ambiente, Jales, SP (G). Brasil, 2007-2008.

Tab.2. Medias para porcentaje de desgrana y incidencia de podridos en racimos de uva cv Niagara Rosada con y sin aplicación de $CaCl_2$ a 10 $g.L^{-1}$ después del almacenamiento a 25°C/70%HR por 3 días o bajo refrigeración (1°C/ 85%HR) por 21 días, seguido de más 3 días bajo condición ambiente (21+3). Brasil, 2007-2008.

Dosis $CaCl_2$ ($g.L^{-1}$)	**Desgrana (%)**			**Podridos (%)**		
	3	**21**	**21+3**	**3**	**21**	**21+3**
Jales - SP						
0	1,07 A	0,82 A	2,81 A	0,41 A	0,34 A	1,03 A
10	0,65 A	0,86 A	3,52 A	0,23 A	0,32 A	1,53 A
Louveira - SP						
0	7,75 A	3,50 A	5,37 A	7,67 A	4,70 A	8,44 A
10	4,37 A	2,26 A	8,54 A	4,76 A	3,74 A	9,82 A
São Miguel Arcanjo - SP						
0	11,84 A	4,18 A	6,89 A	10,23 A	4,08 A	5,96 A
10	10,84 A	4,02 A	6,42 A	7,11 A	3,59 A	5,82 A

Medias seguidas de la misma letra, en la columna, non presentan diferencia significativa entre si ($P \leq 0,05$).

El mayor problema de podrido poscosecha de uva se debe al hongo *Botrytis cinerea*, mientras que la pérdida de agua representa el problema más serio en la preservación de su vida poscosecha. Bajo estos aspectos, CENCI, CHITARRA (1994) demostraron que la aplicación de ANA combinado con $CaCl_2$ en precosecha de uva cv Niagara Rosada redujeran la desgrana y la actividad de enzimas pécticas en comparación con las uvas sin tratar.

BRACKMANN *et al.* (2001) reportaron que la aplicación de $CaCl_2$ puede quitar la cera natural de las bayas o causar daño a las células epidérmicas, predisponiendo ellas a los ataques de hongos, que pueden ser también tenidos en cuenta para el ANA ya que las pulverizaciones se hicieron un día antes de la cosecha.

MOURA *et al.* (2006) evaluaran la aplicación de dosis crecientes de $CaCl_2$ (0, 5, 10, 15 y 20 $g.L^{-1}$) en el envero de la cv Niagara Rosada, con y sin aplicación del ANA (100 $mg.L^{-1}$) un día antes de la cosecha en la región de Jales y encontraran que el uso del ANA fue eficaz para reducir la desgrana y la incidencia de podridos después de un almacenamiento bajo temperatura ambiente o bajo refrigeración, seguido por un período de transferencia, que no se observó cuando se usó sólo $CaCl_2$. Estos resultados también fueron observados en un experimento similar de BENATO *et al.* (2006), realizado en Jundiaí, lo que demuestra la eficiencia del uso del ANA.

Cuanto a las evaluaciones físicas y químicas, tanto para las uvas del cv Centennial Seedless como para las uvas del cv Niagara Rosada, no hubo observadas alteraciones significativas en los valores de pH, SS y de AT, mediante a los tratamientos con ANA y $CaCl_2$.

Sin embargo, algunos autores han reportado poco o ninguna alteración significativa en las evaluaciones físicas y químicas cuando de la aplicación de estos dos productos. CENCI (1994) estudiando la aplicación de $CaCl_2$ con y sin ANA en la precosecha del cv Niagara Rosada observó efectos significativos de los tratamientos en los niveles de AT. En los tratamientos con $CaCl_2$, los contenidos de AT fueran mayores en época de cosecha, y de acuerdo con el autor, esto se debe a un posible retraso en la maduración del fruto. LIMA *et al.* (2000) describieron que, en las uvas maduras, un aumento en el contenido de SS se relaciona generalmente con la pérdida de agua, sin cambios en el peso de solutos por baya. Y el descenso, se explica generalmente por el aumento en el agua por baya, aunque también puede estar asociada con una pérdida de solutos debido a la respiración, el transporte de solutos, la transpiración o del transporte de agua a otras partes de la planta. DETONI *et al.* (2005), relataran que la uva tiene un ejemplo de respiración no climatérico, lo que justifica el mantenimiento o pocas alteraciones en las características del fruto durante el almacenamiento.

CENCI (1994), estudiando tratamientos de precosecha con $CaCl_2$ y ANA obtuve efecto significativo de los tratamientos en el pH de los racimos de uva cv Niagara Rosada. Durante el almacenamiento refrigerado de los frutos, el autor observó una disminución en los niveles de pH. MOURA *et al.* (2006), BENATO *et al.* (2006) y TECCHIO *et al.* (2009) evaluando dosis crecientes de $CaCl_2$ con y sin ANA, aplicadas en la precosecha, en los racimos de uvas cv Niagara Rosada encontraran que los productos evaluados no alteraran el SS, pH y AT.

En general, los resultados obtenidos en el experimento realizado para el cv Centennial Seedless, en Jales, permitieron concluir que la aplicación de ANA, en precosecha, promovió aumento de la desgrana y de la incidencia de podridos, mientras que, en São Miguel Arcanjo, fue mayor la porcentaje de desgrana y de la incidencia de podridos, resultantes de la aplicación de $CaCl_2$. Para los experimentos realizados para el cv Niagara Rosada, en Jales, Louveira y São Miguel Arcanjo, permitieran concluir que la aplicación del ANA, en la precosecha, fue eficaz en la reducción de la desgrana y de la incidencia de podridos, cuando asociados a la refrigeración.

Cuanto a los contenidos de SS, pH y AT, hubo pocas alteraciones significativas como resultado de la aplicación de ANA y $CaCl_2$, para los dos cultivares.

Teniendo en cuenta el efecto del ANA en la reducción de la desgrana y de la incidencia de podridos en los racimos, los mejores resultados se obtuvieran utilizando una dosis de 150 $mg.L^{-1}$.

Se sugiere en investigaciones futuras, el uso de nuevas dosis, especialmente del ANA, pues es probable que una dosis más alta podrá traer resultados positivos para estos cultivares de uva de mesa.

CONCLUSIONES

El acido naftalenoacetico proporcionó aumento en la porcentaje de desgrana y de incidencia de podridos en el cv Centennial Seedless, en Jales, SP, Brasil, mientras que el cloruro de calcio favoreció el aumento de la desgrana y de la incidencia de podridos, en São Miguel Arcanjo, SP, Brasil.

El acido naftalenoacetico fue eficaz para reducir la desgrana y la incidencia de podridos en uva cv Niagara Rosada, cuando asociado a la refrigeración, siendo la dosis de 150 $mg.L^{-1}$ la más eficaz.

Los tratamientos promovieran pocas alteraciones en los sólidos solubles, pH y acidez titulable. No hay interacción significativa del ácido naftalenoacetico con el cloruro de calcio.

AGRADECIMIENTO

A la Fundação de Amparo à Pesquisa do Estado de São Paulo – FAPESP, por la ayuda financiera para el desarrollo de esta investigación.

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Evaluation of Rheological and Sensory Quality of Table Grapes imported

Luis Sousa[1], Nuno Alvarenga[1] and Donato Antonacci[2]

[1]IPB, Rua Pedro Soares, Campus do IPBEJA, Beja, Portugal, luis.peres@esab.ipbeja.pt

[2]ENTECRA, Turi, Bari, Italy, donato.antonacci@entecra.it

ABSTRACT

"Determination of the quality of the imported grapes satisfying the characteristics imposed to its importation".

Introduction

This study aims to evaluate the quality of imported table grapes based on parameters defined as work of compression, hardness, work of penetration, skin resistance to penetration, resistance to penetration inside the berry and even resistance to the detachment of the pedicel.

MATERIAL AND METHODS

Were chosen samples of imported grapes varieties Thompson Seedless and Red Globe reaching the Portuguese market coming from southern hemisphere

A texture analyzer TAHDi (Stable Micro Systems, Godalming, UK), equipped with a 250 N load cell, was used to perform the texture analysis at 20±1 ºC. There different procedures were implemented: (a) Compression test was performed with a 75 mm diameter aluminum cylindrical probe (with a whole berry) at a compression depth of 10 mm, with a crossed speed of 1 mm s^{-1}. From the force vs. time texturograms, two parameters were obtained: hardness (Maximum force in N) and compression work (area in N.mm); (b) Puncture test was performed with a 2 mm diameter aluminum cylindrical probe (with a whole berry) at a penetration depth of 10 mm, with a crossed speed of 1 mm s^{-1}. From the force vs. time texturograms, three parameters were obtained: skin hardness (first peak force in N) and flesh hardness (second peak force in N) and penetration work (area in N.mm); and (c) detachment of the pedicel tension test was performed with a prototype mechanism that coupled to analyzer allows to determinate the detachment tension of the berry at the tension depth of 10 mm, with a crossed speed of 1 mm s^{-1}. From the force vs. time texturograms, the Maximum force in N indicates the detachment of the pedicel tension. For the sensorial analyses, was used the OIV provisional general form for the sensorial analysis of table grapes.

Results and Discussion

In terms of sensorial analysis can be summarized as follows: the Thompson seedless variety presented a differentiation from the Red Globe variety as regards the presence of seeds in the flesh, aromatic intensity, berry crispness, hardness of seeds and that presented a more positive overall evaluation.

In relation to defects caused by cooling, both varieties have a mean similar, around 6 – 7 assessment (scale 1 – 10).

Regarding the rheological evaluation (table 1), in the compression test, we noticed that the Thompson seedless variety has a hardness greater than the Red globe variety, with significant difference ($p < 0.05$). This parameter is in agreement with sensory analysis with regard to the attribute crispness.

Table 1. Average and standard deviation values of Rheological parameters and ANOVA results

	Red Globe	Thompson
Compression work (N.s)	148,82(30,80)b	184,01(32,63)a
Compression hardness (N)	31,38(3,16)b	37,73(8,31)a
Penetration work (N.s)	11,44(3,35)a	7,64(1,41)b
Skin hardness (N)	2,93(0,64)a	2,74(0,53)a
Flesh hardness (N)	2,48(1,41)a	0,59(0,28)b
Pedicel detachment tension (N)	7,37(3,47)a	4,08(1,25)b

$^{a,\,b}$ means in same row with different index means significant differences $P<0.05$ (Scheffé test)

In the penetration test showed that there are no significant differences in relation to skin hardness, although values were higher for the flesh hardness and significant differences in Red Globe variety, justified by the existence of grape seeds.

Regarding the resistance to the detachment of the berry from pedicels, the Red Globe variety was the one that showed higher values of tension to the detachment of the pedicel ($p< 0.05$).

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L'IDENTIFICAZIONE DEI SISTEMI AZIENDALI RAPPRESENTATIVI PER LO STUDIO DEI COSTI DI PRODUZIONE DELL'UVA DA TAVOLA IN PUGLIA

A. Seccia[(1)], D. Antonacci[(2)], V.N. Linsalata[(2)], E. Pomarici[(3)], D. Tosco[(4)]

[(1)] Dip. PROGESA - Facoltà di Agraria - Università di Bari, via G. Amendola, 165/A, 70126 Bari - Italy
seccia@agr.uniba.it
[(2)] CRA, Unità di ricerca per l'uva da tavola e la vitivinicoltura in ambiente mediterraneo
Via Casamassima, 148 – 70010 Turi BA (Italy)
donato.antonacci@entecra.it - vitonicola.linsalata@entecra.it
[(3)] Dipartimento di Economia e Politica Agraria – Facoltà di Agraria - Università degli Studi di Napoli Federico II
Via Università, 96 – 80055, Portici NA (Italy)
pomarici@unina.it
[(4)] Centro per la Formazione in Economia e Politica dello Sviluppo Rurale
Via Università, 96 – 80055, Portici NA (Italy)
d.tosco@unina.it

ABSTRACT

The study of the production costs of table grapes is a topic of growing interest as a result of international competitive environment increasingly affected by the possibility of reducing the costs of production and commercial distribution. The different production contexts, however, make difficult to determine and to analyze costs because the conditions are very different in terms of farm location and organization, grapes varieties, training systems and agronomic techniques of the vineyard. This work focuses on the application of the methodology of representative agricultural systems (RAS) for studying the production costs of farms specialized in the production of table grapes in Puglia, a region that produces about 70% of Italian table grapes. The methodology is based on the European Accounting Data Network (FADN-RICA), and on the EU farm typology classification. Apulian farms specialized in table grapes growing will be divided into homogeneous classes with respect to the production structure and technologies in order to define groups of firms that differ each other for organizational and technological aspects.

RIASSUNTO

Lo studio dei costi di produzione dell'uva da tavola è un argomento di crescente interesse in conseguenza del contesto competitivo internazionale sempre più condizionato dalla possibilità di contenere i costi di produzione e di distribuzione commerciale. L'eterogeneità dei contesti produttivi, però, rende difficile la determinazione e l'analisi dei costi perché molto diverse sono le condizioni in termini di localizzazione territoriale, di organizzazione aziendale, di posizione e gestione agronomica del vigneto, di varietà e forma di allevamento. L'obiettivo del lavoro riguarda l'applicazione della metodologia dei sistemi aziendali rappresentativi (SAR) allo studio dei costi di produzione delle imprese specializzate nella produzione di uva da tavola in Puglia, regione che produce circa il 70% dell'uva da tavola italiana. La metodologia si basa sull'impiego dei dati della rete contabile europea (RICA-FADN), e sui criteri di classificazione tipologica aziendale utilizzati dall'Unione Europea. Le aziende pugliesi specializzate nella produzione di uva da tavola verranno ripartite in classi omogenee rispetto alla struttura aziendale e alle tecnologie di produzione.

1. INTRODUZIONE

L'Italia è fra i principali produttori mondiali di uva da tavola grazie ad una produzione media degli ultimi dieci anni pari a 14,7 milioni di quintali che alimenta, oltre il mercato interno, anche un considerevole flusso commerciale soprattutto verso i paesi europei quali Germania (25%), Polonia (11%), Francia (13%), Belgio e Lussemburgo (5%), Svizzera (5%) con crescita dei mercati dell'Europa orientale e Russia (dati in volume, fonte ICE 2008). Nel 2009 la produzione italiana è stata pari a circa 14,2 milioni di quintali realizzati su una superficie di 70.337 ettari (Istat, 2009).

La Puglia è la regione italiana leader con una superficie di circa 46.000 ettari, pari a oltre il 65% nazionale, e una produzione di oltre 9,4 milioni di quintali, pari al 66% nazionale (Istat, 2009). Il valore della produzione pugliese è pari a 412,7 milioni di euro pari al 11,4 % del valore della produzione agricola pugliese e al 68,5% del valore della produzione di uva da tavola in Italia (Inea, 2008). Le province leader sono Bari e Taranto che detengono rispettivamente il 42% e il 40% della produzione regionale mentre nella provincia di Foggia si produce il 13%. E' possibile distinguere tre aree ben definite: la prima si trova a sud-est del capoluogo di regione, Bari, la seconda a ovest della città di Taranto lungo l'arco ionico mentre la terza è localizzata nel nord della provincia di Bari, lungo la costa adriatica fino a comprendere la parte meridionale della provincia di Foggia. L'unica forma di allevamento adottata per gli impianti di uva da tavola pugliesi è il tendone a doppio impalco in irriguo che può presentare o meno la copertura con film plastico, a sua volta nelle due modalità per l'anticipo della produzione e per il posticipo della raccolta; in caso di assenza di sistemi di forzatura il vigneto viene comunque protetto con reti per ridurre il rischio correlato ad eventi climatici dannosi. Le tecniche di coltivazione protetta sotto rete e/o sotto telo plastico consentono di realizzare un prodotto di migliore qualità e di prolungare il periodo di offerta sul mercato che si estende dalla fine di maggio agli inizi di gennaio. La varietà più diffusa è l'Italia, che copre oltre il 50% della superficie regionale, seguita, a notevole distanza da Victoria, Regina, Red Globe, Cardinal e Matilde. Negli ultimi anni la rapida diffusione delle varietà apirene, caratterizzate da minore produttività a fronte di prezzi di mercato superiori, ha consentito di diversificare l'offerta sia sotto l'aspetto dell'assortimento che del calendario di raccolta superando iniziali diffidenze e difficoltà tecniche.

I produttori pugliesi operano in un mercato nazionale ed internazionale caratterizzato dalla staticità della domanda, anche a causa dell'alta sostituibilità del prodotto, dalla necessità di rispondere alle esigenze delle catene distributive, in termini sia di crescenti standard di sicurezza sanitaria che di ribasso dei prezzi, dall'aumento della concorrenza da parte di produttori di paesi caratterizzati da inferiori costi di produzione e da migliore organizzazione commerciale; di conseguenza si riscontra il livellamento verso il basso dei prezzi di vendita all'origine già caratterizzati da forte ciclicità. In tale contesto, la capacità competitiva delle aziende pugliesi produttrici di uva da tavola e, conseguentemente, la loro capacità di produrre reddito evidenziano criticità sempre crescenti anche a causa dell'incremento dei costi di produzione che stanno deprimendo i margini dei produttori.

Partendo da queste considerazioni, si intende condurre un'analisi della redditività e dei costi di produzione delle aziende pugliesi produttrici di uva da tavola evidenziandone peculiarità e criticità. L'analisi può rappresentare uno strumento di notevole utilità sia per le scelte dell'imprenditore agricolo che per le decisioni di politica agraria ai diversi livelli istituzionali. Gli strumenti di analisi che possono essere adottati per tali obiettivi fanno riferimento ai principi della contabilità tradizionale, come il metodo del conto colturale analitico, oppure a metodologie basate sulla valutazione dei risultati economici a livello di tipologie aziendali ricorrenti in aree omogenee. La metodologia adottata nel presente lavoro fa riferimento alla definizione di sistemi aziendali rappresentativi (SAR), cioè ad aziende "tipo" che siano in grado di rappresentare le caratteristiche medie di un gruppo di aziende risultanti omogenee fra loro con riferimento alla forma di conduzione imprenditoriale, alla natura dei fattori di produzione, alla tecnica di coltivazione adottata, alla dimensione economica e alle scelte commerciali. Tale metodologia è stata esposta, in caratteri generali, con la comunicazione presentata in occasione del 32° Convegno OIV svoltosi a Zagabria nel 2009 (Seccia *et al*., 2009) per cui l'obiettivo specifico del presente lavoro è rappresentato dalla sua applicazione per le imprese specializzate nella produzione di uva da tavola e dal confronto con le imprese che presentano tale coltura nell'ordinamento produttivo, relativamente alla regione Puglia. In particolare, ci si soffermerà sulla prima fase del processo metodologico che prende l'avvio dalla individuazione della base informativa che sarà utilizzata successivamente per la definizione dei gruppi omogenei delle aziende produttrici uva da tavola e dei relativi sistemi aziendali rappresentativi (SAR) con l'integrazione dei criteri di calcolo dei costi di produzione e della redditività dei fattori.

2. MATERIALI E METODI

I dati aziendali utilizzati per le analisi provengono dalla Rete di Informazione Contabile Agricola Europea (RICA-FADN), gestita per l'Italia dall'Istituto Nazionale di Economia Agraria (INEA), il cui campione casuale, a partire dal 2003, è definito con criteri stabiliti in sede comunitaria. La RICA conduce un'indagine campionaria annuale con un'impostazione analoga in tutti i paesi UE pertanto essa rappresenta l'unica fonte armonizzata di informazioni microeconomiche, assicurando la produzione di dati comparabili a livello europeo.

Le aziende pugliesi che hanno aderito alla RICA nel 2007 sono state 911. Ai fini dell'analisi ne sono state individuate 93 nel cui ordinamento produttivo è presente l'uva da tavola, di cui 36, altamente specializzate, sono state utilizzate per il calcolo dei costi di produzione. Sono state considerate specializzate le aziende nelle quali il valore dell'uva da tavola prodotta, in pieno campo o sotto protezione, risulti uguale o superiore al 75% della produzione vendibile (PV) totale aziendale, al netto degli eventuali aiuti pubblici, e la superficie investita in tale coltura sia uguale o superiore al 50% della Superficie Agricola Utilizzata (SAU) totale aziendale.

Gli aspetti economici analizzati riguardano il calcolo del costo di produzione e la stima della redditività dei fattori produttivi conferiti dall'imprenditore.

Ai fini del calcolo dei valori medi, si è proceduto alla stratificazione per classe di SAU sia del campione complessivo delle aziende che producono uva da tavola che del sub-campione delle specializzate, imponendo il vincolo che ciascun gruppo sia formato da almeno cinque aziende.

Per ciascun gruppo è stato calcolato il valore medio del Costo Totale di Riferimento (CTR) quale sommatoria dei costi fissi e variabili, forniti dalla RICA, e delle remunerazioni di opportunità attribuibili ai fattori produttivi conferiti dall'imprenditore. Queste ultime sono ottenute moltiplicando la quantità di ciascun fattore per la relativa Remunerazione Unitaria di Riferimento (RUR). L'insieme di tali remunerazioni è definito Reddito Netto di Riferimento (RNR).

Per il lavoro familiare la RUR coincide con il salario orario medio degli operai agricoli a tempo indeterminato di media qualifica, fissato, per il 2007 e con riferimento alla Puglia, pari a 8,50 euro/ora, al netto degli oneri sociali. Per il capitale di esercizio la RUR coincide con la media del rendimento dei Buoni del Tesoro nel 2007, calcolata pari al 3,6%, al netto delle imposte. Per il capitale fondiario la RUR è fissata pari al 1,7% tenendo conto del rapporto tendenziale tra i canoni di affitto e il valore dei terreni condotti in affitto. In conclusione:

RNR = RUR_lavf*LAVf + RUR_cap*CAP + RUR_capf*CAPf

CTR = CF + CV + RNR

Dove:

- RNR = Reddito Netto di Riferimento
- RUR = Remunerazione Unitaria di Riferimento
- LAVf = Lavoro prestato dall'imprenditore e dai suoi familiari
- CAP = Capitale di esercizio
- CAPf = Capitale fondiario
- CTR = Costo Totale di Riferimento
- CF = Costi Fissi
- CV = Costi Variabili

La redditività complessiva dei fattori IR (Indice di Redditività) è stimata quale rapporto tra il Reddito Netto reale (RN) e il Reddito Netto di Riferimento (RNR), ovvero:
IR = RN/RNR.
Le redditività unitarie effettive sono stimate ripartendo il RN tra i fattori in modo direttamente proporzionale alla composizione del RNR e, per ciascun fattore, dividendo per la quantità impiegata.
Per le aziende specializzate il costo unitario di produzione del prodotto dominante Cupd, ovvero dell'uva da tavola, è calcolato attribuendo allo stesso una quota del CTR aziendale pari al rapporto tra il valore del prodotto dominante PVpd e il valore della produzione totale aziendale PV e dividendo per la quantità del prodotto dominante totale Qpd. Ovvero:

$$CPR = CTR * \frac{PVpd}{PV}$$

$$Cupd = \frac{CPR}{Qpd}$$

3. RISULTATI E DISCUSSIONE

I risultati dell'analisi per le aziende specializzate nella produzione di uva da tavola sono riportati nelle tabelle numero 1 e numero 2 e nella figura 1 mentre per le aziende con ordinamento produttivo comprendente l'uva da tavola si fa riferimento alla tabella numero 3.
Le 36 aziende specializzate del campione RICA presentano un livello di specializzazione colturale molto elevato, passando da 0,97, per quelle della prima e seconda classe di ampiezza, a 0,91 in quelle della terza classe. Sono tutte irrigue e la tecnica di coltivazione sotto protezione è maggiormente sviluppata nelle aziende della terza classe di ampiezza.

In relazione alla dimensione fisica, per entrambe le tipologie aziendali prevalgono le classi dimensionali inferiori: nella classe di ampiezza fino a 5 ettari ricade, infatti, il 44% delle aziende analizzate per il gruppo delle specializzate e il 31% per il gruppo delle non specializzate.
Anche se 33 aziende specializzate su 36 sono a conduzione diretta del coltivatore, la maggior parte fa largo ricorso alla manodopera salariata, poiché il calendario di lavoro prevede un picco di fabbisogno in occasione di operazioni colturali quali la potatura secca, la gestione sia della vegetazione che dei grappoli (diradamento) e la raccolta del prodotto. L'elevato impiego di manodopera, sia famigliare che avventizia, che emerge dall'analisi dei dati per entrambi i gruppi, testimonia il ruolo cruciale esercitato dalla coltura dal punto di vista sociale nelle aree vocate, spesso caratterizzate da scarse alternative occupazionali.
All'aumentare delle classi dimensionali delle aziende specializzate aumenta il valore medio degli investimenti in capitale per macchine, attrezzi e impianti, evidenziato anche dal crescente peso dei costi fissi. Ciò conferma il maggior impegno in termini finanziari da parte nelle imprese di maggiori dimensioni che diversificano la produzione attraverso la scelta di diverse combinazioni fra varietà e tecniche di copertura per l'anticipo e il posticipo della raccolta.
In linea generale, la coltura manifesta una buona redditività, confermando i risultati di precedenti analisi (Cembalo *et al*., 2008), che viene evidenziata sia dai valori dell'Indice di Redditività, IR, che del Reddito Netto, RN. In ambedue i gruppi l'IR, al netto degli aiuti pubblici, è sempre superiore all'unità, tranne nel caso della classe d'ampiezza inferiore a 5 ettari per le aziende non specializzate, e migliora con l'aumento della classe di SAU aziendale soprattutto per i seguenti motivi:

- migliore utilizzo delle risorse disponibili, in particolare del lavoro il cui fabbisogno per ettaro diminuisce con l'aumentare della superficie aziendale;
- maggiore ricorso alla manodopera salariata, il cui costo unitario è inferiore al costo di opportunità della manodopera familiare, che cresce con l'aumento della classe di SAU;
- presenza più estesa della coltura sotto protezione e, quindi, di un prodotto di maggior pregio commerciale; il prezzo medio di vendita, infatti, è leggermente più elevato passando dalla prima alla seconda classe, e cresce in modo significativo con la terza classe.

In particolare, è evidente che a parità di classe di ampiezza le aziende specializzate hanno un migliore IR a causa soprattutto del maggiore reddito netto unitario RN che conferma l'elevata redditività della coltura specializzata con minime differenze fra le classi di ampiezza.
Il rapporto prezzo/costo unitario cresce all'aumentare della classe di ampiezza, partendo da un valore prossimo all'unità per la classe dimensionale più bassa e raggiungendo il valore di 1,18 nella più ampia. Va sottolineato che il costo medio di produzione ha valori elevati, crescenti con la dimensione aziendale, e che le imprese più grandi riescono a spuntare prezzi maggiori, molto probabilmente grazie alla possibilità di diversificazione dell'offerta, in termini sia di gamma varietale che di epoca di raccolta, e di collegamento con i mercati.
La maggiore efficienza nella allocazione dei fattori produttivi per le aziende di maggiori dimensioni, per entrambi i gruppi, è dimostrata dall'andamento crescente della remunerazione unitaria dei tre fattori apportati dall'imprenditore e dalla sua famiglia all'aumentare della classe dimensionale, con l'eccezione della classe da 20 a 50 ettari delle aziende non specializzate.
La Figura 1 evidenzia che il costo di produzione è dominato dal RNR nelle aziende della prima classe (44,9%) e dai costi variabili in quelle della seconda e terza classe di ampiezza (44-48%). Nelle aziende di dimensioni inferiori, infatti, c'è un'alta incidenza dei fattori produttivi forniti dall'imprenditore e dalla sua famiglia per cui la relativa remunerazione è rilevante, in particolare

per il lavoro; nelle imprese di maggiori dimensioni, pur crescendo l'incidenza dei costi fissi, prevalgono quelli variabili e si ridimensiona l'RNR.

Tab.1- Aziende specializzate per la produzione di uva da tavola in Puglia
Costo di produzione e redditività dei fattori produttivi per classe di SAU

Classe di SAU ha	Numero aziende	SAU media az. ha	Fabb. di lavoro ore/ha	CPR euro/ha .000	RN euro/ha .000	IR	Remunerazioni Unitarie LAVf euro/ora	Remunerazioni Unitarie CAP %	Remunerazioni Unitarie CAPf %
<5	16	2,9	705	13,1	6,5	1,10	9,39	4,0	1,9
5 - 10	13	6,8	646	13,8	6,5	1,50	12,73	5,4	2,6
10 – 20	7	13,1	659	14,3	6,1	1,72	14,66	6,2	2,9
Totale	36	6,30	661	13,8	6,3	1,45	12,32	5,2	2,5

Fonte: Ns. elaborazioni su dati Rica 2007

Tab.2- Aziende specializzate per la produzione di uva da tavola in Puglia
Rese produttive, costi unitari e prezzi medi per classe di SAU

Classe di SAU ha	Numero aziende	Capitale macchine euro/ha .000	Resa /ha Q	Prezzo medio euro/q	Costo medio euro/q	Prezzo/ Costo
<5	16	4,7	248,7	50,11	47,86	1,05
5 – 10	13	5,0	257,8	51,36	44,41	1,16
10 – 20	7	7,2	206,5	63,13	53,47	1,18
Totale	36	5,8	235,9	55,10	48,22	1,14

Fonte: Ns. elaborazioni su dati Rica 2007

Tab. 3 - Aziende con ordinamento produttivo comprendente uva da tavola in Puglia
Redditività dei fattori produttivi per classe di SAU

Classe di SAU ha	Numero aziende	SAU media az. ha	Fabb. di lavoro ore/ha	RN euro/ha .000	IR	Remunerazioni Unitarie		
						LAVf euro/ora	CAP %	CAPf %
<5	29	3,1	549	3,6	0,80	6,79	2,9	1,4
5 - 10	23	7,1	559	4,3	1,21	10,26	2	2,1
10 - 20	19	12,8	509	3,8	1,44	12,21	5,5	2,4
20 a 50	14	28,0	364	1,9	1,31	11,11	4,7	2,2
> 50	8	88,0	364	2,3	2,61	22,17	9,4	4,4
Totale	93	17,2	417	2,7	1,53	13,03	5,5	2,6

Fonte: Ns. elaborazioni su dati Rica 2007

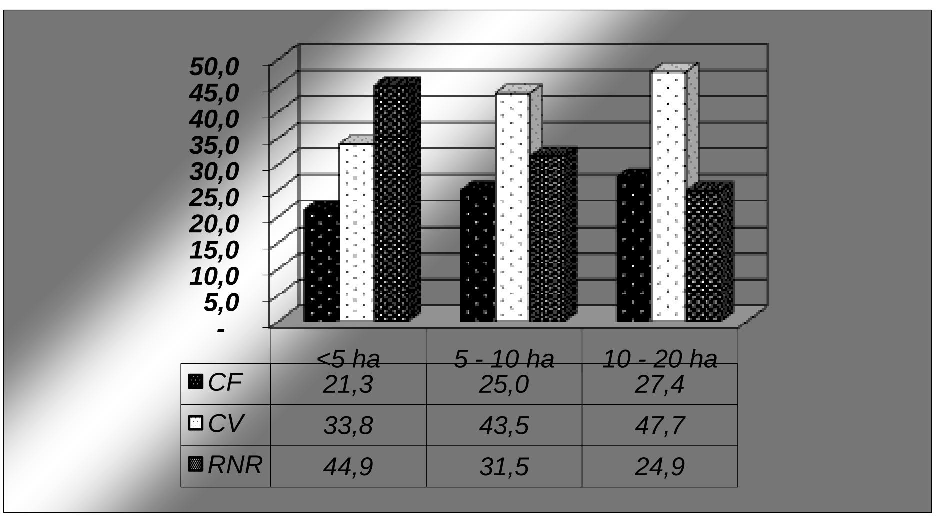

Fig.1 - Aziende specializzate per la produzione di uva da tavola in Puglia
Ripartizione del costo totale di produzione, per classe di SAU aziendale
Fonte: Ns. elaborazioni su dati RICA 2007.

4. CONCLUSIONI

Il presente lavoro riporta la prima fase di un processo metodologico che si basa sull'impiego dei dati della rete contabile europea (RICA-FADN) e sui criteri di classificazione tipologica aziendale utilizzati dall'Unione Europea, con lo scopo di analizzare i costi di produzione e la redditività dei fattori produttivi nella coltivazione dell'uva da tavola.

La descrizione e l'analisi del campione RICA per la Puglia, principale regione italiana per tale coltura, hanno permesso di definire la base informativa utilizzabile per le successive analisi e, in particolare, per pervenire a gruppi omogenei di aziende con la successiva caratterizzazione dei sistemi aziendali rappresentativi (SAR). A tale scopo, i risultati del presente lavoro andranno integrati con un'indagine specifica del campione di aziende che riguarderà la forma di conduzione aziendale, il grado di presenza della coltura negli ordinamenti aziendali, le tecniche produttive, le tipologie di prodotto. Inoltre, nella valutazione delle remunerazioni dei fattori produttivi sarebbe opportuno considerare le differenze nelle figure imprenditoriali.

Le successive analisi saranno condotte facendo riferimento alla nuova tipologia di azienda agricole della Unione Europea, istituita con il Regolamento (CE) n.1242/2008, con l'obiettivo di agevolare l'analisi delle caratteristiche strutturali e dei risultati economici. La nuova tipologia comunitaria delle aziende agricole, che si applicherà a decorrere dall'esercizio 2010, permetterà la costituzione di gruppi omogenei di aziende con riferimento all'orientamento tecnico-economico, alla dimensione economica e alla rilevanza della altre attività lucrative direttamente collegate all'azienda.

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Traditional technologies and history of Georgian wine

Teimuraz Glonti
Institute of Horticultire, Viticultire and Oenology
6 Marshal Gelovani Avenue, 0159, Tbilisi, Georgia
Teimuraz.glonti@gmail.com

Abstract

The main stages of creation and development of Georgian viticulture from VI millennium B.C until today are presented in the article, including the Neolithic, Early, Middle and late Bronze Ages and after the Christianity period. It reviews the main tendencies in the field of viticulture-wine making in XIX-XX centuries, describes the viticulture-wine making technologies used in different regions of Georgia, especially technological specificities of making the Kakhetian type of wine. It is shown that “kvevri” (earthenware buried in soil up to the neck) is a universal, steady vessel for fermentation, formation and storing of high quality traditional Georgian wine.

Resümee

In der Arbeit sind die Hauptetappe des Aufkommens und der Entwicklung der georgischen Weinbereitung vom VI Millennium des alten Jahrhunderts bis Heute, nach neolithischen, frühen, mittleren und späten Bronzezeit, der Folgezeit des Christentums dargestellt; sind auch im Bereich von Weinbau-Weinbereitung die laufende Tendenzen in den XIX-XX Jahrhunderten dargestellt, sind die Technologien der einzelnen Regionen von Weinbau-Weinbereitung Georgiens charakterisiert, besonders die technologische Eigenschaften der Weinbereitung des kakhetischen Typs. Die Hauptaspekte der Weinbereitung. Ist dargestellt, dass für die Bereitung des georgischen, traditionellen, hochwertigen Weins, ist „Kvevri“ (ein Tongefäß, welches im Erde voll eingegraben ist) ein festes universales **G**efäß, wo man Wein gären, fertigmachen und aufbewahren kann.

The history of Georgian wine includes two big periods: before and after the Flood. Results of archeological studies of Shulaveri group (Dangreuli Gora, Imiri, Gadachrili Gora - VI-V-IV millenniums B.C) (Japaridze, 2003; Chilashvili, 2004 A; Rusishvili, 2007 A) indicate about the high level of viticulture – wine making development in southern Georgia before the Flood.

Based on archeological evidences of before the Flood period in Georgia it is possible to come to several important hypothesis and conclusions. The initial process of grape pre-domestication covers the period of VI-V-IV millenniums B.C and is located within the regions of Anatolia, southern Caucasus and Mesopotamia (Forni 2005-2006). However, the cultural grape stones, as well as traces of red wine on clay vessel fragments, are found only in southern Georgia, which indicate about existence of wine making practices at household level during that period. Taking into consideration, that remainders of different varieties of various grain-crops (wheat, barley, oat, peas, etc.) and domestic animals, as well as farming tools are found in the same region, one can certainly conclude about existence of diversified agriculture in southern Georgia in VI-IV millenniums B.C. The archeological findings of Shulaveri also include small clay statues of fertility goddesses which mark the religious consciousness of Georgian ancestor’s tribes. Probably, initial goddess could be considered as a pre-image of the Blessed Virgin.

After the Flood civilization starts its origin just from *before the Flood* (*proto-Iberian, i.e. Mediterranean*) civilization and its commencement is again vine, which is already tamed during the before Flood period; and wine also exists as a household product. “And Noah began working and created vine….. and made wine” (born IX.20) says the Bible. The word – “wine” could be the term inherited from the Proto Iberian world. One may think that Noah did not need to create the new term. In Georgian language wine is called “Gvino”. In Welsh wine is called “Gvin”. Both “Gvino” and “Gvin” possibly are created from one base. Since Welsh language does not belong to the family of European languages, one may assume that welsh are successors of the proto Iberian tribes.

Noah, by grace of God, brought the vine, as well as all other flora and fauna to the ark namely from before the Flood civilization. Where could Noah collect vine and where could he plant it after the Flood? With no doubts, he could collect it where grape was already pre-domesticated and started vine cultivation in the same region, as he was equipped with all knowledge and experience by Grace of God.

According to R. Ramishvili, Noah could plant the vine rather far off the Ararat Mountain, since the valley of Ararat is located as high as at 900-1000 meters above the see level, where ambient temperature in winter falls below -20^0 C and therefore, the climatic conditions is not favorable for vine growing (Ramishvili, 2001). According to P. MacGovern (2007), there is no trace of old vine discovered at the Ararat valley.

All the above mentioned exceeds the bounds of hypothesis and at certain extent allows realistic, logical comprehension of development. The realistic character of such approach is proven by finding of so-called Mtkvari-Araksi culture (IV-III millenniums B.C) during archeological digs in area located between the rivers of Mtkvari and Araksi in southern Georgia.

The early Bronze Age is considered as an initial stage of the start-up of metallurgy (Chilashvili, 2004 B). This period is also characterized by further improvement of crop and livestock farming, means of production, start of copper metallurgy development (Badaani, III millennium B.C) (Rusishvili, 2007 B). Main sphere of metallurgy during this period is processing of bronze, gold and silver. The ceramic production had also developed very rapidly; in this regards, the clay vessel with wide middle part and narrow end, which has a strong resemblance with modern kvevri, deserves special attention. Another important finding is a treasure (including small and big clay vessels) excavated in burial mounds at Trialeti eminence, since according to L. Chilashvili (2004) these stylish clay vessels could be used for wines.

In this regard, one should specifically point out the golden wine bowl, which is made of one piece of gold with double walls and covered with gems (XVIII-XVII centuries B.C.). Also, other golden and silver bowl, styled with various symbolic subject images are discovered in the above mentioned burial mounds. Such wine vessels, found during archeological excavations at various cult places, indicate about the use and devotion of wine vessels in sacred and praying places (Kuptin 1941).

By the end of the II millennium B.C and beginning of the I millennium B.C. a huge progress was made in terms of grape cultivation proved by discovery of bronze vine cutting knifes, hatchets and other tools,. 3000 years ago Kolkhetian wines are mentioned by Homer in “Iliad” when sister of the Kolkhetian king Aieth - Kirike entertained her guests with Kolkhetian wine during 12 months.

Christianity was firstly preached in Georgia by the apostle Andrew the First Called and apostles Matata and Svimon Kananeli. Three centuries later St. Nino equipped with vine-cross led the Christianity into Georgia and it was announced as a state religion. This event had great symbolic meaning as vineyard and vine were conceived not only as a symbols of the God and the Blessed Virgin - Mother of God – but also represented determinative guiding line for Georgians statehood.

Christianity plays very important role in development of Georgian viticulture and wine making. In place of antic, Dionysius' traditions of wine purpose and use, Christianity revived the true sense of the destination and use of wine for the human beings.

Wine making played important role in monastic economical activities. Wine necessary for arrangement of monastic, ritual services was produced in monastery wine-cellars. The viticulture and wine-making was taught in monasteries as one of the disciplines, e.g. in the Academy established at the Ikalto monastery (12^{th} century A.C.). In monastery wine-cellars wine was fermented and formed using traditional method, by kvevris buried in soil.

We should also pay special attention to historically high culture of formation and use of the red wines in Georgia. Out of total 525 vine varieties described in Georgia, 287 breeds are red (54.47%). Depending on geographic location, the proportion of red grape varieties is even higher in particular regions.

There was a tradition in Georgia for making so-called "Zedashe" – wine for oblation to God. This was mainly red wine and was made from high quality self drained sweet. "Zedashe" was kept in the cellar, which was considered as a holy place was blessed by priests. The Eucharist in Georgian Orthodox Church is still received nowadays using the red wine.

19^{th} century is a very important period for development of Georgian viticulture – wine making, though full of serious, painful problems. Despite of popularity of Georgian (particularly Kakhetian) white and red dry table wines in Russian Empire and especially at the Emperor's court, it was decided to change local viniculture / wine making traditions and practices, as well as local grape breeds with European ones. The intention was to substitute earthenware kvevri with European oak fudders, although experts were admitting high technological potential of local endemic breeds (Saperavi, Rkatsiteli, Kakhuri Mtsvane, Kakhuri Mtsvivani) as well as superiority of kvevri buried in the soil upon the fudders and vats spread in the Europe (Lentz, 1846). Kathetian type wine of kvevri had a high reputation by the end of 19^{th} century as well and was exported to Russian Empire in big quantities (Piralov A., Shaverdov S.).

However, in the midst of 19^{th} century, the method of wine making without chacha (so-called European manner) was introduced and practiced quite successfully, though not from the European grape breeds but local endemic varieties. Successful economical performance of some Georgian nobleman should be highlighted here. First of all, it relates to wine cellars of generals - prince Bagration-Mukhraneli and poet Al. Chavchavadze – arranged at modern European level. Also, we should mention Prince Zakaria Jorjadze, who studied the viticulture / wine making in Austria and who's dry table wine was awarded with big golden medal at Brussels exhibition in 1888. In 1907, the natural semi-sweet wine (Racha-Lechkhumi) produced by D. Kipiani, received the main prize "Grand Prix" at the international exhibition in Belgium.

However, despite the big desire and extended efforts of authorities, the European grape varieties could not conquer place in Georgian vineyards, except of few examples in few micro regions. Georgian endemic wine varieties stood up to the severe challenges of the age and proved their superiority in Georgian viticulture and wine-making.

In terms of viticulture / wine-making, as well as from the geographic point of view, Georgia can be divided in eastern (Kakheti, Kartli) and western (Imereti, Samegrelo, Guria, Abkhazia, Racha-Lechkhumi) parts. Environmental conditions in Georgia are very diverse, represented by various climatic zones: subtropical, hot continental, and cold.

The industrial viticulture in Georgia is not presented at higher than 1200 meters above sea level.

The lowest zone of viticulture includes regions located along the Black Sea shore (Abkhazia, Imereti, Samegrelo, Ajara, Guria and some lower areas of Imereti). The vineyards here are cultivated mostly until 400 meters above sea level. This zone is characterized with subtropical climate and high precipitation. The total sum of active temperatures reaches 3500-4000^0.

The middle zone of the mountainous regions is considered as one of the high quality wine making zone in Georgia (Kakheti, Zemo and Kvemo Imereti, Racha-Lechkhumi and most areas of Zemo and Kvemo Kartli). This zone, which is located at 400 to 800 meters above sea level, is characterized with warm, moderate climate and normal precipitation levels. The sum of active temperature is about 3000-3500^0.

The high level is situated at 800-1200 meters above sea level (Akhaltsikhe, Aspindza, Adigeni, Dmanisi, Tianeti, Dusheti, former South Osetia and partially Racha-Lechkhumi).

The wines of eastern Georgia are distinguished with strength, extract content and dark colors, caused by high content of tanning and painting compounds

The wines of western Georgia are softness, lightness, cheerfulness, gentleness and harmonic confluence of compounds.

The distinct diversity between wines of different Georgian regions is caused not only by variety of natural and climatic conditions, but also by grape varieties and specificities of wine formation technologies. High quality Georgian wines were traditionally produced using natural yeasts. During two-three years formation process Georgian wines, processed both with and without chacha, are developing well and gaining rich unique aroma and delicious taste.

Kvevri is traditional Georgian vessel for wine fermentation, formation and storage. The place where kvevri was placed is called “marani”. Two types of maranis are known in Georgia: closed (eastern Georgia) and open (western Georgia). The closed marani is built of stone and kvevris there are buried in the soil up to the neck. The wooden or stone made “satsnakheli” is placed near kvevris, where sweet juice is squeeze out of grapes and flows into the kvevri by special gutter. The open marani or “chur-marani” is located in open area and surrounded by tall broad-leaved tree, which create certain conditions for shading and ventilation of buried “churis” (kvevri is called “churi” in the western Georgia). In some regions of western Georgia chur-marani is built of wood as well, where satsnakheli was placed together with churis.

The Georgian wine making is especially distinguished by the fact that wine is produced mostly from local endemic grape varieties. 525 vine varieties, which are sorted out on a regional basis, are described in Georgia. Eastern Georgian breeds are mostly concentrated in Kakheti and Kartli with certain spread of varieties based on specifics of the region. The following grape varieties, which are producing high quality wines and are well adapted to local climatic and soil conditions are spread in Kakheti: Rkatsiteli, Saperavi, Kakhuri Mtsvane, Khikhvi, Kisi, Kakhuri Mtsvivani, Bideshuri (unfortunately, the area of spread of latter three varieties, especially of Kakhuri Mtsvivani, is very limited nowadays). Chinuri, Goruli Mtsvane, Tavkveri, Shavkapito and some other breeds are mostly spread in Kartli.

The high level folk selection process, which took place during the centuries, has determined differentiation of Georgian vine breeds taking into consideration local climatic and soil conditions and the potential of each breed. As a result, we have arrived to the wide range of wine varieties of white and red grapes from Kakheti, Kartli, Imereti, Samegrelo, Abkhazia, Guria-Ajara and Racha-Lechkhumi. In turn, it created a rich bouquet of high quality, original table dry and naturally semi-sweet white and red wines, which are diversified not only at regional, but also at individual micro-zone and even micro-district level.

Georgian dry and red vintage wines were well represented and played important role in the wine assortment in Soviet Union both in terms of quality and variety of brands.

The traditional assortment of Georgian wines was created as a result of revival-preservation and use of local traditional wine making technologies and their enrichment with modern ones.

Traditional technologies of Georgian wine making mostly include three categories: Kakhetian wines (fermentation and formation with complete chacha), Imeretian (partial formation with chacha) and naturally semi-sweet wines. The naturally sparkling wines were also known in Georgia.

Kakheti is specifically notable among other Georgian regions for different types of highest quality wines, and especially Kakhetian type of wine, which is traditionally produced in kvevris (earthenware buried in soil up to the neck) by fermentation and formation of squeezed grapes with complete chacha during 5 months. The highest quality Kakhetian wine is formed only in Kakheti, specifically in certain micro-zones from grapes of Rkatsiteli, Saperavi, Kakhuri Mtsvane, Khikhvi, Kisi and Kakhuri Mtsvivani. During the fermentation and formation processes Kakhetian wine is enriched with various volatile, aroma-forming and phenolic compounds of hard parts of the grape - skin, skeleton and pips, which in turn ensures high antioxidant activity, healing, dietary and nutritional value of the product.

Imereti is considered as on of the big wine making regions in Georgia, where various high quality wines are produced. Imeretian type of wine is processed with partial chacha (up to 10%, without skeleton. While formed, Imeretian wines are developing pleasant, strong bouquet and taste. High quality Imeretian wines are produced from Tsolikouri, Tsitska and Krakhuna varieties.

Georgian natural sweet wines are known from the ancient period and have important place in the assortment of Georgian wines. Naturally semi-sweet wine is produced from grapes with high sugar content harvested in late fall. Traditionally sweet juice of grapes was fermented in kvevris with chacha. The wine was separated from chacha when the latter was sunk to the bottom of kvevri at the residual sugar content of 4-5%. The bulk of the liquid and squeeze remainder was place in kvevri and hermetically sealed by clay. Naurally semi-sweet wines were produced in different regions of the eastern and western Georgia (Kakheti, Racha-Lechkhumi, Guria, etc.). Traditionally, for production of the natural sparkling wines grapes were moved from lowland areas to higher elevations and fermented in kvevris. Due to the relatively cold climate conditions the fermented was not complete and after opening the kvevri wine was sparkling, cheerful and semi-sweet. The natural semi-sweet wines were also produced in Kartli, Imereti, Guria.

In XX century, the gradual process of site-specific wine production was introduced and practiced, which resulted in broadening of industrial production of the highest quality table dry and semi-sweet wines. Thus, well-known brands of “Tsinandali”, “Gurjaani”, “Napareuli”, “Tibaani”, “Rkatsiteli”, “Tsarapi”, “Mukhranuli”, “Atenuri”, “Tsolikouri”, “Tsitsqa”, “Khvanchkara”, “Ojaleshi”, “Chkhaveri”, “Mukuzani”, “Akhasheni”, “Teliani” and others were introduced to the market.

Although, during the Soviet period there was no official site-specific accountable categorization of wines, however in reality such approach was actually adopted and implemented. Georgia was supplying the Soviet market with high quality table dry, semi-sweet and strengthened white and red wines, which were awarded with many highest prizes at various international exhibitions and competitions.

By the end of XIX century, creation of private cooperatives and partnerships took place in various regions. In 1919, during the first democratic Republic of Georgia, the council of Georgian viticulture/wine making congress established the magazine “Vazi da Gvino” (“Vine and Wine”), and the first law on wine making was adopted. However, after Soviet occupation in 1921, such efforts were stopped and the state began direct management of the viticulture/wine making industry. Fortunately, following restoration of Georgian independence in 1992, the printing of the magazine was renewed and the “Georgian law on vine and wine” was adopted.

In 70th of XX century, the negative tendencies and events in Georgian viticulture and wine-making were revealed, which were further aggravated in 90th. Inadequate economic decisions/practices during transformation to the new political formation caused creation of unmanageable processes in Georgian viticulture / wine-making. Out of various negative tendencies, the most important was commissioning of the absolutely unwarrantable campaign against kvevri and introduction / use of European aroma-forming yeasts in Georgian wine-making. As a result, from one side, traditional Georgian wine making culture was diminished and diversity of Georgian wines was down graded, and from the other side, huge limitations were imposed to production of high quality Kakhetian wine of kvevri. During the process of long-term formation Georgian wines are developing rich aroma and taste, therefore due to introduction of yeasts from essentially different regions they are loosing its’ unique qualities. Georgia always possessed fine cultures of domestic yeasts for different regions and vine breeds, and this direction should be revived and developed by all means. Application of the foreign aroma-forming yeasts in Georgian wine-making is not acceptable. As for the kvevri made Kakhetian type of wine, in case of fermentation with natural yeasts it gains higher quality and content.

To date, the main tasks for the modern Georgian oenology is purposeful development and confluence of rich traditions and new researches, which should be done through maximal revealing of the potential of Georgian vine breeds, preservation and creative application of the traditional technologies.

Georgian vine breeds, Georgian wine making technologies, with there ancient and rich history, do not represent only Georgian people’s not made by hands treasury, but also belong to the world viticulture and oenology.

Conclusions

Following analysis of the above mentioned issues one may conclude the following:

- Georgia is a cradle of cultural oenology.
- The oenology as a productive industry of after the Flood period – Early, Middle and Late Bronze Ages - is a result of inherited development of the wine making practices of before the Flood (proto-Iberian, i.e. Mediterranean) Age, and is a new qualitative leap in history of oenology.
- Georgian wine making is based on local, endemic varieties of vine, which in combination with diversity of climatic and soil conditions created a solid foundation for development of

high quality, diverse wine making technologies and established a rich bouquet of Georgian wines.
- Traditionalism was and is a major feature of Georgian oenology.

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Vini bianchi georgiani in anfora.
Caratteristiche organolettiche e analitiche di vini ottenuti con differenti tecniche di vinificazione delle uve.

Georgian white wines in amphoras.
Organoleptic and analytical properties of wines obtained with different winemaking techniques.

D. Lanati[1], D. Marchi[1], G. Mazza[1]
[1]Enosis srl, via per Cuccaro 19 – Cascina Meraviglia, 15043 Fubine (AL), Italy
direzione@enosis.it

RIASSUNTO

Sono stati prodotti sei vini bianchi da uve Rkatsiteli secondo l'antica tecnologia Kakhetiana che impiega le anfore, sia in fase di fermentazione e macerazione, sia in affinamento. Un primo gruppo di vini è stato vinificato utilizzando lieviti selezionati con una lunga macerazione delle bucce, mentre un secondo gruppo è stato ottenuto impiegando lieviti indigeni ed una macerazione anche con i raspi. Nei vini fermentati con i raspi si osserva un più alto rapporto tra flavani reattivi alla vanillina e proantocianidine e al contempo un elevato valore dell'indice di gelatina e dei tannini reattivi alla siero albumina bovina, dati che indicano una maggior presenza di tannini astringenti. L'analisi del profilo aromatico dei vini, ha portato alla identificazione di 65 composti nella frazione libera e 35 in quella legata. Tutti i vini sono caratterizzati da una elevata presenza di terpeni e norisoprenoidi, provenienti dalle uve di origine. I vini ottenuti dalla macerazione con i raspi presentano delle note amare e tanniche più marcate rispetto a quelli ottenuti dalla macerazione delle bucce

SUMMARY

Six white wines were produced from the Rkatsiteli grapes, based on the ancient Kakhetian technique, which involves the use of amphoras, during the fermentation as well as the maceration phase. One group of wines was produced using selected yeasts and a long maceration of grape skins and another one was produced with indigenous yeasts and maceration with both grape skins and stalks, while aging phase is the same for all wines. In wines fermented with grape stalks, we can also observe a higher ratio between the flavans reactive to vanilline and the proantocyanidins and at the same time a high value in the gelatin index and of tannins reactive to the bovine sieroalbumine. This information shows a higher rate of astringent tannins. In aromatic profile analysis of the wines, 65 and 35 components have been identified in the free and bound fractions, respectively. All the wines contain a high percentage of varietal terpens and norisoprenoids. The wines produced from maceration with grape stalks are more bitter and contain a higher percentage of tannins.

INTRODUZIONE

La Georgia è un paese di antichissima tradizione vitivinicola e rappresenta, al tempo stesso, una riserva unica di specie autoctone. Degli oltre 525 vitigni esistenti, 38 sono quelli commercialmente coltivati mentre i vitigni internazionali sono quasi del tutto assenti.
Nella fertile regione del Kakheti si concentra oltre il 60% della produzione vitivinicola della Georgia. Qui vengono coltivate sia uve bianche delle cv Rkatsiteli, Mtsvane, Kisi, sia uve rosse tra cui spicca la cv Saperavi dalla quale si ottengono vini ben strutturati, fortemente tannici e dal colore intenso.

In questi ultimi anni, si sta cercando di riscoprire o meglio ancora di far conoscere fuori dai confini georgiani l'antica tradizione legata ai vini delle anfore (kvevri). Si tratta di una tecnica di vinificazione e invecchiamento del vino in anfore interrate che risale agli albori della storia del vino, e dal Caucaso, dove è nata, si è poi estesa in epoche successive all'Egitto e alla Grecia. Il vino non subisce alcun trattamento e si mantiene stabile dopo un periodo di invecchiamento in anfora di circa 5 mesi.
In questo lavoro sono stati analizzati sei vini bianchi ottenuti da uve Rkatsiteli secondo l'antica tecnologia Kakhetiana che impiega le anfore, sia in fase di fermentazione e macerazione, sia in affinamento.

MATERIALI E METODI

Le uve della cv Rkatsiteli sono state raccolte ad Alaverdi, località sita nella regione georgiana del Kakheti. La vinificazione delle uve è stata condotta presso la cantina del monastero di Alaverdi, mentre tutti i campioni di uve e di vini sono stati analizzati presso i laboratori di Enosis. Le analisi delle uve e dei vini sono state effettuate secondo i metodi descritti in letteratura (Di Stefano et al.1989; Harberstone et al. 2002). I composti volatili delle uve e dei vini sono stati identificati e quantificati mediante GC-MS; maggiori dettagli sulle procedure utilizzate vengono forniti in Mazza et al. 2002, 2006.

RISULTATI E DISCUSSIONE

Le uve raccolte avevano un contenuto zuccherino di 255 g/L un pH di 3.48 ed una acidità totale di 5.6 g/L. Il mosto da esse ottenuto è stato vinificato nelle anfore allestendo tre prove (B1, B2 e B3) con le sole bucce utilizzando lieviti selezionati ed altre tre prove (BR1, BR2 e BR3) con i graspi e le bucce impiegando lieviti autoctoni. A fine fermentazione le anfore sono state riempite fino alla sommità e tappate ermeticamente. Dopo un periodo di affinamento di sei mesi i vini risultano stabili e non necessitano di alcun trattamento; a questo punto sono stati travasati in altre anfore separandoli dalle fecce. I dati della Tab. 1 si riferiscono ai dati analitici dei vini dopo sei mesi di affinamento nelle anfore. Tutte le fermentazioni sono andate a buon fine con ambedue i tipi di lievito utilizzati ed i vini hanno raggiunto gradazioni alcoliche intorno al 15% di alcol svolto e con un pH molto elevato. Quelli macerati con le sole bucce mostrano valori medi leggermente più bassi di pH, di potassio e di calcio.

Tabella 1 : Parametri analitici dei vini dopo 6 mesi di affinamento

	u.d.m.	B1	B2	B3	BR1	BR2	BR3
alcol svolto	%vol	15,12	15,06	14,97	14,67	15,14	15,02
acidità totale	g/L	4,8	4,7	4,8	4,5	4,4	4,4
pH	-	3,83	3,87	3,85	3,91	3,89	3,9
acidità volatile	g/l	0,67	0,62	0,75	0,69	0,66	0,67
zuccheri riduttori	g/L	1	1	1	1	1	1
alcol complessivo	%vol	15,12	15,06	14,97	14,67	15,14	15,02
rame	mg/L	0,10	0,10	0,13	0,11	0,13	0,14
ferro	mg/L	0,99	0,87	0,72	0,87	0,89	0,78
potassio	mg/L	1164	1257	1190	1332	1250	1257
calcio	mg/L	55	57	47	56	64	67
$D.O._{420}$ componente gialla	$D.O._{1cm}$	0,930	0,901	1,249	1,064	1,129	1,088

Le differenze più evidenti tra i due gruppi di vini B e BR si riscontrano a carico di altri parametri maggiormente legati all'astringenza dei vini stessi. La Tab. 2 ci mostra che per i vini macerati con i graspi e le bucce (BR1, BR2 e BR3) si ottiene un più alto valore dell'indice di gelatina, ma soprattutto il dato della BSA è quello che meglio evidenzia questo maggiore apporto di tannini astringenti nei vini di questo gruppo rispetto a quelli macerati con le sole bucce. Anche i valori di V/P seguono lo stesso andamento tranne che per il campione B2 che mostra un valore simile al campione BR1. La rappresentazione grafica delle Fig. 1-3 evidenzia chiaramente tali differenze.

Tabella 2 : Parametri analitici correlati all'astringenza dei vini.							
	u.d.m.	**B1**	**B2**	**B3**	**BR1**	**BR2**	**BR3**
tannini reattivi alla BSA	mg/L	934	1293	1197	1443	1371	1586
V/P	-	1.39	1.94	1.34	1.92	2.42	2.26
indice di gelatina	%	90.6	91.8	91.2	92.5	92.7	92.6

Note: V = flavani reattivi alla vanillina; P = proantocianidine

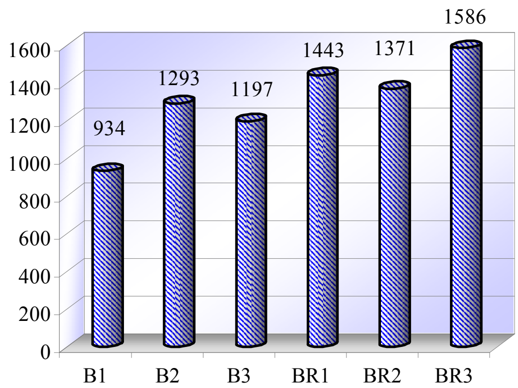

Figura 1: Tannini reattivi alla BSA

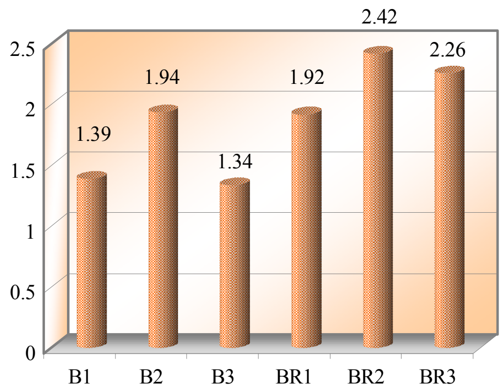

Figura 2: V/P

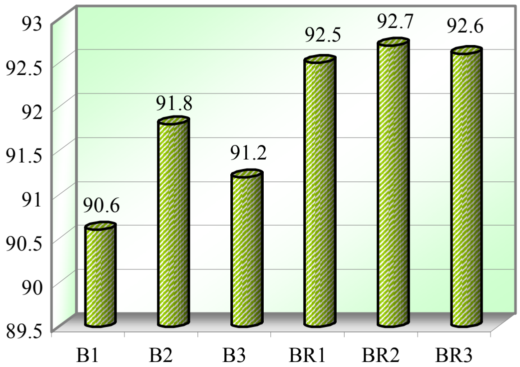

Figura 3: Indice di Gelatina

Nella Tab. 3 vengono evidenziate le analisi delle catechine, degli acidi idrossicinnamici e dei flavonoli. La differenza più significativa tra i due gruppi di vini esaminati si osserva a carico dei flavani reattivi alla vanillina con valori più elevati per i vini BR vinificati con bucce e graspi, mentre per gli altri parametri non si osserva una tendenza netta.

L'analisi organolettica dei vini ha chiaramente messo in evidenza differenze marcate tra i due

Tabella 3 : Parametri relativi ai composti fenolici						
	B1	**B2**	**B3**	**BR1**	**BR2**	**BR3**
indice di flavonoidi totali (mg/L (+)catechina)	1596	1731	1672	1970	1940	1925
indice di flavani reattivi vanillina (mg/L (+)catechina)	726	1002	650	1024	1213	1201
ac.idrossicinnamici (mg/L ac. caffeico)	144,2	140,9	127,4	138,2	149,2	120,9
flavonoli totali (mg/L quercetina)	61,5	58,5	60,9	57,7	65,3	56,2

gruppi di vini, soprattutto al gusto. All'assaggio, i vini del gruppo BR, vinificati con i graspi e le bucce ed il contributo dei lieviti indigeni, si presentano molto più astringenti con delle note amare più marcate rispetto ai vini fermentati con le sole bucce, in accordo con i dati analitici ottenuti. Dal punto di vista aromatico, va rimarcato il fatto che, pur in presenza di una notevole concentrazione in terpeni, l'espressione aromatica legata a questi composti si avverte in modo piuttosto blando in tutti i campioni esaminati, a causa delle interazioni tra le diverse molecole presenti nei vini.
Il profilo aromatico delle uve della cv. Rkatsiteli (Tab. 4) mostra un ottimo potenziale aromatico complessivo con un elevato tenore in terpeni, tra cui spiccano il linalolo e il geraniolo. Anche i norisoprenoidi, tra cui emergono il 3-oxo-α-ionolo e il vomifoliolo, sono

Tabella 4 : Composti presenti nella frazione legata dell'uva della cv Rkatziteli (conc. μg/Kg)			
ALCOLI E BENZENOIDI		**TERPENI**	
esanolo	81,0	trans-furanlinalolo ossido	14,9
alcool benzilico	1237,9	cis-furanlinalolossido	16,5
2-fenil etanolo	329,5	linalolo	371,0
4-vinil guaiacolo	399,1	α-terpineolo	15,7
4-vinil fenolo	455,5	trans-piranlinalolossido	5,0
vanillina	28,4	cis-piranlinalolossido	4,2
acetovanillone	178,3	nerolo	22,5
zingerone	68,0	geraniolo	108,1
diidroconiferilalcool	61,6	2,6-dimetil-3,7-ottadien-2,6-diolo	37,1
3,4,5-trimetossifenolo	149,1	2,6-dimetil-1,7-ottadien-3,6-diolo	21,9
TOTALE	**2988,4**	OH-citronellolo	7,3
NORISOPRENOIDI		8-OH-didrolinalolo	16,5
3,4-diidro-3-oxoactinidolo I	13,5	trans-8-OH-linalolo	115,6
3,4-diidro-3-oxoactinidolo II	34,2	OH-geraniolo	31,3
3,4-diidro-3-oxoactinidolo III	26,1	cis-8-OH-linalolo	229,7
3-idrossi-β-damascone	81,3	p-ment-1-ene-7,8-diolo	71,2
megastigma-7-en-3,9-diolo	91,3	**TOTALE**	**3018,4**
3-oxo-α-ionolo	633,0		
3-idrossi-7,8-diidro-β-ionolo	64,9		
3-idrossi-β-ionone	37,3		
blumenol C	43,6		
3-idrossi-5,6-epossi-β-ionone	31,3		
3-idrossi-7,8-deidro-β-ionolo	72,3		
vomifoliolo + grassopperchetone	662,2		
TOTALE	**1790,9**		

presenti in gran quantità; l'alcol benzilico e i 4-vinilfenoli sono i composti più rappresentativi tra gli alcoli e benzenoidi.
Se esaminiamo l'andamento dei composti volatili della frazione libera dei vini (Tab. 5) notiamo subito come sia fortemente ridimensionata in tutti i campioni la presenza degli esteri di fermentazione, soprattutto gli acetati. Gli esteri degli acidi organici, in particolare il dietil succinato, aumentano considerevolmente a causa dell'invecchiamento chimico del vino che si manifesta in modo più pronunciato nel vino B3, mentre mostra il valore più basso nel campione B1 dello stesso gruppo.
L'idrolisi spontanea dei composti legati libera una elevata concentrazione in terpeni e norisoprenoidi nella frazione libera con una importante presenza di alcoli terpenici monoidrossilati, come il linalolo, il geraniolo, il nerolo ed il citronellolo. Quest'ultimo composto deriva dal geraniolo e si forma durante la fermentazione per l'azione dei lieviti. La presenza dei dioli terpenici è molto consistente ma meno importante dal punto di vista aromatico trattandosi di composti ad elevata soglia olfattiva. Appare rilevante anche la presenza dei norisoprenoidi, anche se quantitativamente meno evidente di quella dei terpeni, ma con un alto tenore in 3-oxo-α-ionolo e vomifoliolo.

Tabella 5 : Composti presenti della frazione libera dei vini (conc. in μg/L)

VINO	B1	B2	B3	BR1	BR2	BR3
ESTERI E ACETATI						
isoamil acetato	96,3	74,3	110,7	84,5	83,7	90,1
etil esanoato	71,4	61,7	80,5	81,4	83,6	88,0
etilottanoato	64,4	44,5	85,4	58,9	63,3	70,7
etil decanoato	30,1	22,6	34,2	29,0	35,5	31,3
2-feniletilacetato	40,4	30,2	106,8	25,6	109,4	60,7
TOTALE	**302,6**	**233,4**	**417,6**	**279,5**	**375,5**	**340,9**
ESTERI DI ACIDI ORGANICI						
isoamil lattato	243,4	237,6	300,2	251,4	244,4	277,1
metiletil succinato	83,2	106,1	180,0	142,3	106,7	127,6
dietil succinato	9269,4	10239,3	16725,5	12069,7	11982,2	12747,8
dietil malato	261,2	201,4	183,8	220,9	186,5	111,9
dietil-2-OH-glutarato	274,2	339,0	252,8	224,5	272,5	226,8
monoetil-2-OH-glutarato	248,8	161,3	160,9	144,5	166,3	159,3
etil-3-fenil-2-OH-propionato	368,6	476,0	455,7	497,2	554,9	518,5
dietil piroglutammato	84,4	24,8	28,9	17,8	19,6	22,5
TOTALE	**10833,3**	**11785,5**	**18287,9**	**13568,3**	**13533,2**	**14191,5**
ALCOLI E BENZENOIDI						
esanolo	431,5	420,6	457,2	469,1	463,6	463,4
cis-3-esenolo	9,5	9,9	11,4	11,8	11,1	6,8
alcool benzilico	289,8	282,2	320,8	444,8	399,8	318,1
2-fenil etanolo	34696,2	36216,1	38878,7	39162,5	39637,8	41692,3
4-vinil guaiacolo	153,0	198,2	69,1	153,9	214,2	153,3
4-vinil fenolo	21,7	38,3	22,9	22,1	22,0	19,7
diidroconiferilalcool	145,1	24,9	29,9	88,7	94,2	216,5
3,4,5-trimetossifenolo	67,4	25,6	69,9	116,9	97,7	107,8
TOTALE	**35814,3**	**37215,9**	**39859,9**	**40469,9**	**40940,4**	**42977,9**
TERPENI						
trans-furanlinalolo ossido	10,3	14,2	12,6	44,6	29,3	14,7
cis-furanlinalolossido	44,0	38,3	40,0	116,8	96,2	50,7

linalolo	338,2	387,6	367,1	392,1	284,6	311,8
α-terpineolo	83,2	98,0	84,7	76,3	77,2	63,8
trans-piranlinalolossido	16,7	20,1	21,5	24,7	25,7	18,8
cis-piranlinalolossido	30,6	40,3	21,2	76,0	62,2	40,7
citronellolo	45,5	59,7	59,3	50,9	58,6	54,6
nerolo	17,2	23,2	17,4	26,8	15,5	14,5
geraniolo	49,4	76,7	51,9	69,1	56,5	48,6
2,6-dimetil-3,7-ottadien-2,6-diolo	91,0	129,8	101,6	86,1	91,0	90,4
2,6-dimetil-7-otten-2,6-diolo	28,0	28,5	35,7	23,0	24,3	21,8
2,6-dimetil-1,7-ottadien-3,6-diolo	31,6	29,0	42,9	31,1	29,8	26,8
OH-citronellolo	55,3	45,3	50,0	43,2	41,9	23,6
8-OH-didrolinalolo	23,7	25,4	20,7	14,4	19,6	6,4
trans-8-OH-linalolo	54,0	90,4	59,3	67,3	55,6	57,1
OH-geraniolo	39,6	48,5	33,3	30,6	22,2	33,3
cis-8-OH-linalolo	163,7	169,6	179,9	155,0	126,0	107,3
p-ment-1-ene-7,8-diolo	88,2	39,3	53,4	66,8	55,3	61,0
TOTALE	**1210,2**	**1363,8**	**1252,6**	**1394,7**	**1171,5**	**1045,9**
NORISOPRENOIDI						
megastigma-7-en-3,9-diolo	49,7	42,8	62,3	51,3	39,6	48,7
3-oxo-α-ionolo	200,8	251,0	180,1	295,0	245,3	221,1
3-idrossi-7,8-diidro-β-ionolo	22,5	21,5	20,3	24,6	23,5	24,9
3-idrossi-β-ionone	35,0	36,8	28,8	75,9	58,9	62,4
3-idrossi-7,8-deidro-β-ionolo	36,2	31,0	42,5	39,0	37,2	37,2
vomifoliolo	129,5	62,0	53,2	78,4	67,9	58,3
TOTALE	**473,6**	**445,1**	**387,3**	**564,2**	**472,4**	**452,6**
LATTONI						
γ-butirolattone	1831,3	1610,7	1694,2	1733,1	1757,2	1607,4
γ-nonalattone	21,3	26,3	24,6	21,4	21,4	30,5
pantolattone	21,0	15,4	19,0	14,6	17,5	16,3
δ-decalattone	11,3	15,1	13,4	10,1	8,0	13,9
sherry lattone 1	226,1	132,6	196,2	147,4	156,8	197,1
sherry lattone 2	210,5	192,6	197,6	172,9	176,3	192,2
TOTALE	**2321,5**	**1992,6**	**2145,0**	**2099,5**	**2137,1**	**2057,5**
ACIDI						
acido isovalerianico	436,0	370,9	376,2	310,8	313,6	292,0
acido esanoico	695,4	860,2	940,1	912,8	847,8	891,7
acido ottanoico	590,7	767,5	820,2	725,3	852,6	783,0
acido decanoico	105,8	115,2	135,8	142,3	192,9	94,1
acido esadecanoico	140,3	168,4	98,3	125,0	95,2	84,5
TOTALE	**1968,1**	**2282,3**	**2370,6**	**2216,2**	**2302,0**	**2145,4**
ALTRI COMPOSTI						
acetoino	138,5	161,9	10,0	5,1	12,9	39,0
3-etossi-1-propanolo	224,9	174,2	207,4	129,3	121,9	121,2
3-OH-etil butirrato	153,6	172,4	159,9	177,6	149,8	146,7
2-OH-etil esanoato	0,0	119,4	133,3	139,9	159,1	116,1
4-OH-etil butirrato	7913,8	7930,0	7010,3	7280,7	8306,9	7910,3
3-metiltio-1-propanolo	74,1	65,0	26,7	127,7	128,1	91,9
N-3-metilbutil acetamide	605,2	608,9	722,0	479,7	363,8	484,4
N-2-feniletilacetamide	116,1	236,9	277,7	196,0	135,8	192,9

Il 2-feniletanolo raggiunge concentrazioni molto elevate in tutti i vini analizzati ma per i vini del gruppo BR, vinificati con bucce e graspi, i valori risultano leggermente più elevati.
Tuttavia non si osservano marcate differenze tra i due gruppi di campioni B e BR, nelle diverse classi di composti identificati.
Nella frazione legata dei vini (Tab. 6) sono ancora presenti in concentrazione elevata molti terpeni e norisoprenoidi, che rappresentano un potenziale aromatico residuo importante per i vini durante l'invecchiamento. Infatti gran parte di questi composti si idrolizzeranno spontaneamente e passeranno nella frazione libera. Molto interessante la concentrazione in linalolo e geraniolo tra gli alcoli monoidrossilati.

Tabella 6 : Composti presenti nella frazione legata dei vini (conc. in μg/L)

VINO	B1	B2	B3	BR1	BR2	BR3
ALCOLI E BENZENOIDI						
esanolo	68.0	34.7	41.3	60.9	51.6	45.6
alcool benzilico	45.1	38.3	45.0	34.7	54.1	41.4
2-fenil etanolo	56.7	75.4	53.4	66.3	87.5	70.6
4-vinil guaiacolo	263.0	205.8	197.0	215.3	271.3	235.2
4-vinil fenolo	12.8	10.3	8.7	14.3	17.2	16.2
vanillina	20.3	23.3	18.7	18.2	20.2	16.2
diidroconiferilalcool	102.4	115.4	85.0	139.3	111.4	104.6
TOTALE	**568.3**	**503.2**	**723.9**	**549.1**	**613.2**	**529.8**
TERPENI						
trans-furanlinalolo ossido	46.7	58.3	41.2	47.9	53.4	40.8
cis-furanlinalolossido	122.9	137.3	103.7	118.6	138.5	105.9
linalolo	242.9	224.4	265.0	281.9	326.2	225.9
α-terpineolo	48.0	45.3	43.6	34.9	48.1	36.8
trans-piranlinalolossido	14.4	10.1	14.1	10.8	17.1	13.1
cis-piranlinalolossido	23.3	17.4	22.1	16.6	28.0	21.4
nerolo	17.6	28.8	10.8	9.4	16.6	12.7
geraniolo	66.1	47.0	49.7	40.5	59.3	49.9
2,6-dimetil-3,7-ottadien-2,6-diolo	31.1	23.5	32.1	31.3	26.3	20.1
2,6-dimetil-7-otten-2,6-diolo	19.2	14.1	19.8	22.0	21.7	16.6
OH-citronellolo	16.2	17.4	16.7	15.3	29.5	22.6
8-OH-didrolinalolo	5.9	10.7	5.2	6.9	4.9	3.7
trans-8-OH-linalolo	116.4	92.4	118.1	131.1	133.7	141.7
OH-geraniolo	30.8	31.8	38.8	53.9	69.8	59.0
cis-8-OH-linalolo	72.4	56.2	72.2	21.2	80.8	83.9
p-ment-1-ene-7,8-diolo	29.3	30.7	31.7	14.3	48.0	44.0
TOTALE	**903.1**	**845.3**	**884.7**	**856.5**	**1128.0**	**918.3**
NORISOPRENOIDI						
3,4-diidro-3-oxoactinidolo I	5.3	6.3	6.2	5.3	7.9	7.8
3,4-diidro-3-oxoactinidolo II	22.4	17.6	20.9	13.2	32.6	41.9
3,4-diidro-3-oxoactinidolo III	20.4	16.0	19.7	21.0	31.2	30.1
3-idrossi-β-damascone	65.6	68.3	67.4	61.9	88.8	80.9
megastigma-7-en-3,9-diolo	55.3	64.6	62.9	66.2	74.8	72.1
3-oxo-α-ionolo	386.6	377.1	334.7	324.2	551.8	525.0
3-idrossi-7,8-diidro-β-ionolo	50.2	52.2	39.6	40.2	89.7	86.7
3-idrossi-β-ionone	16.3	17.7	10.7	30.6	62.2	61.8

blumenol C	88.5	91.8	81.0	107.6	151.5	160.5
3-idrossi-7,8-deidro-β-ionolo	56.1	50.0	49.2	59.0	67.4	62.7
vomifoliolo + grassopperchetone	110.1	126.0	110.8	202.6	248.0	228.6
TOTALE	**953.0**	**956.6**	**850.8**	**1038.5**	**1569.3**	**1522.8**

CONCLUSIONI

La vinificazione tradizionale in anfore rappresenta la massima espressione della vinificazione naturale, senza utilizzo alcuno di coadiuvanti normalmente utilizzati nei moderni sistemi di vinificazione. Tutti i vini sono stabili e mantengono le loro caratteristiche peculiari anche dopo l'affinamento senza la necessità di interventi ulteriori. Tuttavia, va rimarcato il fatto che sotto l'aspetto organolettico questi vini bianchi sono molto particolari e si presentano con forti colorazioni ambrate, mentre al gusto sono più vicini ai vini rossi piuttosto che a quelli bianchi.

Se, invece, ci soffermiamo sulle differenze riscontrate tra i due gruppi di vini esaminati, notiamo che le diversità analitiche più significative si riscontrano a carico dei parametri legati all'astringenza. Tale evidenza viene confermata dall'esame organolettico dei vini che indica come quelli del gruppo BR siano notevolmente più astringenti di quelli del gruppo B. Questi ultimi, vinificati con le sole bucce, sono meno aggressivi e meno tannici e risultano più accettabili ad un consumatore poco abituato a vini bianchi così forti e diversi da quelli normalmente presenti sul mercato.

Dal punto di vista aromatico, le differenze tra i due gruppi di vini sono meno evidenti e si osserva sempre una scarsa incidenza degli aromi fruttati sul bouquet dei vini che in affinamento tendono a perdere buona parte della freschezza tipica dei vini bianchi. Al tempo stesso, una forte idrolisi dei composti varietali presenti nelle uve di origine, arrichisce la frazione libera di numerosi terpeni e norisoprenoidi che rendono unica e inimitabile la complessità aromatica di questi vini. E' importante sottolineare il fatto che le uve utilizzate per le prove possedevano un ottimo potenziale aromatico complessivo che ha permesso ai vini di mantenere, in affinamento, un potenziale aromatico residuo ancora elevato. Non si sono apprezzate differenze significative nell'utilizzo di lieviti selezionati rispetto a quelli indigeni.

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CARATTERISTICHE SENSORIALI ED AROMATICHE DI VINI ELABORATI TRAMITE MACERAZIONI LUNGHE

R. Ferrarini[1], G. Zanella[1], C. Carbognin[1], G.Versini[2], B. Fedrizzi[2]

[1] Dipartimento di Scienze, Tecnologie e Mercati della Vite e del Vino – Università di Verona
Via della Pieve 70, 37029 S. Floriano (VR), Italy
roberto.ferrarini@univr.it
[2] Unione Italiana Vini Soc. Coop. , Verona, Italy.

RIASSUNTO
Molti dei vini tipici della Georgia sono prodotti con metodiche di macerazione pellicolare protratta per tempi assai lunghi e spesso condotta con l'utilizzo di vasi vinari tradizionali, quali anfore di terracotta, e in assenza di anidride solforosa e altri coadiuvanti.
I vini ottenuti con questa tecnica sono fortemente caratterizzati, diversificati e riconoscibili. Soprattutto nel caso dei vini bianchi i prodotti risultano arricchiti in composti fenolici (catechine, proantocianidine, acidi cinnamici) che appaiono caratterizzanti, anche sotto il profilo della riconoscibilità varietale, e di possibile valenza salutistica. Sempre l'elevata presenza di composti fenolici a carattere antiossidante, preconizza l'uso di questa procedura per produzioni vitivinicole sostenibili anche in regioni diverse da quella di origine.
Queste tecnologie sono oggi, in Italia, particolarmente apprezzate per il loro richiamo emozionale, per la riscoperta delle tradizioni enologiche del passato, ma anche come reale possibilità tecnologica di diversificazione e caratterizzazione della produzione di vini stabili e longevi.

ABSTRACT
Many typical Georgian wines are produced using long time skin contact, often conducted with the use of traditional tanks, as jars of clay, and in absence of sulphur dioxide and other adjuvants.
The wines made with this technique are strongly characterized, diverse and recognizable.
Especially in case of white wines, they are enriched in phenolic compounds (catechins, proanthocyanidins, cinnamic acids) which are characterizing for the variety and probably of healty importance.
The high presence of phenolic antioxidants, recommends the use of this procedure in the sustainable wines production also in regions that are different from the area where the technique has been firstly developed.
These technologies are nowadays, in Italy, well valued for their emotional appeal, for the re-discovery of the winemaking traditions of the past, the technological diversification of production and the characterization of stable, long-living wines.

INTRODUZIONE
La macerazione pellicolare di uve bianche condotta per tempi lunghi sta riscuotendo un certo interesse anche fra i produttori italiani. La tecnica, che pone le sue origini nelle tradizioni enologiche Georgiane, caratterizzate da macerazioni con le parti solide dell'uva per tempi prolungati e dall'utilizzo di anfore in terracotta senza l'impiego di coadiuvanti.
Precedenti studi, ("Studio delle caratteristiche dei vini bianchi elaborati con tecniche arcaiche di macerazione" R. Ferrarini et al., OIV 2009),

hanno dimostrato la grande influenza della tecnica sulle caratteristiche chimico-fisiche, in particolare per quanto concerne il contenuto in catechine, proantocianidine ed acidi cinnamici, i quali risultano più presenti nei vini macerati rispetto a vini testimoni vinificati tradizionalmente.
Anche dal punto di vista sensoriale si sono riscontrati effetti positivi, diversificando il prodotto sia per quanto riguarda aspetti olfattivi che gustativi, e conferendo maggior longevità al vino.

Sulla base di queste premesse si è ritenuto interessante approfondire le conoscenze sui vini elaborati mediante questa tecnica, in particolare per quanto concerne gli aspetti aromatici.
E' stata inoltre condotta un'analisi sensoriale allo scopo di verificare, dopo diversi anni di sperimentazione, l'effettiva diversificazione del vino prodotto con queste tecniche, nonché di indagare sulle caratteristiche sensoriali acquisite nel corso dell'affinamento.

MATERIALI E METODI

I vini, derivanti da macerazioni lunghe effettuate su cv Garganega, presi in esame per questa indagine sono stati prodotti nelle annate 2008 e 2009 nell'area del Soave DOC, e 2007, 2008 e 2009 presso la DOC Custoza.
Tutte le uve utilizzate per queste esperienze erano caratterizzate da un ottimo stato sanitario e da una buona maturazione tecnologica.
La vinificazione prevedeva una macerazione del pigiato privo di raspi per quattro mesi in assenza di anidride solforosa (Ferrarini *et al.*, 2009).
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 (Versini *et al.*, 1999)
Metanolo, acetaldeide e acetato di etile sono stati determinati sul distillato mediante iniezione diretta in GC-FID.
Le analisi sensoriali sono state condotte attraverso test triangolare e determinazione del profilo sensoriale attraverso un panel di 10 giudici addestrati.
Infine, per il vino ottenuto da macerazione lunga con Garganega di Custoza prodotto nel 2007, l'analisi sensoriale è stata abbinata ad una analisi di piacevolezza. Inoltre, attraverso un differente panel di assaggiatori addestrati, composto di 13 soggetti, è stato condotto uno studio emozionale (per la scheda emozionale utilizzata si veda Ferrarini et al. 2009 e 2010) composto di tre 3 fasi:

- assaggio *blind*, valutazione emozionale con assaggio senza alcuna informazione sul vino in esame;
- valutazione di aspettativa (*expectation*), aspettativa emozionale dopo aver ricevuto informazioni sul tipo di vino e sul processo di vinificazione e aver preso visione della bottiglia e dell'etichetta;
- assaggio *lebelled*, valutazione emozionale con degustazione del prodotto e indicazione del vino assaggiato.

Il significato emozionale attribuito al vino e l'attribuzione di piacevolezza sono stati poi approfonditi mediante focus group.

RISULTATI E DISCUSSIONE

Analisi aromatiche

Per quanto concerne i vini elaborati da macerazione per 4 mesi di uve Garganega, ottenute nella zona di Soave e Custoza, si evidenzia in maniera marcata il notevole decremento, fra gli analiti in forma libera dosati, dei composti a nota di frutta bianca, ossia gli acetati di alcoli superiori (da ca. 4700 a 330 μg/L nel confronto 2009; da ca. 1660 a ca. 400 μg/L nel 2008) e gli esteri etilici di acidi grassi dal C_4 al C_{10} (da ca. 3600 a 970 μg/L nel 2009, da ca. 2500 a ca. 950 μg/L nel 2008), risultando più elevati i loro livelli nei vini testimone, vinificati mediante vinificazione in bianco tradizionale. Quali notevoli supporter del sentore di frutta bianca vi sono poi gli esteri etilici dei 2- e 3-metilbutirrati che si producono nel tempo per reazione chimica, come il 2-feniletilacetato di etile.
Le differenze del contenuto di composti di origine fermentativa risultano appiattite nei vini del 2007, a causa di una diminuzione di questi composti nel corso del tempo.
Accanto al rilevante incremento nei vini macerati di alcuni ariletanoli, in particolare: alcool omovanillico e il tirosolo, nonché di acetovanillone e di altri analoghi arilchetoni e della vanillina con la siringaldeide, osserviamo quello dei monoterpeni 8-idrossilinalolo cis e trans, del 3-cheto-α-ionolo fra i norisoprenoidi, del furaneolo e del norfuraneolo fra i derivati furanoidi, e di altri

composti minoritari.
Nei particolari, tabella 1, permette di avanzare le seguenti considerazioni:

- i tenori di lattato di etile, lattato di isoamile e di dietilmalato indicano che le prove del 2009 e i testimoni delle annate 2008 e 2007 hanno fatto solo una parziale malolattica rispetto alle altre. Per il dietilsuccinato si evidenzia una variazione fra le annate in funzione sia della diversa produzione di acido succinico che di un diverso decorso della malolattica, pur trattandosi in parte di una sintesi da reazione chimica, quindi da doversi ritenere più avanzata nei campioni del 2008 e del 2007.
- Nel caso di altri idrossiesteri, il 3-idrossibutirrato di etile, di ca. il 30% maggiore nelle prove macerate rispetto al test solo nel 2008, ha comunque un tenore prossimo fra le tre annate e probabilmente caratterizzante il vino Garganega. Il 4-idrossibutirrato di etile è maggiore nelle prove macerate rispetto alla tradizionale, di ca. il doppio nelle prove 2009 e di ca. 5 volte in quelle 2008, risultando i tenori massimi abbastanza simili, mentre il γ-butirrolattone, tendente all'equilibrio con il 4-idrossibutirrato, di etile è ca. il triplo nelle macerate 2008 rispetto a quella corrispondente 2009. Si può prospettare una formazione biologica complessiva di intensità diversa per le tre annate, così come evidenzia lo spostamento dell'equilibrio verso il γ-butirrolattone nelle prove del 2008 e 2007, che risulta più basso nei vini testimoni più invecchiati e con valori maggiori nei macerati. Per il 2-idrossiisovalerato di etile e il 2-idrossi-4-metilpentanoato di etile, a tenori sensibilmente minori degli altri esteri commentati, le variazioni di livello paiono rispecchiare quelle del lattato di etile.
- Alcoli fermentativi:
    - alcool 2-feniletilico: tutte le prove del 2008 e 2007 hanno tenori almeno doppi (ca. 45 mg/L) rispetto al test da vinificazione tradizionale (ca. 18 mg/L). Nel 2009 i contenuti sono simili fra vini macerati e testimoni, con ca. 35 mg/L. I livelli di interesse sensoriale sono a ca. 30-40 mg/L nei vini bianchi;
    - tirosolo ed il triptofolo, derivati dalla riduzione di corrispondenti amminoacidi (di cui il primo di valenza salutistica quale antiossidante ed il secondo favorente la sonnolenza), mostrano un comportamento diverso: il tirosolo aumenta nettamente nelle prove macerate, mentre il triptofolo ha livelli elevati nelle vinificazioni in bianco, ma anche tendenzialmente in alcune delle macerate.
    - nel caso del metionolo il suo contenuto, nei vini macerati prodotti nella zona del Soave, raddoppia nelle prove 2009 mentre quadruplica in quelle del 2008. Nei vini prodotti nella regione di Custoza il contenuto di questo composto si rivela di molto superiore ai precedenti. Questo comportamento era atteso dalla letteratura poiché il tenore è in antitesi alla limpidezza dei mosti. I valori maggiori sono prossimi o pari a ca. metà quelli di soglia olfattiva (sentore da vegetale cotto). Analoga tendenza attesa in funzione opposta all'illimpidimento dei mosti riguarda la N (3-metilbutil) acetammide e la N (metionil) acetammide.
- Piroglutammato di etile, responsabile della nota da brodo/lievito, proviene da esterificazione di acido di derivazione amminoacidica (acido glutammico e glutammina) che è generalmente favorita dal contatto con i lieviti. I valori trovati parrebbero non in questa direzione risultando un livello decisamente minore nelle prove macerate. Questo potrebbe significare che la presenza delle parti solide, a contatto con la feccia di macerazione, non implementi la produzione di questo composto.
- Alcoli a C6:
    - l'esanolo è marcatamente minore nelle prove non macerate, risultando tuttavia i valori delle macerate non elevati, approssimabili ancora a quelli di vinificazioni in bianco.
    - Anche il cis 3-esenolo tende ad incrementare nelle prove macerate. In tutti casi il trans 3-esenolo supera il cis 3-esenolo, fatto che conferma una peculiarità della Garganega già osservata da Moret et al. (1984).

- Lattoni:
  - il γ-nonalattone, responsabile della nota di cocco, incrementa di ca. 5 volte nel raffronto fra vinificazione tradizionale e con macerazione lunga, raggiungendo anche i 20 μg/L;
  - il γ-decalattone è invece in tracce ≤ 1 μg/L;
  - per quanto riguarda il 4-carboetossi-γ-butirrolattone in abbinamento al dietil 2-idrossiglutarato, se ne ha un maggiore livello nelle prove non macerate: vi è un maggior tenore di 4-carboetossi-γ-butirrolattone rispetto alla forma aperta, come atteso da letteratura, e con un incremento relativo maggiore di quest'ultima nell'invecchiamento, come osservato nei prodotti 2008 e 2007.
- Monoterpeni:
  - il livello di linalolo varia da ca. 4 a 8 μg/L con tenori maggiori nelle prove del 2008 macerate, probabilmente anche in ragione del pH maggiore;
  - l'α-terpineolo è maggiore, come atteso per sua derivazione dal linalolo, nei campioni del 2008 e 2007, variando da ca. 3 a 9.5 μg/L;
  - il citronellolo (anche fino a 12 μg/L), nerolo e geraniolo (insieme, anche fino a 11 μg/L) paiono maggiori nelle prove macerate per un maggior tenore nelle bucce, così come l'ossido di linalolo piranico trans, il maggiore fra gli ossidi.
  - L'Ho-diendiolo è ca. triplo nelle prove del 2008 (fino a ca. 33 μg/L) rispetto ai livelli di ca. un quarto nel 2009 (probabile effetto annata).
  - Infine l'interessante situazione del trans e cis 8-idrossilinalolo, quest'ultimo molto maggiore nei vini da macerazione lunga rispetto ai testimoni con incrementi da 5 a 15 volte fino a ca. 160 μg/L, soprattutto nel 2008 [livelli maggiori di isomero cis si riscontrano anche fra gli agliconi da forme legate nel confronto fra i due tipi di vinificazione].
- Benzenoidi varietali, trattasi dell'alcool benzilico, dei fenoli, quali fenolo, o- e p-cresolo, guaiacolo, eugenolo; del metil vanillato, metil salicilato, aceto-, proprio- e butirrovanillone, aceto-, proprio- e butirrosiringone, alcool omovanillico, vanillina, siringaldeide.
  - Per l'alcool benzilico abbiamo un forte incremento con la macerazione anche fino a 1100 μg/L nel 2008 (620 μg/L nel 2009) a fronte di meno di 100 μg/L nei non macerati.
  - Fra i fenoli aventi spesso apporto sensoriale anche a tenori inferiori a 10 μg/L, c'è in generale un incremento nel passaggio da vinificazione tradizionale e i vini elaborati tramite macerazioni lunghe, da ca. 6 a ca. 20 μg/L.
  - I vinifenoli (4-vinilfenolo e 4-vinilguaiacolo), attribuiti al metabolismo degli acidi idrossicinnamici da parte dei lieviti, hanno livelli da ca. 20 a 70 μg/L, ma non necessariamente in relazione al tipo di macerazione.
  - Gli etilfenoli (4-etilfenolo e 4-etilguaiacolo) non sono presenti in quanto derivati da Brettanomyces, che qui non hanno operato.
  - Fra i vanillil e siringilderivati, abbiamo una prevalenza dei primi con il tenore più elevato per l'alcool omovanillico anche fino a 1600 μg/L nelle prove macerate (ca. 100 μg/L nei test), quindi per l'acetovanillone con analoga tendenza in relazione alla tecnologia fino a ca. 350 μg/L (propio- e butirrovanilloni non superano i 20 μg/L), la vanillina (non oltre i 15 μg/L), mentre la siringaldeide è sui 50 μg/L nei macerati del 2008 e 2007, con un contenuto più elevato per il 2009 della zona di Custoza. I proprio- e i butirrosiringoni risultano a meno di 5 μg/L.
  - Anche l'alcool diidroconiferilico tende ad aumentare nelle prove macerate, così come pare per l'alcool coniferilico.
  - Merita attenzione la situazione del salicilato di metile a nota di miele di castagno sui 50 ppb: questo valore è prossimo a quelli misurati in due prove macerate del 2008;
- Norisoprenoidi: consideriamo fra questi β-damascenone, TDN, VTP, actinidoli e 3-cheto-α-

ionolo.

  - I primi quattro non paiono dipendere dalla macerazione, ma forse dall'annata;
  - TDN e VTP sono a valori alquanto bassi, non oltre 1 μg/L, per i vini delle annate 2009 e 2008, mentre si può osservare un loro incremento durante l'invecchiamento per l'annata 2007;
  - gli actinidoli sono, come atteso, più alti nelle prove del 2008 e 2007 per la loro formazione per via chimica, ma non oltre i 33 μg/L.
  - E' evidente l'aumento deciso del 3-cheto-α-ionolo (dalla nota di tabacco/speziato) nelle prove macerate raggiungendo anche ca. 110 μg/L;
- Furaneolo, omofuraneolo e norfuraneolo paiono aumentare nelle macerazioni lunghe del 2007 e 2008 raggiungendo valori di possibile contributo sensoriale per il furaneolo. Questo composto aumenta anche nel vino da macerazione del 2009, mentre vanno con tendenza opposta i vini testimoni.
- E' stato quantificato anche il p-cumarato di etile con tenori maggiori, comprese tra ca. 120 e 180 μg/L, nei vini da vinificazione in bianco e con sensibile calo in quelli da macerazione lunga (da ca. 60 a 100 μg/L): può prospettarsi una tendenza all'esterificazione simile a quella che avviene per gli esteri etilici di acidi grassi ad opera dei lieviti.

Analisi sensoriali ed emozionali dei vini ottenuti con macerazione lunga e dei relavi testimoni ottenuti con vinificazione tradizionale (DOC di Soave e DOC di Custoza)

I risultati del test triangolare evidenziano che tutti i prodotti a lunga macerazione (sia della DOC di Soave che di Custoza) sono sensorialmente distinguibili dai relativi testimoni.

Per quanto riguarda i profili sensoriali (Figura 1 e 2) va evidenziato per la del 2008 della DOC di Soave e per tutte e tre le prove della DOC di Custoza (annate 2007, 2008, 2009) l'apporto importante conferito dalla tecnica a lunga macerazione alle caratteristiche di balsamico, mandorla, speziato (in linea con le analisi aromatiche) e astringente.

Relativamente meno importanti per quanto riguarda il sentore di mandorla e di speziato le differenze tra prova a macerazione lunga del 2009 della DOC di Soave e il relativo testimone; invece, non vi è alcuna differenza per quanto riguarda il sentore di balsamico.

Dal punto di vista gustativo si può notare l'emergere di una nota amara per le prove a macerazione lunga della DOC di Soave (Figura 1) nel confronto con i relativi testimoni, differenza comunque più contenuta nell'annata 2009. Tuttavia, nelle prove a lunga macerazione della DOC di Custoza, l'amaro presenta valori medi molto bassi e le differenze con i relativi testimoni sono molto lievi o nulle.

Rilevante inoltre la persistenza che caratterizza generelmente i vini a macerazione lunga rispetto ai testimoni, eccenzion fatta per la prova 2009 della DOC di Soave, la quale comunque presenta un livello di persistenza non marcatamente inferiore al testimone.

Si evidenzia tuttavia una penalizzazione nei sentori floreali e di frutta bianca per i vini a lunga macerazione prodotti nella DOC di Custoza.

Per quanto riguarda lo studio di piacevolezza ed emozionale va evidenziato che generalmente in sede di studio sensoriale, gli assaggiatori spesso attribuivano una certa originalità al prodotto ottenuto con macerazione lunga. Anche per tale motivo, abbiamo realizzato uno studio approfondito sull'aspetto della percezione del prodotto del vino a lunga macerazione del 2007 di Custoza in raffronto con il relativo testimone.

Per quanto riguarda il giudizio di piacevolezza percepita, possiamo dire che all'assaggio in sede sensoriale (pertanto da considerarsi come fase *blind*) il vino ottenuto da macerazione lunga risulta penalizzato rispetto al testimone. Infatti, mentre il testimone riporta una media di giudizio di piacevolezza (sempre range 1-100, come per la sensoriale) di 46.22, il macerato presenta media 23.67. Tuttavia è interessante evidenziare che nella fase *expectation* dello studio emozionale il macerato è stato definito maggiormente "curioso", "interssante" e "passionale" rispetto al testimone. Dal focus group è emerso che a connotare l'alta aspettativa e l'attrazione verso il vino macerato è stata proprio l'originalità attribuita al particolare processo di vinificazione. Il focus group ha messo, infatti, in evidenza che la macerazione lunga viene associata al concetto di una "affascinante storia del vino e delle scelte del produttore", intesa come processo di lavorazione delle uve, evoluzione, tempo di invecchiamento.

Dunque l'aspetto caratteristico e distintivo della vinificazione con macerazione lunga ha determinato alti livelli di aspettativa e e richiamo emozionale.

Figure 1:DOC di Soave anno 2008 e 2009. Profili sensoriali dei vini ottenuti da vinificazione tradizionale (testimoni) e delle prove a macerazione lunga

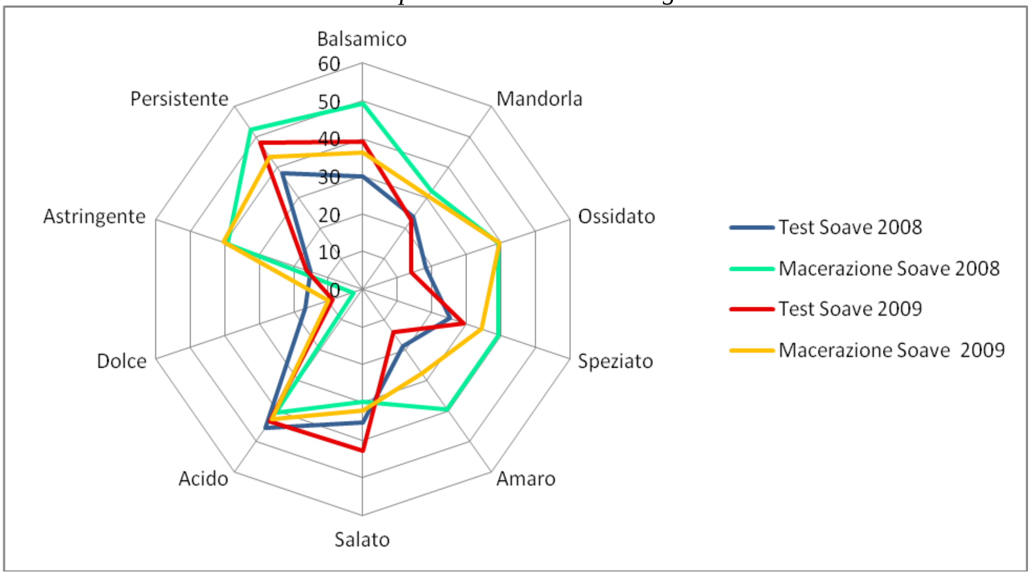

Figure 2:DOC di Custoza anni 2007, 2008 e 2009. Profili sensoriali dei vini ottenuti da vinificazione tradizionale (testimoni) e delle prove a macerazione lunga

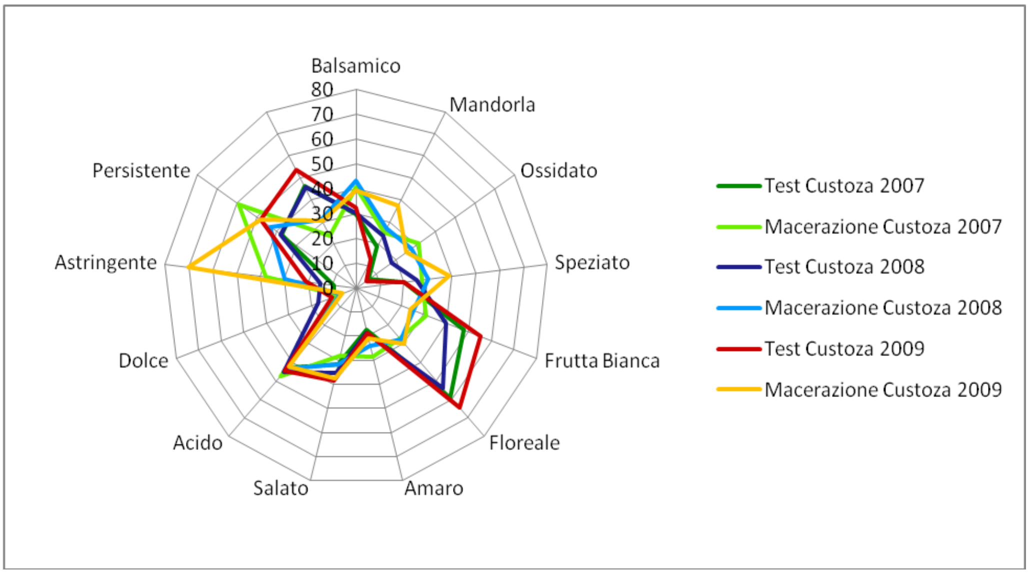

Tabella 1: confronto della composizione aromatica di vini testimone, vinificati attraverso vinificazione tradizionale, e vini vinificati con macerazione lunga di 4 mesi.

	VINI TESTIMONE			MACERAZIONI LUNGHE				
	Soave 2009	Soave 2008	Soave 2007	Soave 2009	Custoza 2009	Soave 2008	Custoza 2008	Custoza 2007
Esil Acetato	67,5	51,4	11,8	5,7	1,8	15,2	6,2	15,5
Isoamil Acetato	3.778,0	1.375,4	375,8	216,3	148,8	289,4	105,4	191,4
Beta-Feniletil Acetato	739,5	171,7	42,5	19,4	27,7	30,6	14,0	27,4
Etilfenil Acetato	2,8	4,1	4,1	6,5	8,0	3,3	9,1	13,4
Etil Butirrato (C4 Etile)	373,0	210,7	393,1	146,0	88,7	133,3	168,4	168,5
Etil Caproato (C6 Etile)	937,7	651,8	1.434,9	352,1	253,5	330,2	432,8	518,4
Etil Caprilato (C8 Etile)	1.641,7	1.103,7	1.855,5	367,6	243,7	444,8	470,8	555,5
Etil Caprinato (C10 Etile)	616,6	494,2	449,3	104,8	77,1	98,8	73,5	219,9
9-Decenoato di Etile	13,0	29,7	8,6	1,8	1,8	2,2	4,0	6,7
3-Idrossi Butirrato di Etile	264,4	259,5	235,3	217,4	247,8	359,7	395,1	307,7
4-Idrossi Butirrato di Etile	2.378,7	564,7	214,9	4.543,1	3.772,5	3.624,7	614,4	833,4
Etil-2-Idrossi-Iso-Valerico	2,4	4,9	8,1	2,3	8,1	23,3	22,0	21,9
Etil-2-Idrossi-4-Metil-Pentanoato	34,5	50,9	61,1	29,3	67,8	142,9	147,2	89,7
DiEtil Succinato	506,3	2.655,6	4.363,1	711,8	1.476,0	8.174,4	4.464,5	15.451,8
Etil Lattato	3.047,7	4.990,1	8.758,2	4.662,9	16.823,4	19.295,3	31.855,5	38.803,4
Isoamil Lattato	10,1	20,2	15,8	25,4	356,5	277,6	376,0	337,8
Etil Succinato (+ Isoamile)	8,3	18,5	15,6	12,7	24,4	143,6	43,1	110,0
Dietil Malato	355,6	4.944,2	5.892,5	234,2	1.137,5	323,6	1.155,7	903,4
Metil Vanillato	2,6	3,9	3,8	4,5	4,0	5,2	5,9	10,3
Etil Vanilato	4,2	1,8	2,1	5,6	5,7	7,9	6,7	15,0
Cinammato di Etile	0,5	0,6	0,0	1,2	0,0	0,6	0,0	0,0
Salicilato di Etile	0,0	0,3	0,0	0,7	0,3	1,8	0,6	0,0
Salicilato di Metile	1,4	6,4	0,0	9,3	4,2	41,0	8,6	9,8
Esanolo	207,0	355,8	424,0	1.167,3	1.032,3	848,0	1.551,6	1.720,6
Trans-3-Esenolo	21,7	39,2	26,4	52,8	31,1	21,3	32,5	59,6
Cis-3-Esenolo	4,6	4,7	9,7	11,4	14,7	9,1	16,8	26,5
2-Esen-1-olo	1,2	1,6	1,4	4,0	6,5	2,5	3,7	5,4
Alcool Benzilico	48,6	90,4	44,3	619,4	340,3	1.108,3	539,8	545,9
Alcool Beta-Fenil Etilico	36.846	17.980	15.172	35.016	73.895	47.061	41.633	54.646
3-Metil-Tio-Propanolo (Metionolo)	653,6	388,1	175,3	1.388,5	3.684,5	1.897,2	1.655,8	2.862,2
Furfuril Alcool	8,3	13,6	12,1	14,1	590,5	53,0	19,0	19,7
Alcool Omovanillico	67,2	119,7	86,3	879,1	1.315,2	1.585,3	1.380,5	1.606,5
1-Octen-3-olo	0,0	0,0	0,0	4,7	3,5	4,5	6,5	5,6
Linalolo	4,2	4,2	1,9	3,9	6,6	8,6	4,6	3,1
HO-Trienolo	0,0	1,5	0,7	0,0	0,0	2,2	2,5	1,7
Alfa-Terpineolo	1,2	3,3	3,9	1,8	2,7	4,3	6,2	9,5
Citronellolo	2,4	2,4	0,0	8,2	5,3	12,3	2,3	1,4
Nerolo	0,0	0,0	0,0	3,2	2,2	3,8	1,1	2,4
Geraniolo	3,2	0,7	0,0	4,8	4,7	6,9	1,6	2,1
Trans-Linalolo Ossido (A)(Fur)	0,0	0,0	0,0	0,0	2,4	0,0	2,3	5,6

	VINI TESTIMONE			MACERAZIONI LUNGHE				
	Soave 2009	Soave 2008	Soave 2007	Soave 2009	Custoza 2009	Soave 2008	Custoza 2008	Custoza 2007
Cis-Linalolo Ossido (B)(Fur)	0,0	0,0	0,0	0,0	1,7	0,0	1,3	2,8
Trans-Linalolo Ossido (C)(Pir)	1,6	1,9	0,0	7,4	4,4	8,5	7,6	8,5
Cis-Linalolo Ossido (D) (Pir)	0,0	0,0	0,0	0,0	0,0	0,0	1,8	1,2
HO-Diendiolo 1	8,4	18,9	4,9	8,6	6,2	33,3	19,3	10,1
HO-Diendiolo 2	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
EnDiolo	3,4	7,7	9,7	4,9	15,1	5,5	14,6	30,6
8-IdrossiLinalolo trans	13,1	16,9	4,1	16,6	7,0	20,0	10,2	17,2
8-IdrossiLinalolo cis	10,0	12,7	9,4	50,5	32,7	140,7	59,1	44,7
4-Terpineolo	1,0	0,0	0,0	1,0	0,0	0,4	0,0	0,0
Para - Cimene	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
Terpinolene	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
4-Vinil-Guaiacolo	69,6	34,4	55,0	19,3	32,7	39,3	37,8	51,3
4 Vinil Fenolo	35,5	7,1	4,4	4,2	8,6	33,2	5,5	5,6
Eugenolo	2,5	3,9	2,4	6,3	8,2	9,0	4,9	3,7
Guaiacolo	0,3	0,3	2,2	1,2	1,7	1,1	1,0	1,3
Orto-Cresolo	0,6	0,7	0,7	3,9	2,0	4,4	2,9	4,5
Para- Cresolo	0,7	0,4	0,8	1,8	1,6	1,9	2,0	2,9
Vanillina	3,2	4,7	7,0	9,3	93,3	16,8	8,1	18,9
Aceto Vanillone	201,9	192,5	178,8	319,5	259,4	344,6	193,0	357,7
Fenolo	1,1	1,5	1,7	5,2	3,3	4,1	4,0	6,2
Fenil Acetaldeide	9,6	13,6	17,9	10,2	28,7	11,1	32,7	20,9
Benzaldeide	3,9	4,6	4,0	12,9	4,9	17,4	92,4	19,5
Gamma Nonalattone	3,2	3,3	2,6	19,3	11,0	15,5	9,8	13,5
Gamma-Deca Lattone	0,0	0,0	0,0	0,0	0,9	0,0	1,0	1,0
g-ButirroLattone	759,3	818,1	683,9	1.001,4	1.525,2	3.303,5	1.542,6	2.220,1
Acido Butirrico	541,9	573,0	803,2	374,1	283,6	447,4	522,1	452,8
Ac Iso-Valerianico	376,5	232,2	178,8	351,2	380,0	425,4	339,8	414,9
Acido Caproico (C 6)	2.507,1	2.057,7	3.431,2	1.192,8	947,5	1.399,0	1.723,5	1.522,5
Acido Caprilico (C 8)	5.657,3	4.058,5	6.794,8	1.421,5	1.172,8	2.031,2	2.259,7	2.233,8
Acido Caprico (C 10)	2.279,7	1.732,7	2.432,7	225,4	253,4	329,4	337,2	1.071,2
Acido Omovanillico	22,7	12,6	21,5	40,7	114,7	62,2	61,5	46,4
N-(3 MetilButil) Acetamide	59,0	65,8	43,8	594,4	1.061,6	273,3	3.974,4	2.227,5
Beta Damascenone	7,8	4,3	2,0	4,7	2,4	5,3	1,7	1,8
Alfa-Ionone	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
Beta-Ionone	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
TDN	0,5	1,2	1,8	0,3	0,3	0,3	0,5	2,0
Etossi TDN	0,0	0,3	0,6	0,0	0,0	0,0	0,0	0,0
Vitispirano 1	0,0	1,0	1,8	0,0	0,0	0,0	1,5	5,2
Vitispirano 2	0,0	0,6	1,4	0,0	0,0	0,0	1,2	3,8
Actinidoli Etossi Derivati 1	0,0	1,0	2,1	0,0	0,0	0,0	1,4	3,1
Actinidoli Etossi Derivati 2	0,0	0,6	1,5	0,0	0,0	0,0	0,8	1,9
Actinidoli 1	1,1	5,6	10,4	1,2	3,3	2,1	10,1	17,0
Actinidoli 2	2,5	10,8	19,5	2,9	7,4	5,0	19,7	33,1
3-oxo-alfa-ionolo	22,6	23,7	32,5	114,1	67,7	85,3	69,2	94,8

	VINI TESTIMONE			MACERAZIONI LUNGHE				
	Soave 2009	Soave 2008	Soave 2007	Soave 2009	Custoza 2009	Soave 2008	Custoza 2008	Custoza 2007
Furfurolo	6,6	15,8	47,6	3,5	46,2	1,6	61,3	98,1
Siringaldeide	2,2	1,9	9,8	4,2	146,7	40,5	8,2	13,6
5-Metil Furaldeide	0,0	1,1	2,8	0,0	23,6	0,0	1,5	3,2
Furaneolo	7,3	6,8	4,2	15,5	13,7	18,3	8,9	10,9
Omo furaneolo	9,6	12,9	4,5	4,4	13,9	15,8	10,4	13,4
Nor Furaneolo	121,4	117,3	57,6	53,8	72,5	242,0	83,2	61,4
PiroGlutammato di Etile	842,4	5.601,1	6.125,1	91,8	471,1	315,7	559,2	1.881,5
p-Hydroxycinnamic acid, ethyl ester	121,4	181,6	0,0	97,1	10,5	58,2	208,9	44,8
Propiovanillone	2,1	3,4	4,1	11,6	25,7	12,4	7,0	8,1
Butirrovanillone	1,8	4,6	26,1	7,2	99,5	17,2	28,9	107,7
Acetosiringone	1,9	2,3	5,0	5,8	9,9	7,2	6,5	8,3
PropioSiringone	1,3	1,2	1,8	4,6	10,7	4,2	3,0	4,1
ButirroSiringone	0,9	0,8	2,1	1,2	32,7	1,1	4,7	6,0
Tirosolo	149,5	80,4	394,2	331,9	1.456,4	313,4	174,1	1.151,7
Triptofolo	1.981,7	1.586,4	1,5	613,5	364,8	2.643,2	3.385,3	349,9
TPB	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
Diidro Conifeil Alcool	37,9	21,5	38,4	69,2	193,6	104,6	103,2	82,7
Coniferil Alcool	2,0	1,0	0,0	7,4	0,0	8,7	0,0	0,0
Vomifoliolo	20,9	21,4	44,3	34,9	52,4	36,6	36,0	62,4
Dietil 2 Idrossi Glutarato	77,8	567,7	702,5	49,9	420,9	389,0	944,0	1.118,3
4-CarboEtossi ButirroLattone	412,0	840,9	805,8	264,6	752,5	552,2	1.275,5	1.346,7
Etil-2-Metil-Butanoato	1,0	9,1	12,1	0,7	6,7	0,8	11,4	28,9
Etil-3-Metil-Butanoato	9,0	14,9	32,4	4,0	8,3	8,0	25,9	52,4
Metionil Acetamide	2,0	2,5	5,8	17,3	41,8	10,4	169,4	114,2

CONCLUSIONI

Complessivamente si può osservare come i vini ottenuti con macerazione lunga risultino, sotto l'aspetto aromatico, caratterizzati da composti di origine evolutiva, come 3-cheto-α-ionolo, salicilato di metile, fenoli, lattoni e actinidoli, e sensoriale, con note balsamiche, speziate e di mandorla.

Questa tecnica comporta però ridotta presenza di composti aromatici di origine fermentativa, quali acetati ed esteri etilici, importanti in quanto responsabili delle note di frutta bianca e floreale.

L'analisi aromatica evidenzia che la macerazione lunga riprofila il vino, suggerendo quindi di includere altri descrittori sensoriali generalmente non impiegati per i vini ottenuti da Garganega con vinificazione classica (in bianco), quali ad esempio sentori di miele o note di cocco.

Inoltre, l'analisi sensoriale ed emozionale mette in risalto l'apporto della macerazione lunga quale tecnica in grado di dare forte indentità al prodotto.

Interessante è quindi indagare ulteriormente la tecnica di vinificazione a macerazione lunga, per la messa a punto di prodotti di qualità che rispondano così anche da un punto di vista sensoriale e della piacevolezza complessiva all'alta aspettativa emozionale suscitata dal prodotto ottenuto con questa tecnica arcaica; soprattutto alla luce che essa può prestarsi all'elaborazione di vini stabili nel tempo senza l'uso di coadiuvanti e in particolare di anidride solforosa,

Ricerca condotta con il contributo della Fondazione Cariverona nell'ambito del progetto "Vignealte 2".

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Authors: N. Ebelashvili, L. Mujiri., L. Shubladze,
N. Chkhartishvili, T. Asashvili
Organization: Institute of Horticulture, Viticulture and Oenology
Address: # 6 Marshal Gelovani Av. 0159, Tbilisi, Georgia

nana-ebelashvili@hotmail.com

CLAIM

Theme title: New products, new technologies, new challenges
Sub-theme: Enology - New oenological practices and their application with regard to consumer demands

Type of presentation: Oral

Language: English

Title: Red Wines Concentrated with Polyphenols

Abstract

Numerous researches have evidenced that only those red wines which contain high level of polyphenols have antioxidant effect. We have elaborated and offered the processing technology of dry red wines concentrated with polyphenols. The objects of research were dry red wine samples made from Saperavi grape variety grown in four different regions of Georgia: test sample – using the technology developed by us; control sample– using the existing technology. By means of the HPLC analysis we determined the total amount of catechins, phenolcarbonic acids, flavonols, resveratrol and vanillin aldehyde. Total amount of phenols was determined by the Folin-Ciocalteau reactive. It has been established that compared with control the total amount of phenols in test samples is high by 50%, of catechins by 72-139%, of phenolcarbonic acids by 55-104%, of flavonoles by 3- 4 times; the amount of vanillin aldehyde - by 1.5-2.9 times. The results of wine tasting assessment are higher compared with the control.

La demande accrue sur les vins rouges sur le marché mondial s'explique par son activité antioxydante. Les nombreux recherches démontrent que cette activité n'est propre que dans les vins où la teneur en polyphénols est haute. Nous avons élaboré et recommandons une technologie de production des vins rouges secs concentrés en polyphénols. Pour cette étude, nous avons utilisé les échantillons des vins rouges variété Saperavi de provenance de quatre différent régions de Géorgie: essai – par procédé élaboré ; référence – par procédé usuelle.. Nous avons constaté que par rapport aux références, les échantillons expérimentaux contiennent davantage : phénols totales de l'ordre de 50% ; celui des catéchines – de 72 a 139% ; la somme des acides phénolcarboniques– de 55 a 104% ; flavonoles 3 – 4 fois; aldéhyde de vanilline – 1,5 – 2,9 fois. Les résultats d'analyse organoleptique des échantillons expérimentaux sont avérés plus hauts par rapport aux référence.

Introduction

Polyphenols of red wines have been found to have strong antioxidant effect which is evidenced by numerous studies (Pace-Asciak et al.,1995; Miyagi,.Miwa , 1997; Flesch et al.,1998; Golde et al.,1999; Knekt, 2000; Verts, Litvak, 2001; Verts, Litvak, 2003; Oganesyants et al., 2003; Corder, 2006; Shalashvili et al, 2007). The increased demand for red wines in the world market is attributed to their antioxidant effect. The data obtained by the researchers show that only the red wines which have high level of polypehol content reveal antioxidant effect. The polyphenol content of red wines depends on grape variety, grape growing site, winemaking technology (Valuiko, 1973; Kishkovskii, Skurikhin, 1976 Durmishidze, Khachidze, 1985; Mayen et al, 1995; Versari et al. 1999;; Servili et. al, 2000; Gomez-Plaza et al., 2001; Bavaresco, 2003; Papadopoulau et al. 2005; Prajitna et al.,2007; Roussis et al, 2008).

The development of winemaking technology of red wines concentrated with polyphenols is regarded to be a topical issue in winemaking.

We have elaborated and offered production technology of dry red wines concentrated with polyphenols.

The purpose of this study is to examine phenol components and organoleptic characteristics in red wines prepared by the technology offered by us and the existing technology.

Material and Methods

The objects of research were dry red wine samples made from Saperavi grape variety (vintage 2008) originating from four different wine regions of Georgia (Gurjaani, Kvareli, Telavi, Sagarejo): test – using the technology of polyphenols concentration developed by us (Ebelashvili et al. 2009); control – using the existing technology (Valuiko,1973).

In research objects the determination of catechins, phenolcarbonic acids, flavonols and vanillin aldehyde 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 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 (gallic, chlorogenic, vanillic, caffeic, p-coumaric, sinapic, t-cinnamic acids, (+) – catechin, (-) – epicatechin, and vanillin aldehyde); 360 nm (quercetin, quercetin-3-β-D-glucosid, kaempherol and ellagic acids); 310 nm (resveratrol). 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 (Servili et al. 2000; Bonerz et al. 2008).. The total amount of phenols was determined by Folin-Ciocalteau reactive (Valuiko, 1973).

Results and Discussion.

The obtained data are given in Tabs 1, 2 and illustrated in Figs. 1, 2. Wine samples prepared from Saperavi grape variety grown in the mentioned four regions show high level of (+)-catechin, (-) epicatechin and sinapic acid. In research objects the total of (-) epicatechin is

Table 1.

Polyphenol components in research objects prepared from Saperavi grape variety grown in different regions.

Polyphenol components mg/dm^3	Kvareli		Gurjaani		Telavi		Sagarejo	
	control	test	control	test	control	test	control	test
Total of polyphenols	3130	4688	3208	4870	3160	4795	3130	4701
Total of catechins	162.22	387.858	257.22	541.57	173.208	297.77	191.103	400.063
Total of phenolcarbon acids	268.127	416.636	296.492	604.789	284.671	536.034	288.901	462.404
Total of flavonoles			21.867	72.984	13.818	58.564		
(+) - catechine	46.378	165.644	102.036	223.391	67,710	121.982	67.585	177.656
(-) - catechine	115.842	222.214	155.176	318.175	105.498	175.786	123. 518	222,407
Sinapic acid	215.731	347.233	237.711	513.639	250.157	478.124	228.644	403.806
Vanillic acid	1.567	7.696	3.665	12.988	2.701	5.557	0.225	11.560
Caffeic acid	7.083	16.428	2.682	34.098	2.495	5.388	9.932	18.199
t-Cinnamic acid	8.331	9.277	7.502	17.549	7.049	16.869	8.074	9.907
Ellagic acid	–	4.106	1.598	5.341	0.147	3.802	–	4.449
Vanilline aldehyde	1.940	2.830	3.649	6.619	2.025	4.787	1.565	4.524

Table 2.

Wine tasting results in research objects.

Region	Research objects	Wine tasting results, in points
Kvareli	control	8.35
	test	8.45
Gurjaani	control	8..36
	test	8.54
Telavi	control	8.27
	test	8.49
Sagarejo	control	8.22
	test	8.39

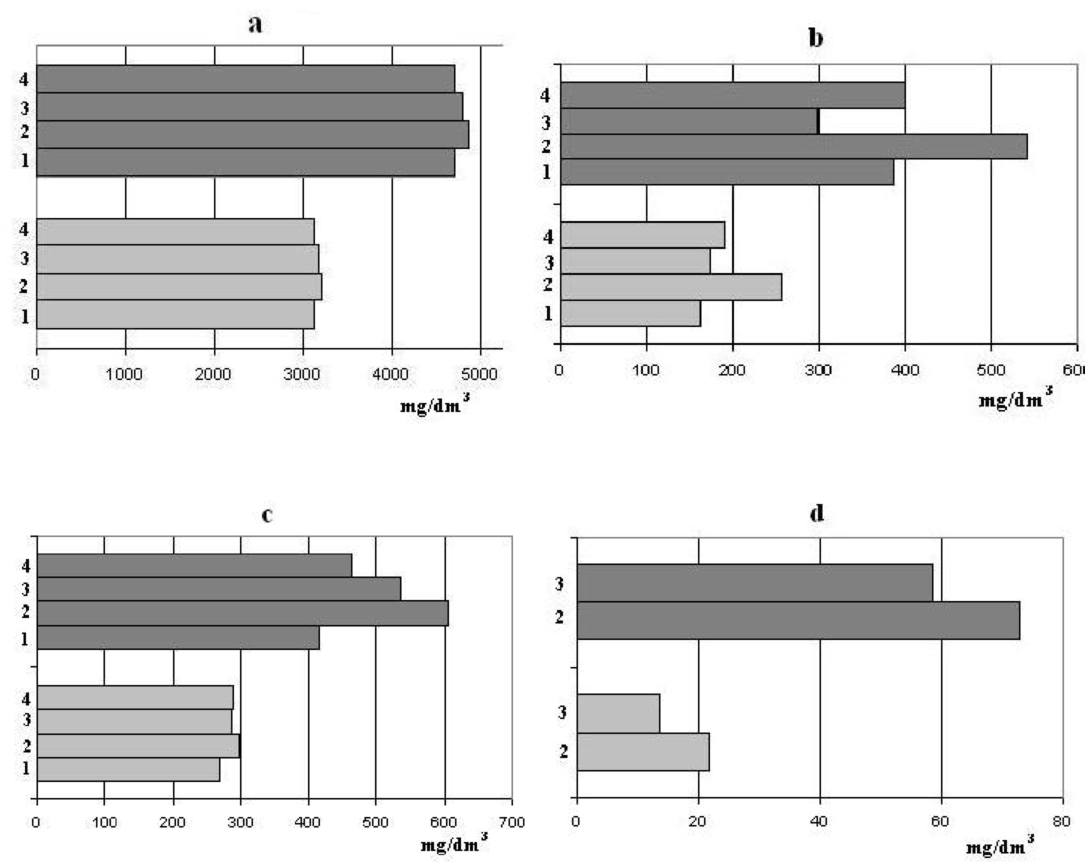

Figure 1. The total of common phenols (a), catechines (b), phenolcarbon acids (c) and flavonoles (d) in research objects prepared from Saperavi grape variety grown in different regions.

1 – **Kvareli**
2 – **Gurjaani**
3 – **Telavi**
4 – **Sagarejo**

Test

Control

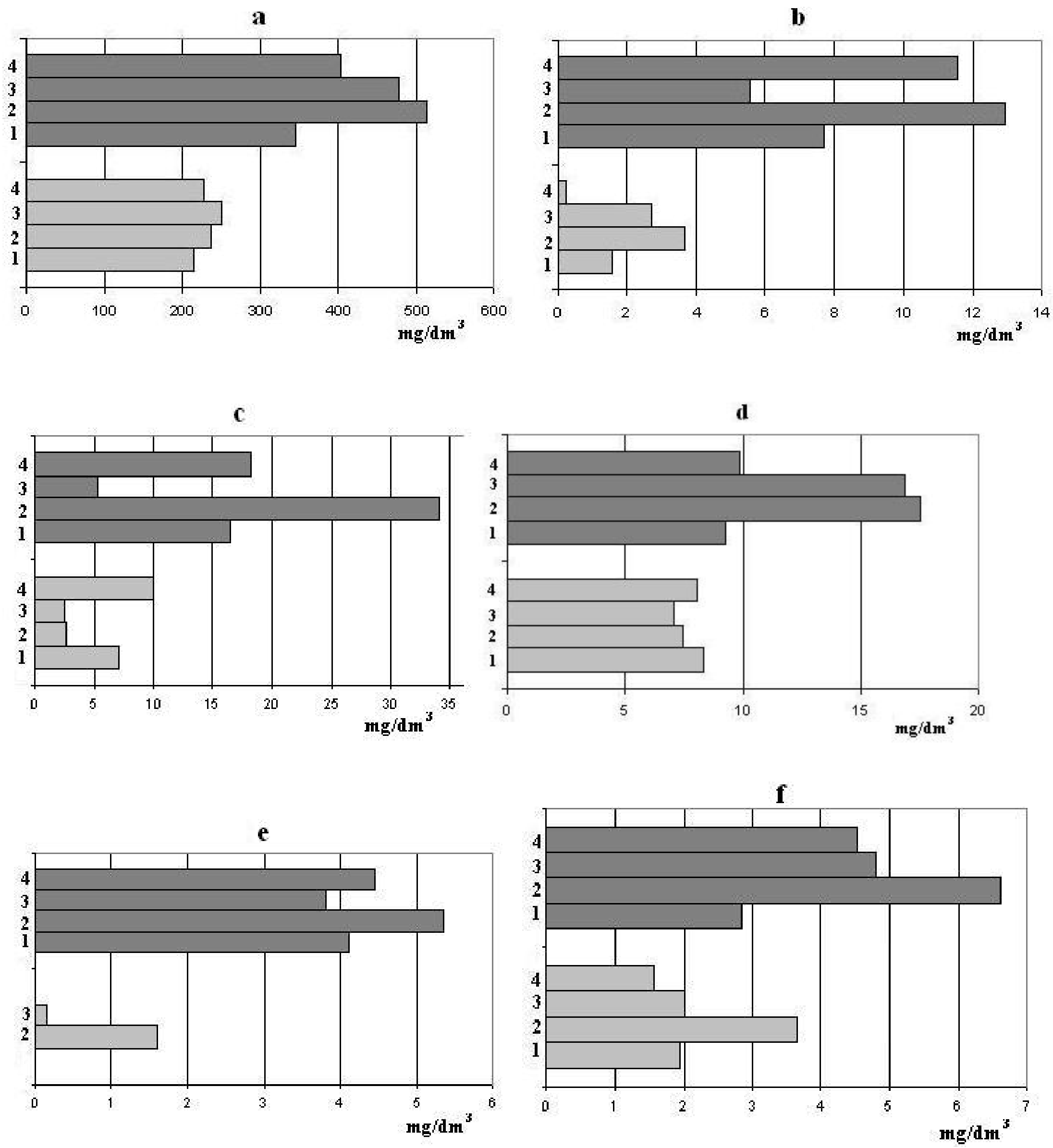

Figure 2. The amount of sinapic (a), vanillic (b), caffeic (c), cinnamic (d), ellagic (e) acids and vanilline aldehyde (f) in research objects prepared from Saperavi grape variety grown in different regions.

1 – **Kvareli**
2 – **Gurjaani**
3 – **Telavi**
4 – **Sagarejo**

Test

Control

higher than that of (+)-catechin. Cinapic acid makes 80% of total phenocarbonic acids. In wine samples (control) prepared by the existing technology the total of catechins amount is within the range of 162-257 mg/dm^3.

Catechins amount in commercial red wines prepared from Cabernet Sauvignon sold in US supermarkets was studied by Ritchey and Waterhouse (1999). According to their data the amount of these components in ultra-premium wines of Cabernet Sauvignon is far low compared with Saperavi wine samples (control) prepared with the use of standard technology. The total of catechins is also far higher in the same wine samples of Saperavi compared to the Hungarian wines Kadarka and Lemberger (Bonerz et al., 2008) as well as Spanish wines prepared from Cabernet Sauvignon and Tempranillo (Mayen, et al., 1995).

In Bordeaux wines catechins concentration makes 94 mg/l, epicaterchins – 48 mg/l (Bourzeix, et al. 1986).

The analysis of the obtained results has shown that phenolic content of the test samples compared with controls sharply increases.

The total mass concentration of general phenols in wine test samples (4688; 4870; 4795; 4701 mg/dm^3) in comparison with controls (3130; 3206, 3160; 3130 mg/dm^3) increases by 50%. In the test samples an increase in monomeric phenolic components is significantly higher in test samples compared with control.

It has been established that the total of catechins in test samples (387.858; 541.57; 297.77; 400.063 mg/dm^3) compared with controls (162.22; 257.22; 173.208; 191.103 mg/dm^3) is higher by 72 -139 %.

The total of phenolcarbonic acids in test samples (416.636; 604.789; 536.034; 462.494 mg/dm^3) in comparison with controls (268.127; 296.492; 284.671; 288.901 mg/dm^3) increases by 55-104%.

In test samples in comparison with control the total of flavonoles is three or four times higher (72.984; 58.565 mg/dm^3 vs 21.867; 13.818 mg/dm).

The amount of resveratrol in test samples prepared from Saperavi grape variety grown in Sagarejo and Kvareli regions was up to 2 mg/dm^3, whereas in control it made less than 1 mg/dm^3. Resveratrol was not found in control samples prepared from Saperavi grape variety grown in Telavi and Gurjaani regions but in test samples its content was less than 1 mg/dm^3.

Ellagic acid in wine control samples prepared from Saperavi grape variety grown in Kvareli and Sagarejo regions was recorded as a trace whereas in test samples their amount was 4 mg/dm^3.

The amount of kaemferol in research objects was recorded as a trace.

In test samples the total of catechins grows at the expense of the increase of both forms; the total of phenolcarbonic acids increases mainly at the expense of increasing sinapic, vanillic, caffeic, cinnamic and ellagic acids; those of flavonoles – at the expense of quercetin-3-β-D-glucosid.

According to literature data phenolcarbonic acids together with antioxidant ability determine sort peculiarities and influence on the formation and typicalness of a wine. Caffeic acid has bacteriocide action which is so high that its activity is often expressed by certain unit of penicillin (Valuiko, 1973;).

We have established an increase in the amount of vanilline aldehyde in wine test samples compared with control samples (2.830; 6.619; 4.787; 4.524 mg/dm^3 vs 1.940; 3.649; 1.565 mg/dm^3) by 1.5 - 2.9.times. As is known aromatic aldehydes exercise a significant influence on sensor indices of wine, among which vaniline aldehyde has stronger and nicer aroma (Valuiko, 1973; Kishkovskii., Skurikhin, 1976 ; Rodopulo, 1983).

The indicated increase in the content of catechines, phenolcarbonic acids, flavonoles and aromatic aldehyde – vanillin in test samples of red wines must be explained by the application of the technology developed by us which provides far better extraction of these components from grape pulp in the process of alcoholic fermentation compared to the existing technology.

According to the data of degustation commission the test samples were distinguished with much more fullness, harmony, brighter bouquet, sort aroma and wine tasting assessment which was in 0.1-0.22 points more compared with control samples.

Conclusions

The analysis of the obtained data has evidenced that the application of the technology of making red wines offered by us in comparison with the existing one gives the possibility of much more extraction of phenolic components. The mentioned increase of the amount of monomeric phenolic compounds enhances the antioxidant activity and improves sensory characteristics of wines. The technology offered by us was transferred to the National Centre of intellectual property (Sakpatenti) to obtain a patent.

Acknowledgements

This research was financially supported by: BPG #07/08. Donor Organization: CRDF, GRDF, GNSF.

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THE DEVELOPMENT AND IMPROVEMENT OF WINE TECHNOLOGY BASED ON THE EFFECTIVE USE OF THE SOURCE FRUIT, ENERGY AND MATERIALS

A. M. Avidzba

National Institute for Vine and Wine "Magarach"
of the National Academy of Agricultural Sciences of Ukraine
31 Kirov St., 98600 Yalta, Crimea, Ukraine
e-mail: magarach@rambler. ru

ABSTRACT

The technology and equipment to produce yeast autolysates destined for the use in the production of different types of still and sparkling wines and brandy spirits were developed at the National Institute for Vine and Wine "Magarach".

An installation for the yeast mass cavitation treatment is viewed as a constituent element of wine production technology as well as for the cold prepation of bentonite suspension with the aim to clarify and stabilize wines and wine materials, for the cold preparation of syrups, for the preparation of tirage and dosage liqueurs during the production of sparkling and champagnized wines and for the preparation of syrups on the basis of brandy spirits during the production of cognacs and brandies.

RÉSUMÉ

Les chercheurs du Centre National de Recherches sur la Vigne et le Vin "Magarach" ont élaboré une méthode de production, ainsi que l'équipement correspondant, pour la production des autolysats de levures de qualité, qui conviennent pour l'élaboration de différents types de vins, traquilles comme effervescents, ainsi que d'alcools de brandies.

L'installation pour le traitement de cavitation de la masse lévurienne est destinée à être inclue dans la technologie de la production du vin en tant qu'élément technologique principal. Elle est également utilisée pour la préparation de la suspension de bentonite à froid, dans le but de la clarification et de la stabilisation des vins et des vins de base, pour la préparation des sirops à froid, des liqueurs de dosage et de tirage au cours de l'élaboration des vins effervescents et champagnisés, ainsi que des sirops à base d'alcools de brandies au cours de l'élaboration des Cognacs et des brandies.

INTRODUCTION

A study of technological processes of Ukrainian wine production indicates that many of those intended to treat wines are associated with excessive energy consumption justified by the need to achieve stable and typical final products. The use of yeast autolysates is an effective technological approach enabling the wine-maker to exert influence on the formation and maturation of wine materials in a way leading to the improvement of their quality and typicality.

In 1926, the French scientist Martini was the first to point at the positive influence of yeast autolysis on the maturation of bottle champagne. The biochemical role of the autolytic processes during champagnization was substantiated by Oparin et al. in 1943-47. Yeast autolysis was further studied by Sisakian, Rodopulo, Popova, Agabaliants (1964), Loza, Nilov, Datunashvili (1964), Sarishvili (1982), Avakiants (1982) and other researchers. Yeast autolysates and preparations on their basis are widely used throughout the world with the aims to regulate the fermentation processes during the production of wine materials and in the production of sparkling wines. The technology has been attempted in Ukraine yet is still hampered by the time- and energy-consuming preparation of lysate materials.

Autolysis (in yeasts in our case) is the self-destruction of the cell's components by various enzymes after the cell has died. The death of the cell may be rapid, without changes in its constitution, as when it is heated or slow, leading to necrobiosis.

Autolysis is especially intense if the cell dies rapidly and the intracellular components are still active. The process is most favored by the temperatures of 40-45^{o}C and involves hydrolysis of cellular proteins by enzymes while the medium is enriched with self-formed amino acids, vitamins, micro-elements and other cellular products. The termination of the autolytic processes which occur in the cell is synchronous with the inactivation of the proteolytic enzymes.

The direction of the autolytic processes and the composition of yeast autolysates are temperature-dependent. Yeast autolysis is slow when the wine is aged at 10-15^{o}C while the heating of the wine at 40-45^{o}C results in a rapid death of yeast cells. A wide range of products are secreted by yeast cells into the wine in the course of autolysis, including enzymes (proteolytic enzymes, β-fructofuranosidase, dehydrogenases), nitrogenous substances (proteins, peptides, nucleic acids), phosphoric compounds, lipids, polysaccharides, aroma-forming components (esters, terpenoids, fatty acids), etc. Fermentative reactions occur in the cytoplasm and on individual organoids of the cells as promoted by active hydrolases and oxidoreductases present in the cells. This means that the autolyzing yeast cells enter as the centers of the fermentative reactions in the wine.

A long-term contact of the wine with the lees, the addition of yeast autolysates and the use of yeast enzyme concentrates and preparations help enrich the wine with yeast autolysates. The long-term contact of the wine with the lees needs much heed and care to avoid unwanted consequences, such as the yeasty and the hydrogen sulfide off-odors. Unfortunately, this cannot be circumvented in many cases, and that is why this effective technological approach is not in wide use in Ukrainian wine-making.

The problem is that the deaths of individual yeast cells are not synchronous with each other throughout the total yeast mass that contains living cells, dead cells and those in the states of autolysis and necrobiosis. Besides, the gradual autolysis of yeast cells dramatically increases the risk that the forein microflora, including lactic acid and acetic acid bacteria, will develop.

A rapid death of yeast cells is promoted by the heating of the wine at 60^{o}C. However, this also leads to the inactivation of the enzyme systems of the cells. As a result, the yeast extract instead of the autolysate is raised, with only a limited value for wine-making.

Thus, it may be concluded that the rapid death of yeast cell throughout the total yeast mass is a necessary prerequisite for obtaining a quality autolysate, with high enzyme activities and the least possible damage of the yeast cells to avoid the enrichment of the wine with cellular polisaccharides.

Material and methods

A mass of yeast from the champagnization of a sparkling material and the material itself were used. Proteinase activities (those of acid, alkali and neutral proteinases) and protein mass concentration were determined by the modified method of Anson and the method of Lowry, respectively. The visual evaluation of the state of the yeast was by digital optical microscopy. The yeast mass was processed on an experiment installation for cavitation treatment via disintegration with the rotory working organ.

Results and discussion

The scientific and technological standards of the wine industry arise from the possibility to use modern technological equipment. The technology for the accelerated production of yeast autolysates requires a brand-new device on the basis of yeast mass disintegration using intensified cavitation processes.

The possibility was studied to find out whether the yeast mass may be processed on a newly-designed installation with the aim to produce autolysates destined for the use in the production of different types of wines, and experiment data was obtained, enabling conclusions as to their commercial production.

The cavitation effect has led for the first time to an instantaneous death of yeast cells. In this case, the constitution of the cell is not changed after it has died as necrobiosis does not occur and its enzyme systems inherent in living cells remain active.

To be mentioned is that, with the use of our installation, the heating of the yeast mass during the cavitation treatment is not dependent on an outer heat-transfer agent and is enabled by the intensification of both liquid friction arising from the rotation of the working organ of the machine and the cavitation effect.

Since the highest activities of the yeast cell's proteolytic enzymes occur at $40^{o}C$, the process was controlled by following the rise in the temperature of the yeast mass.

The first-generation yeast mass from the champagnization of the above-mentioned sparking material was diluted with the latter to achieve the concentration of 15-20% and processed on our disintegration installation taking account of its temperature every 5 min. The process was terminated when the yeast mass had a temperature of $40 \pm 2^{o}C$.

The yeast mass obtained was held at $35^{o}C$ to allow autolysis. The sampling was on a daily basis. The samples were observed with microscope to determine the state of the cells and the levels of proteinase activities (those of acid, alkali and neutral proteinases).

One hour after the treatment, all yeast cells were dead, with not more than 30% of the dead cells in the control. The yeast mass was maintained at $35^{o}C$ for a period of one day, after which the separation of the undamaged cell membranes from the cell contents was observed. Following five to eight days, the majority of the cells were in the state of distortion of their membranes and became compacted in an orderly mass, with the cell contents secreted into the medium. The control showed single damaged membranes, without the distortion and the compaction of the cells.

The changes in proteolytic activities of the enzymes present in the yeast mass were followed. Proteolytic activities increased ten-fold on an average one day after the caviation treatment, remained so for two days and went down in a uniform fashion over a period of five days to the level twice as large as that of the control.

The data arising from digital optical microscopy and proteolytic activities of the yeast mass leads to a conclusion that the cavitation treatment entails the death of yeast cells mostly without the damage of their membranes. As a result, an activation of the proteolytic enzymes occurs and the autolytic processes are induced throughout the yeast mass. This was accompanied by a four-fold reduction in protein mass concentration since the proteolytic enzymes passed into the wine and proteins hydrolysed to amino acids.

Of importance from a science and technology point of view is the fact that autolysis may be induced in yeast in any physiological condition, at any technologically appropriate stage of the vinification process and for a period of not more than ten days. As a result, the medium is enriched with the autolyzed intracellular liquid, without the products arising from the destructed cell membranes.

The yeast mass was processed on our newly-designed installation for cavitation treatment which is ready for commercial manufacture and introduction.

The design features of our installation are as follows. Its working organ is a particularly designed disintegrator that, provided the cyclical pumping-in of the product, enables it to be processed in the cavitation mode, which leads to a total death of yeast cells and their autolysis. An effective bentonite activation without the need for it to swell is another strong point. The installation may also be used for the cold preparation of quality sugar syrups based on various media, being a universal polyfunctional system capable to find an answer to different technological problems of wine-making.

Conclusions

The technology and equipment to produce yeast autolysates destined for the use in the production of different types of still and sparkling wines and brandy spirits enable the following advantages:

- the possiblity of an accelerated production of lysate wine materials on the basis of effective processing of the yeast mass followed by its autolysis;
- the possiblity of an accelerated cold production of bentonite suspension based on various media, including wine material, without the use of water;
- a two-to-three fold reduction of the doses of ingredients;
- continuous precessing of the must and wine materials;
- at least a ten-fold reduction of energy consumption associated with the processes;
- an improved filtrability of materials that have been subject to the treatment;
- a reduction in the duration of cold treatment to three to five days during the batch production;
- a reduction in the duration of potassium ferrocyanide treatment to three to five days;
- guaranteed cloud stability of wine materials;
- the possiblity to process both ordinary and check wine materials using the “bentonite-gelatin” scheme without changes in their color and characteristics due to the absence of the “stripping effect”;
- the possiblity of the cold preparation of syrups, including those based on brandy spirits, as well as tirage liqueur and dosage liqueur.

The above-indicated technology and equipment will lead to reduced energy consumption associated with wine production, improved quality and competitivity of wines and other vintage products and upgraded technological standards of the wine industry.

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REDUCTION DE L'IMPACT SUR L'EFFET DE SERRE PAR L'ECO-CONCEPTION DES CAVES : CONTEXTE ET APPLICATION

ROCHARD J., VALLET C., LABBE P.
Institut Français de la Vigne et du Vin
joel.rochard@vignevin.com

RESUME

La construction d'un bâtiment viticole ou d'une cave et le choix des équipements associés à la conception des équipements de l'ouvrage suppose une réflexion approfondie concernant notamment les aspects économiques, qualitatifs, la sécurité des utilisateurs. Au-delà de l'aspect fonctionnel, la prise en compte du développement durable impose une réflexion relative à l'impact de la conception et du fonctionnement des caves sur l'effet de serre.
Autrefois tous les moyens naturels qui permettaient de bénéficier de la fraîcheur ou de la chaleur étaient utilisés
La conception des bâtiments, associant une bonne isolation éventuellement complétée de solutions originales (toits ou murs végétalisés, puits canadiens…) et les énergies alternatives (solaire, géothermie, biomasse…) s'intègre dans cette dynamique de conception écologique des caves.
Ces aspects, ainsi que l'intégration paysagère contribue à valoriser l'image environnementale de la cave. Des démarches originales pionnières, peuvent s'intégrer, au-delà des choix architecturaux, dans une démarche de communication et de valorisation des vins.
Par ailleurs, la réglementation, les normes évoluent au cours des prochaines années, ce qui justifie d'anticiper les exigences environnementales, afin d'éviter des modifications de mise aux normes coûteuses.

SUMMARY

The construction of a viticultural building or wine cellar and the choice of machinery associated with the design of facilities imply in-depth reflection, particularly with regard to the economic and qualitative aspects as well as the safety of those using the machines. As well as the practical side, taking sustainable development into account requires a reflexion about impact of the cellar's design and operation on the greenhouse effect.
In the past, natural means were always used to take advantage of coolness and warmth.
Building design, involving efficient insulation and sometimes completed with original solutions (green roofs or walls, underground heat exchangers, etc.) and alternative energies (solar, geothermal, biomass...) is part of this eco-design process for wine cellars.
While the various traditional methods cannot be used in every situation, eco-technological progress, besides economic measures, means that it is possible to envisage developing.
These aspects, together with landscape integration, contribute to promoting the cellar's environmental image. It is possible to incorporate an original and pioneering approach, besides architectural choices, in communications and wine promotion measures.
At the same time, experts are predicting a significant rise in the price of water and energy, which will contribute to raising wine production costs.

INTRODUCTION

La construction d'un bâtiment viticole ou d'une cave et le choix des équipements associés à la conception des équipements de l'ouvrage suppose une réflexion approfondie concernant notamment les aspects économiques, qualitatifs, la sécurité des utilisateurs Au-delà de l'aspect fonctionnel, la prise en compte du développement durable impose une réflexion notamment sur l'utilisation de l'énergie, la gestion de l'eau. Ces aspects, ainsi que l'intégration paysagère contribue à valoriser l'image environnementale de la cave. Des démarches originales pionnières, peuvent s'intégrer, au-delà des choix architecturaux, dans une démarche de communication et de valorisation des vins.

I Eco-conception et développement durable

Comme le souligne B. Peuportier, « *la notion d'éco-conception des bâtiments fait appel aux éco-techniques dans le domaine des économies d'énergie et d'eau, des énergies renouvelables, de la gestion des déchets, des matériaux à moindre impact, en incluant des aspects de confort et de santé* ». Architecture solaire, solaire passif, bioclimatique, haute qualité environnementale, basse consommation, énergie positive, zéro émission, sont autant de termes usités pour définir des projets avant-gardistes.

Le développement durable s'impose à tous les secteurs d'activité. La définition de ce concept pour la filière viticole a été formalisée par une résolution de l'Organisation Internationale de la Vigne et du Vin.
« *L'approche globale à l'échelle des systèmes de production et de transformation des raisins, associant à la fois la pérennité économique des structures et des territoires, l'obtention de produits* de *qualité, la prise en compte des exigences d'une viticulture de précision,* des risques *liés* à *l'environnement,* à *la sécurité* des *produits* et *la santé* des *consommateurs et la valorisation des aspects patrimoniaux, historiques, culturels, écologiques* et *paysagers.* »

L'intégration du concept de développement durable au sein de la filière viticole associe en premier lieu une adaptation des itinéraires viticoles et œnologiques aux contraintes environnementales, mais également, avec une vision sur le long terme, une prise en compte notamment des aspects énergétiques dans la conception des exploitations et des caves. En effet, l'impact environnemental lié à la conception des bâtiments et équipements associés est assez faible comparativement aux itinéraires viticole et œnologiques mais tes choix liés à la construction engagent l'exploitation et la cave pour plusieurs décennies.

Par ailleurs, il est probable, qu'à l'image des normes de sécurité du personnel, la réglemention et les normes évoluent au cours des prochaines années, ce qui justifie d'anticiper les exigences environnementales, afin d'éviter des modifications ultérieures couteuses. Parallèlement les experts prédisent une augmentation significative du coût de l'énergie au cours du XXIème siècle.

Mise en œuvre de l'Eco-conception

- **Notion d'énergie renouvelable**

La crise de l'énergie a mis en évidence à partir des années 1970 la nécessité de diversifier l'approvisionnement énergétique. Par ailleurs la combustion d'énergie fossile contribue à accentuer l'effet de serre à l'origine d'un probable réchauffement climatique. Les énergies renouvelables utilisent des sources inépuisables d'énergies d'origine naturelle : rayonnement solaire, vents, cycles de l'eau et du carbone dans la biosphère, flux de chaleur interne de la Terre, effet de l'attraction lunaire et solaire sur les océans. Elles s'opposent ainsi aux énergies minières et fossiles dont les stocks, forcément limités, se sont constitués lors de la formation du système solaire (uranium, thorium) ou au cours des âges géologiques, à partir d'une fraction infime de la biomasse terrestre qui a pu se fossiliser (charbon, pétrole, gaz naturel). Les énergies renouvelables intègrent également les flux de déchets de l'activité économique qui peuvent donner lieu à une valorisation énergétique : déchets de l'agriculture et de l'exploitation forestière, part combustible ou fermentescible des déchets industriels et des ordures ménagères.
Comme le souligne B. CHABOT (cf. figure 1), les énergies renouvelables doivent mettre à notre disposition une diversité de services énergétiques : éclairage, chaleur et climatisation, force motrice pour la production de biens et de services, transport de personnes et de marchandises, traitement et transmission de l'information, communication...

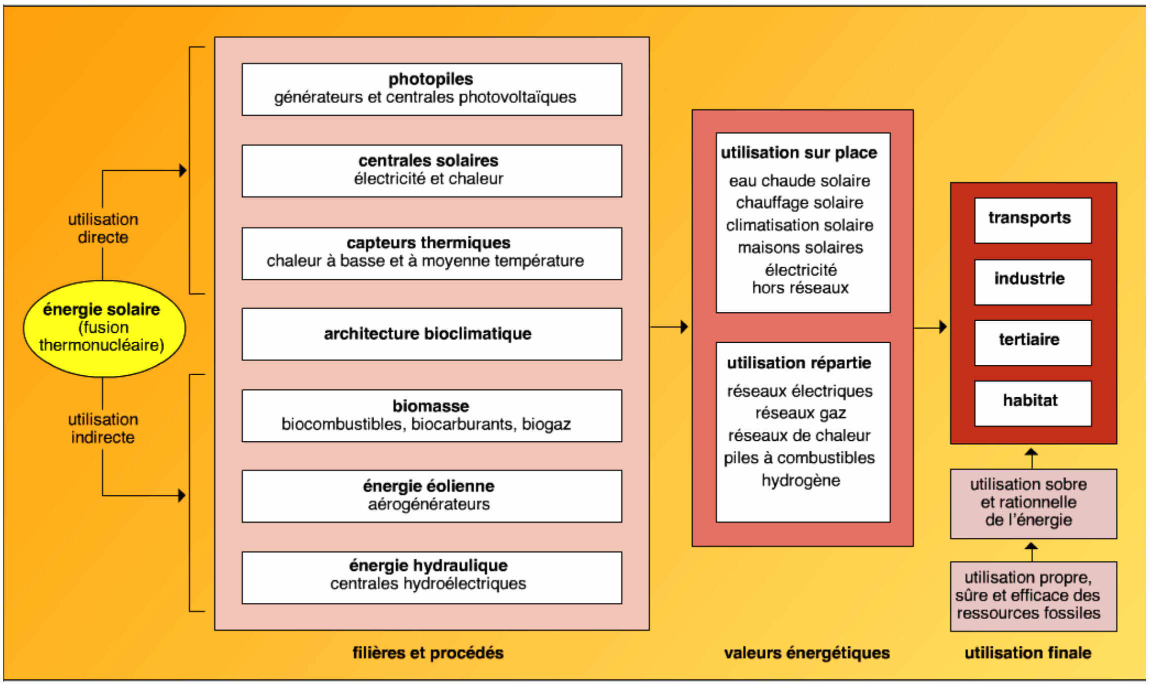

Figure 1 : Schéma d'intégration des énergies renouvelables
(source : B. CHABOT « Energies renouvelables » Encyclopedia Universalis, 2008)

Les énergies renouvelables nécessitent néanmoins des ressources en capital, en matières premières et en main-d'œuvre pour être exploitées. Leur exploitation suppose une démarche globale de maîtrise de l'énergie fondée sur deux principes :

- L'**efficacité énergétique**, qui permet d'assurer ces services prioritaires en consommant le moins possible d'énergie primaire, (ressources énergétiques fossiles ou renouvelables). Cet impératif suppose d'utiliser des appareils et des procédés à haut rendement. Par exemple, le choix de lampes à fluorescence plutôt qu'à incandescence permet de diviser par cinq la consommation d'électricité à niveaux d'éclairages équivalents.

- Le **respect de l'environnement**, qui implique le recours à des sources d'énergie qui soient le plus possible compatibles avec l'environnement local, régional et planétaire et dont l'exploitation puisse se faire à la fois dans les pays industrialisés et en développement, c'est-à-dire adaptée aux exigences économiques actuelles et ne s'accomplisse pas au détriment des générations futures. Le choix d'isolants écologiques (chanvre, coton recyclé...) et du bois dans la construction des bâtiments s'intègre également dans une approche durable.

ENERGIE SOLAIRE

L'énergie solaire permet d'assurer des conditions de température favorables à la vie et anime les cycles de l'eau, des vents et du carbone dans la biosphère. Mais elle peut aussi être utilisée directement pour produire de la chaleur à différents niveaux de température pour divers usages : chauffage et climatisation de locaux, séchage de produits agricoles, production d'eau chaude et de vapeur, production d'électricité par le biais de cycles thermodynamiques. La production d'électricité peut être obtenue directement par la conversion photovoltaïque de l'énergie solaire au moyen des cellules solaires (cf. figure 2 et 3).

Figure 2 : Panneaux solaires de production d'eau chaude en Inde

Figure 3 : Panneaux photovoltaïques (Lieu : Cave coopérative les Coteaux du Pic à Pic Saint-Loup)

BIOMASSE

La biomasse est formée de l'ensemble des organismes vivants sur les continents et dans les océans, qu'ils soient des micro-organismes, des plantes ou des animaux. Grâce à la photosynthèse, mettant en jeu les molécules de chlorophylle, les plantes utilisent l'énergie solaire pour décomposer l'eau (H_2O) qu'elles contiennent dans leurs cellules et le dioxyde de carbone (CO_2) de l'atmosphère pour les transformer en matières végétales, principalement des hydrates de carbone (sucres) et de la cellulose :

$$CO_2 + H_2O + \text{énergie solaire} \Rightarrow \text{matières végétales} + O_2$$

Le compostage, est, dans une certaine mesure une filière de valorisation énergétique. En effet, le compost limite l'impact énergétique des engrais, dont la production exige beaucoup d'énergie.

L'utilisation la plus courante de la biomasse à des fins énergétiques intervient soit directement sous forme de « biocombustibles » comme le bois, les sarments, le marc, soit après l'avoir transformée en « biogaz » (mélange de méthane CH_4, un peu de CO_2 et d'autres gaz) ou en biocarburants (alcool). Le CO_2 émis lors de la combustion ou de l'oxydation et refixé par les plantes lors de leur croissance, ce qui contrairement aux énergies fossiles, n'impacte pas la concentration en CO_2 de l'atmosphère.

Le gisement mondial de biomasse est important et peut permettre une utilisation énergétique à grande échelle, en substitution de combustibles fossiles. La valorisation énergétique des sous-produits et déchets s'intègre dans ce bénéfice écologique. Par contre la mobilisation des surfaces agricoles, au détriment de la production alimentaire et soumise à un débat éthique en liaison avec les besoins alimentaires.

GEOTHERMIE

Comme le précisent J. GOGEL et J. VARET, le flux géothermique de la terre est généralement beaucoup trop faible pour être utilisé directement avec un procédé « haute température » sauf lorsqu'il se trouve concentré dans une source thermale ou une chambre magmatique superficielle avec son système hydrothermal associé (cf. figure 4).

Sous le nom d'« énergie géothermique », on exploite selon différentes modalités, la chaleur du sous sol par l'intermédiaire le plus souvent d'une circulation d'eau (cf. figure 4 de géothermie très basse énergie par forage). Ce n'est donc pas une énergie renouvelable au sens stricte lorsque l'on exploite des sites conductifs. Mais les sites favorables de ce type sont très abondants, et, si nécessaire, il sera éventuellement possible de déplacer en profondeur la zone de production pour poursuivre l'exploitation.

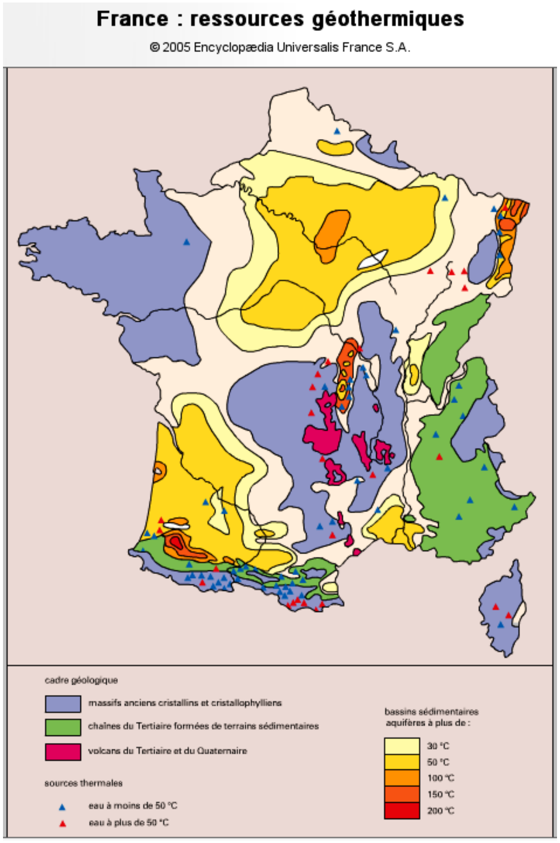

Figure 4 : Carte des ressources géothermiques en France (source : GOGEL J. et VARET J., Géothermie, Encyclopédia Universalis, 2008)

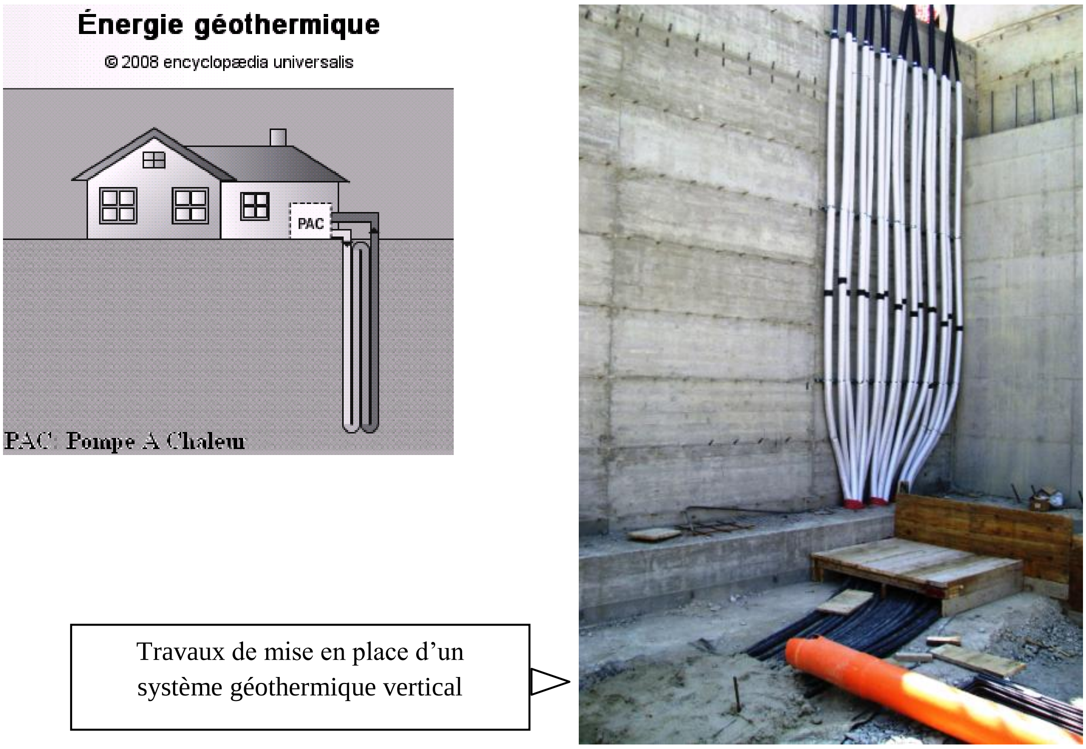

Figure 5 : Schéma de géothermie très basse énergie par forage (source : GOGEL J. et VARET J., Géothermie, Encyclopédia Universalis, 2008)

L'application la plus classique consiste à exploiter la chaleur diffuse en implantant dans la terre, verticalement ou horizontalement, des capteurs chargés d'un fluide caloporteur. Associés à une pompe à chaleur, ils offrent pratiquement les mêmes avantages. La profondeur d'une sonde verticale est généralement comprise entre 30 et 150 mètres. Le dispositif peut être installé dans n'importe quel milieu géologique (cf. figure 5). Selon la nature du sous-sol, il est possible d'utiliser différents dispositif : aiguilles, serpentins, forages. Le « puits canadien » s'inspire du même principe, mais en utilisant un flux d'air grâce à des canalisations horizontales enterrées dans le sol.

- **Les isolants écologiques**

Les maîtres d'ouvrages ont cherché à améliorer les écobilans de l'isolation avec des matériaux moins toxiques, recyclés ou recyclables, peu consommateurs d'énergie, tout en gardant des performances supérieures ou égales aux isolants classiques. La contamination potentielle de l'atmosphère interne (micro-organismes et micropolluants) doit être prise en compte dans le choix des isolants. Les isolants écologiques sont des matériaux de construction qui répondent à la fois aux critères techniques habituelles de construction mais aussi à ces critères environnementaux, tout au long de leur cycle de vie. Les caves sont des milieux aux caractéristiques particulières : humidité importante, présence d'organisme xylophage, renouvellement régulier de l'air, inertie olfactive nécessaire… c'est pour cela que tous les isolants ne sont pas adaptés au secteur viticole. Les isolants écologiques sont d'origine minérale, végétale ou animale et leurs caractéristiques sont différentes (cf. figure 6).


Laine de roche


Laine de verre


Perlite expansée


Vermiculite


Laine de lin


Laine de mouton


Laine de chanvre


Panneaux de cellulose


Liège


Panneaux de bois feutré

Figure 6 : Diversité des isolants thermiques (Source : Energie plus)

CONCLUSION

Au cours du XXIème siècle, la filière viticole, a progressivement intégré l'évolution technologique issue des progrès et innovations de l'industrie. Les faibles coûts de l'énergie ont contribué à développer des itinéraires viticoles et œnologiques qui impactent le bilan carbone®. Ainsi l'éco-conception est également un enjeu pour l'image d'une entreprise. De nombreuses caves assurent l'accueil de visiteurs qui seront de plus en plus attentifs aux initiatives pionnières dans le domaine de l'éco-construction des caves et la mise en œuvre de technologies écologiques innovantes.
Concernant l'énergie, tous les moyens naturels qui permettaient autrefois de bénéficier de la fraîcheur ou de la chaleur étaient utilisés (caves souterraines, ouverture des celliers pendant l'hiver, orientation du soupirail en fonction de l'exposition ou des vents dominants). Si ces différentes méthodes traditionnelles ne peuvent pas être mises en place pour toutes les situations, le progrès des écotechnologies, au delà des mesures d'économie, permet d'envisager le développement d'énergies alternatives (solaire, géothermie, biomasse….).
La conception des bâtiments, associant une bonne isolation éventuellement complétée de solutions originales (toits ou murs végétalisés, puits canadiens…) s'intègre dans cette dynamique de conception écologique des caves.

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Determination of Geographical Origin of Brazilian Wines Using Joint Isotopic and Mineral Elements

Dutra, S.V.; Adami, L.; Marcon, A.R.; Carnieli, G.; Roani, C.; Spineli, F.; Leonardelli, S; Munaro, D; Vanderlinde, R
Universidade de Caxias do Sul (UCS), Instituto de Biotecnologia, Rua Francisco Getúlio Vargas, 1130, 95070-560, Caxias do Sul, Brasil; LAREN – Laboratório de Referência Enológica, Av. da Vindima, 1855, Caxias do Sul, Rio Grande do Sul, Brasil

ABSTRACT

The vast expanse of land and the diversity of climates of Brazil allows the production of grapes and wines in different regions and which have their own special characters. In this work were studied wine varieties Cabernet Sauvignon and Merlot from three wine regions of southern Brazil, aiming to distinguish between them in relation to the geographical origin of grapes, using isotopic analysis by mass spectrometry isotope ratio (IRMS) and minerals by atomic absorption. The analysis of $\delta^{18}O$ of water, magnesium, rubidium and litium were the most efficient to differentiate the three regions. Could classify a rating of 80% of wines using isotopic and mineral analysis.

RESUMEN

La grande extensión de tierra y la diversidad de climas del Brazil permiten la produción de uvas y vinos en diferentes regiones y que tienen tipicidad proprias. En esto trabajo fueran estudiados vinos de las variedad Cabernet Sauvignon y Merlot, de tres regiones vitivinicolas del sur del Brazil com el objetivo de diferenciarlos con relación al origen de las uvas usando espectrometría de masa de isótopos y la determinación de los minerales por Espectrofotometria del Absorción Atómica. Las analises de $\delta^{18}O$ de la aqua, los minerales rubidio, magnesio y litio fueram las mas eficientes para diferenciar las tres regiones. Fue posible la classificacion de 80% de los vinos usando análisis isotópicas y minerales.

INTRODUCTION

In Brazil, the cultivation of vines for the production of wine is concentrated in the South, especially in the Serra Gaúcha, responsible for 90% of wine production in the country. In recent decades, there were two new centers of production, the Campanha and the Serra do Sudeste. The Serra Gaúcha, with a latitude of 29° S is a mountainous region cultivation of the vine concentrates on altitude 400-600 meters. The Campanha is situated at an average latitude of 31° with border to Uruguay, with altitudes between 100 and 200 meters. The Serra do Sudeste presents undulating topography or heavily undulating with an altitude of 200 to 400 meters (Miele & Miolo, 2003).

Knowledge of the geographical origin of foods, is considered an addicional guarantee of their quality, authenticity and typicality. Several studies were conducted to determine the geographical origin of different food products such as tea (Fernández-Cáceres *et al*., 2001), olive oil (Angerosa *et al*., 1999) and rice (Kelly *et al*., 2002).

The isotope ratio analysis can provide information on the source of a chemical compound. The natural abundance of stable isotopes of elements H, C and O showed small but measurable variations due to fractionation that occurs during the chemical and physical processes of the natural cycles. These processes result in enrichment or depletion of the heavier isotopic form (Klein & Klein, 1982). The biological isotopic fractionation provides an unequal distribution of isotopes lighter and heavier between the reactants and reaction products. In most cases, the lighter isotopes are preferentially used and the heavier fraction are enriched in the uneven fraction of substrate (Morasch *et al.,* 2001). The magnitude of isotopic fractionation is affected by regional differences, which imply changes in parameters such as humidity, temperature, winds, weather conditions, among others (Brause *et al*., 1984). The isotopic measurements can provide information on the botanical and geographical origin (Calderone *et al*., 2007).

The determination of minerals in wines are used to distinguish the geographical origin as well as to detect tampering and forgery thereof. The composition of minerals in wines depends on the viability of these elements in soil, fertilization practices, conditions of processing and location of the vineyard (Nuñez, 2000; Iglesias, 2007). The minerals allow for a differentiation according to geographical origin due to the direct relationship with the soil composition (Frias *et al*., 2003). The minerals content and trace elements of wines of the Boehmia region (Czech Republic) were used to differentiate samples from different regions. The best elements for this identification were aluminum, barium, calcium, potassium, lithium, magnesium, manganese, molybdenum, rubidium, strontium and vanadium (Sperková and Suchánek, 2005). Martin *et al*. (1995), determined the different producing regions of France using stable isotope analysis and a greater number of variables such as barium, strontium, magnesium, iron, aluminum and rubidium.

This study aimed to determine the gregraphical origin of Merlot and Cabernet Sauvignon wines from Serra Gaúcha, Serra do Sudeste and Campanha, the main producing areas of southern Brazil.

MATERIAL AND METHODS

Collection of samples and microvinification

Samples of grape varieties Cabernet Sauvignon (50 samples) and Merlot (50 samples) were collected in southern Brazil, in areas of Serra Gaúcha, Serra do Sudeste and Campanha, 2007 vintage. The microvinification and analysis were performed at the Laboratory of Enologic Reference (LAREN) the Secretaria Agriculture, Livestock, Fisheries and Agribusiness of Rio Grande do Sul, in collaboration with the Brazilian Institute of Wine (IBRAVIN) and the University of Caxias do Sul (UCS). Samples of grape (5 kg) were stalked and crushed manually and microvinification were held in stainless steel tanks (10L), coupled with valve Muller.

Analysis of Carbon Isotopic Ratio of Ethanol and Oxygen Water

The values of $^{13}C/^{12}C$ isotope ratio of samples were obtained in relation to the international standard PDB ($\delta^{13}C$) according to methodology described by the Ministry of Agriculture through the Normative Instruction No. 4 of 02.05.2001 (BRAZIL, 2001).

The values of isotope ratio $^{18}O/^{16}O$ water were obtained in relation to the international standard V-SMOW ($\delta^{18}O$) as official methodology of the Ministério da Agricultura through the Normative Instruction No. 2 of 02.02.2006 (BRAZIL, 2006).

Determination of Minerals

Some minerals were determined directly in atomic absorption spectrometer Perkin Elmer Aanalyst 800, according to the Perkin Elmer guidelines.

Statistical Analysis

The results were statistically analyzed using SPSS 15.0 for Windows. The standard error was used instead of the standard deviation, since the number of samples at each location and variety were not equal. To evaluate the interaction between variety and location of production in relation to variables isotope of oxygen, carbon and mineral analysis was used Tukey analysis. The discriminant analysis was used to differentiate the regions through a multivariate model.

RESULTS AND DISCUSSION

Isotopic Ratio $^{18}O/^{16}O$ of Water and $^{13}C/^{12}C$ of Ethanol of Wines

The analysis of $^{18}O/^{16}O$ water independent of the variety studied was effective in differentiating the three regions studied, but there was no statistical difference among varieties (Table 1). The samples of Serra Gaucha showed average values of $\delta^{18}O$, followed by samples of the Campanha and Serra do Sudeste. According Brause *et al.*, 1984, this avriation can be explained due to differences in weather conditions such as humidity, temperature, winds. The $\delta^{18}O$ o values obtained in this study were lower than those cited by Day *et al.* (1994), in comparison samples of grapes from five regions of France (average 3.8 ‰) and those written by Matin *et al.,* 1999 also for French wines, but the region of Bordeaux (1,7 a 4,5‰).

Tab. 01 – Values of isotope ratios $^{18}O/^{16}O$ (‰) of wine water and $^{13}C/^{12}C$ (‰) of ethanol of the wines Cabernet Sauvignon and Merlot of the different studied regions

Regions	$^{18}O/^{16}O$ (‰)									$^{13}C/^{12}C$ (‰)								
	Merlot			Cabernet Sauvignon			Total			Merlot			Cabernet Sauvignon			Total		
	Mean		Standard error	Mean		Standard error	Mean		Standard error	Mean		Standard error	Mean		Standard error	Mean		Standard error
Serra Gaúcha	0.38^{A}	±	0.15	-0.28^{A}	±	0.18	0.05^{A}	±	0.12	-27.73^{Aa}	±	0.06	-28.52^{Ab}	±	0.08	-28.12^{A}	±	0.06
Serra do Sudeste	-2.34^{C}	±	0.21	-2.17^{C}	±	0.25	-2.28^{C}	±	0.16	-28.75^{Ba}	±	0.15	-29.82^{Bb}	±	0.3	-29.11^{B}	±	0.18
Campanha	-1.35^{B}	±	0.39	-1.24^{B}	±	0.3	-1.29^{B}	±	0.24	-28.90^{Ba}	±	0.13	-29.37^{Bb}	±	0,11	-29.17^{B}	±	0.09
Total	-0.23	±	0.16	-0.64	±	0.15	-0.44	±	0.11	-28.08^{a}	±	0.07	-28.82^{b}	±	0.07	-28.45	±	0.05

Means followed by different capital letters in the column and means followed by different letters in the line differ significantly by ANOVA complemented by the Test of Multiple comparisons tukey significance level de 5%.

The values $^{18}O/^{16}O$ of wine water when compared to wines from different European countries, classified according to the climate (Breas *et al.,* 1994, showed lower than those in warm climate countries, whose average value was 5.2‰ and also lower than the countries of temperate climate (average 2.3‰). However, our average results are close to those found for countries with cold climates (-0.7‰). The climate in the three regions studied in this work is classified as temperate, but in the 2007 harvest was an average precipitation greater than the median of the last 10 years, reducing the oxygen isotopic values. The precipitation from January to mid February was lower when compared to the period of February to the end of March in the three regions studied. The collection of samples in the Serra Gaúcha began before the regions of the Campanha and Serra do Sudeste. In the Serra Gaúcha the largest number of samples was collected before the rainy season (mid-February) whereas the other regions the samples were collected after this period, which probably explains the higher value $\delta^{18}O$ of water of Serra Gaúcha wines compared to other regions (Table 01).

According to Martin *et al*., (1999) the climatic influences are most marked in the distinction between hasvest production than growing regions. The $\delta^{18}O$ values are modified by climatic conditions during ripening and harvest of the grapes (Ogrinc *et al,* 2001). On $\delta^{18}O$ of water are typical of the other hand, negative values of surface water, and wine may indicate the addition of exogenous water or climatic conditions and rainfall during the particular vintage (Rossmann *et al*., 1996).

On the other hand, negative values of $\delta^{18}O$ of water are typical of surface water, and wine may indicate the addition of exogenous water or climatic conditions and precipitation during the particular vintage (Rossmann *et al*., 1996).

The average value of the $\delta^{13}C$, independent of the variety, distinguished the samples from Serra Gaúcha of the other regions studied, with average. The cv Merlot had an average total was significantly higher than the Cabernet Sauvignon regardless of the region studied. Rossmann *et al*. (1996) found average values of δ^{13} C higher for the cv Merlot in relation to the varieties studied, this difference can be explained by the influence of genetic variability in certain biochemical reactions that occur in plants and cause different fractionations of carbon isotopes during fixation of CO_2.

The average values of the $\delta^{13}C$ found in this study are lower than those obtained by Martin *et al*. (1999), in a study of wines from different regions of Europe, which had average values between -25.8 to -27.1‰, but remain within the range of values obtained by Rossmann *et al*. (1996) who found values of $\delta^{13}C$ of -23 to -29,00‰ in Italy -24 to -29‰ in France and from -23 to -30 in Germany.

Mineral content in the wines from different regions studied

Regardless of variety, Serra do Sudeste had a mean total magnesium, significantly higher, differing Campanha and the Serra Gaúcha (Table 2). Regardless of region, the variety Cabernet Sauvignon, had a total mean significantly higher than the Cv Merlot. These values agree with those found in other studies realized with Brasilian wines where they found levels ranging from 21.00 to 111.00 mg L-1 (Silva *et al*., 1998) and from 44.30 to 124.10 mg L-1 (Daudt *et al*., 1987b). Frías *et al*., 2001 differed geographically spanish wines from the Canary Islands using only magnesium and sodium.

Tab. 02 – Values of Magnesium and Rubídio (mg.L^{-1}) of the wines Cabernet Sauvignon and Merlot of the different studied regions

Regions	Magnesium									Rubidium								
	Merlot			Cabernet Sauvignon			Total			Merlot			Cabernet Sauvignon			Total		
	Mean		Standard error	Mean		Standard error	Mean		Standard error	Mean		Standard error	Mean		Standard error	Mean		Standard error
Serra Gaúcha	79.68^{Cb}	±	0.87	80.30^{Ca}	±	1.02	79.99^{C}	±	0.67	6.95^{Bb}	±	0.25	7.99^{Ba}	±	0.23	7.47^{B}	±	0.18
Serra do Sudeste	90.94^{Ab}	±	3.03	98.52^{Aa}	±	4.70	93.47^{A}	±	2.62	8.47^{Ab}	±	0.29	9.72^{Aa}	±	0.20	8.88^{A}	±	0.25
Campanha	86.52^{Bb}	±	1.40	89.22^{Ba}	±	1.38	88.08^{B}	±	1.00	4.45^{Cb}	±	0.22	5.50^{Ca}	±	0.27	5.06^{C}	±	0.19
Total	82.19^{b}	±	0.79	83.70^{a}	±	0.91	82.96	±	0.60	6.50^{b}	±	0.21	7.31^{a}	±	0.20	6.92	±	0.15

Means followed by different capital letters in the column and means followed by different letters in the line differ significantly by ANOVA complemented by the Test of Multiple comparisons tukey significance level de 5%.

The determination of rubidium, regardless of variety, allow the differentiation of wines from three regions studied, samples from the Serra do Sudeste had a higher average, differing from Serra Gaúcha and the Campanha. Besides differentiating regions, the rubidium values allow to differentiated varieties, the Cv Cabernet Sauvignon, regardless of region, had a total mean significantly higher than the Cv Merlot. Frias *et al*. (2003) were able to differentiate Spanish wines of the Canary Islands through the rubidium, obtaining values of 0.33 mg.L^{-1} to 2.88 mg.L^{-1}. Latorre *et al*. (1994) also obtained a good classification of Spanish wines from the region of Galicia, through the levels of lithium and rubidium.

Tab. 03 – Values of Manganes e Calcium (mg.L^{-1}) of wines Cabernet Sauvignon and Merlot of the different studied regions

Regions	Manganese									Calcium								
	Merlot			Cabernet Sauvignon			Total			Merlot			Cabernet Sauvignon			Total		
	Mean		Standard error	Mean		Standard error	Mean		Standard error	Mean		Standard error	Mean		Standard error	Mean		Standard error
Serra Gaúcha	2.21^{Ba}	±	0.13	1.84^{Aa}	±	0.08	2.03	±	0.08	55.14^{Ab}	±	1.29	68.97^{Aa}	±	1.5	62.06	±	1.10
Serra do Sudeste	1.96^{Ba}	±	0.1	1.27^{Aa}	±	0.12	1.73	±	0.11	48.61^{Aa}	±	2.49	51.13^{Ba}	±	8.89	49.45	±	3.24
Campanha	3.62^{Aa}	±	0.33	2.44^{Ab}	±	0.15	2.94	±	0.18	59.12^{Aa}	±	1.44	60.72^{Ba}	±	1.75	60.04	±	1.17
Total	2.51	±	0.13	2.00	±	0.07	2.25	±	0.08	55.51	±	0.99	65.78	±	1.23	60.75	±	0.85

Means followed by different capital letters in the column and means followed by different letters in the line differ significantly by ANOVA complemented by the Test of Multiple comparisons tukey significance level de 5%.

The results of manganese (Table 03) allowed differentiation of the region's of Campanha for the other regions, only for the variety Merlot, in Cv Cabernet Sauvignon no significant difference between regions were show. In the region of Campanha the average of Cv Merlot was significantly higher than the Cabernet Sauvignon. The total average levels of manganese, found in this work are consistent with those reported for Brazilian wine business, whose contents varied from 1.9 to 3.30 mg.L^{-1} (Rizzon & Miele, 2002), 2.1 to 2.8 mg.L^{-1} (Daudt *et al*., 1987a) and from 0.70 to 4.10 mg.L^{-1} (Silva *et al*., 1999). The manganese content can be influenced by the fermentation process also may be a particular feature of a given region of production (Daudt *et al*., 1987b). Normally the source of this mineral is associated with soil and pesticide treatments used on the vine (Silva *et al*., 1999).

The levels of calcium (Table 03) allowed the different wines of the region of Serra Gaúcha compared with other regions, only to Cv Cabernet Sauvignon. In this region the average of Cv Cabernet Sauvignon was significantly higher than the Cv Merlot. The average levels of calcium found in this work, ranging from 48.61 mg.L^{-1} to 69.0 mg.L^{-1}, being lower than described for commercial Brazilian wines by Rizzon & Miele (2002), whose average content was 74.0 mg.L^{-1}. The lower values of calcium in our study may be associated with the fact that these wines are elaborated under microvinification conditions in the laboratory, and were not subjected to treatment with calcium carbonate and bentonite. According Daudt *et al*. (1987b) factors like grape variety, soil type, climatic conditions, technological processing of must and wine filtration process, bottling, storage temperature during storage and aging, influence of calcium content in wines.

Tab. 04 – Values of Litium (µg.L^{-1}) of the wines Cabernet Sauvignon e Merlot of the different studied regions

Regions	Merlot			Cabernet Sauvignon			Total		
	Mean		Standard error	Mean		Standard error	Mean		Standard error
Serra Gaúcha	4.11^{Bb}	±	0.13	4.43^{Ba}	±	0.15	4.27^{B}	±	0.10
Serra do Sudeste	5.36^{Ab}	±	0.40	6.24^{Aa}	±	0.29	5.65^{A}	±	0.30
Campanha	5.05^{Ab}	±	0.17	5.96^{Aa}	±	0.25	5.59^{A}	±	0.17
Total	4.42^{b}	±	0.11	4.96^{a}	±	0.14	4.70	±	0.09

Means followed by different capital letters in the column and means followed by different letters in the line differ significantly by ANOVA complemented by the Test of Multiple comparisons tukey significance level de 5%.

The determination of lithium, regardless of variety, allow the differentiation of the Campaign and Serra do Sudeste in the Serra Gaucha (Table 4). Besides differentiating regions, there was statistical difference between the varieties, Cv Cabernet Sauvignon, regardless of region, hard amean total significantly higher than the Merlot.

Through discriminant analysis of the experimental wines was possible to developed a statistical model to differentiate between geographical regions, using the region as the dependent variable and independent variables isotopic values and minerals. This model allowed a rating of 80% of all wines. All wines Serra do Sudeste were correctly classified (100%), followed by the Campanha (83.3%) and Serra Gaúcha (75.5%).

Conclusion

The determination of the isotopic ratio of carbon and oxygen and minerals can help in the characterization of wines from a particular region. The analysis of $\delta^{18}O$ of water, magnesium, lithium and rubidium were theδ most efficient to differentiate the three regions studied. The values of $\delta^{13}C$ of ethanol, magnesium, lithium and rubidium showed differences between varieties. The results of isotopic analysis of mineral elements and allowed a good classification of wines from different studied regions.

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Phenolic profile and tannin perception of Bordeaux red grapes for three concecutive vintages (2006, 2007 and 2008).

Kleopatra CHIRA and Pierre-Louis TEISSEDRE
UMR 1219- Œnologie, Institut des Sciences de la Vigne et du Vin, Faculté d'Œnologie, 210 chemin de Leysotte, CS 50008 33882 Villenave d'Ornon Cedex France

*Corresponding author: **pierrelouis.teissedre@u-bordeaux2.fr**

ABSTRACT

Cabernet-Sauvignon (CS) and Merlot (M) tannins and anthocyanins composition as well as tannin perception have been studied for Bordeaux grapes for three consecutive vintages (2006, 2007 and 2008). Proanthocyanidin monomers and oligomers des procyanidines were identified and quantified by HPLC-UV. Percentage of galloylation (%G), percentage of prodelphinidins (%P) as well as mean degree of polymerization (mDP) were determined by HPLC-UV-MS. Sensory analysis concerning astringency and bitterness intensity was also performed. The anthocyanic composition of skins extracts was analysed by HPLS-MS. Depending on variety and vintage, grape quality is evaluated. We observe that the mDP, % G, % P, astringency and bitterness intensity vary significantly depending on variety and tannin origin (seeds or skins). In seeds, the mDP varies between 2.2 and 8.8 (CS) and between 2.0 and 3.6 (M). In skins, mDP varies between 7.8 and 30.0 (CS) and between 4.3 and 35.4 (M). Astringency intensity in skins shows a greater variability (1.9-7.0, CS and 1.8-6.0, M) than in seeds (2.7-7.0, CS, and 2.9-6.0, M). 2006 seeds (CS) are more astringent and bitter. 2007 seeds (M) have the highest % G (33.34) and are perceived bitter. Acetylated anthocyanins values are two times highest for CS (6.99 mg/g), while M presents (3.31 mg/g) coumarylic anthocyanin levels higher than CS (1.99 mg/g).

KEYWORDS: grapes, wine, Cabernet-Sauvignon, Merlot, tannins, tannin perception, anthocyanins, vintage, HPLC/UV-MS, Bordeaux.

INTRODUCTION

Merlot (M) and Cabernet Sauvignon (CS) phenolic composition of grapes and wines has a great interest in Bordeaux wine region, as they considered being the emblematic cultivars of this region. Among red wine phenolic compounds, proanthocyanidins and anthocyanins are essential for color, astringency and bitterness (Ribéreau-Gayon, 1963; Glories, 1978; Arnold *et al.*, 1980). Grape-based proanthocyanidins contain the flavan-3-ol subunits (+)-catechin (C), (-)-epicatechin (EC), (-)-epicatechin-3-O-gallate (ECG), and (-)-epigallocatechin (EGC) (Czochanska *et al.*, 1979; Romeyer *et al.*, 1985; Prieur *et al.*, 1994; Souquet *et al.*, 1996). Skin proanthocyanidins differ from those found in seed, in that skin tannin contain prodelphinidins and have a higher degree of polymerization and a lower proportion of galloylated subunits (Somers, 1971; Gawel, 1998; Santos-Buelga ;Scalbert, 2000). Astringency and bitterness are two major characteristics in wine quality definition.

The quality of red wines is also dependent on their color, which is caused by anthocyanins and their polymers. Wine phenolic composition and sensory perception of Gamay, Dornfelder (Preys *et al.*, 2006), Syrah (Gawel *et al.*, 2007), as well as of various Italian grape varieties (Boselli *et al.*, 2004) and Croatian (Budić-Leto *et al.*, 2008) were studied. Similarly, these characteristics (phenolic composition and sensory perception) have been established for Cabernet Sauvignon and Merlot wine obtained from Washington State (Landon *et al.*, 2008). Thus, the purpose of our study is to examine Bordeaux grapes (Cabernet Sauvignon and Merlot) tannin and anthocyanin composition as well as their tannin perception

MATERIALS AND METHODS

The study was carried out with samples from Bordeaux vineyards. They are situated in Pauillac, Margaux, Saint Emilion, Saint Emilion and Côtes de Bourg. The vineyards are all planted with *V.vinifera* L. cv. Cabernet Sauvignon (CS) and Merlot (M). Grapes were collected at maturity in 2006, 2007 and 2008. Tannin extracts of seeds and skins were obtained as described previously (Chira *et al.*, 2009). Anthocyanin extracts were acquired according to Sriram *et al.*(1999) Proanthocyanidins monomers and oligomers were identified and quantified (Chira *et al.*, 2009). The proanthocyanidin mDP concentrations were quantified by phloroglucinolysis (Drinkine *et al.*, 2007). Anthocyanins identification and quantification [cyanidin-3-*O*-glucoside (Cy), delphinidin-3-*O*-glucoside (Dp), paeonidin-3-*O*-glucoside (Pn), malvidin-3-*O*-glucoside (Mv), petunidin-3-*O*-glucoside (Pt) acetylated glucosides (acet) and coumarylic glucosides (coum) of Pn and Mv was also carried out (Burns *et al.*, 2002). Sensory analysis was also conducted, twelve judges from the Oenology department of Bordeaux University participated in the sensory analysis. They were trained to evaluate astringency and bitterness of tannin extracts. All the judges were asked to hold each sample in their mouth, spit it out and rate the astringency and bitterness intensity using a 0-7 point scale. Statistical data analysis was performed using Analysis of Variance (ANOVA) using Statistica V.7 (Statsoft Inc., Tulsa, OK, US).

RESULTS AND DISCUSSION

Catechin monomers (C, EC and ECG) and proanthocyanidin oligomers (dimmers: B1, B2, B3, B4, trimmer: T) in CS and M grape skin and seed tannin extracts were identified and quantified at harvest (Tab. 1). Skin and seed proanthocyanidin profile for both varieties differed by their low amounts of galloylated derivatives and higher mDP (Sun *et al.*, 1999; Monagas *et al.*, 2003; Vidal *et al.*, 2003). Tab. 1 shows the variables that are significantly different ($p \leq 0.05$) between varieties and vintages for both skins and seed extracts.

Tab. 1: Proanthocyanidin composition of the seed and skin extracts of each variety and vintage.

	Seed extract						
	Variety*			Vintage**			
Variables	CS n=15	M n=15	p	2006 n=10	2007 n=10	2008 n=10	p
C	9.90 ± 0.67 a	6.72 ± 1.07 b	0.0398	6.67 ± 0.54 a	14.37 ± 3.39 b	3.63 ± 0.09 a	0.0000
EC	5.26 ± 1.42 a	6.58 ± 0.98 a	0.1140	5.53 ± 0.98 a	9.61 ± 1.82 c	2.62 ± 0.24 b	0.0000
ECG	0.98 ± 0.26 a	1.21 ± 0.53 a	0.3242	0.60 ± 0.21 a	2.45 ± 0.68 b	0.22 ± 0.00 a	0.0000
Sum of monomers	16.14 ± 2.35 a	14.51 ± 1.28 a	0.5154	12.80 ± 0.93 a	26.43 ± 2.62 b	6.47± 0.29 a	0.0000
B1	1.73 ± 0.53 a	1.41± 0.39 b	0.0267	3.77 ± 0.38 a	0.67 ± 0.16 b	0.27 ± 0.07 c	0.0000
B2	2.92 ± 1.24 a	1.93± 0.42 a	0.1377	1.14 ± 0.16 a	5.25 ± 0.77 b	0.89 ± 0.06 a	0.0000
B3	1.08 ± 0.34 a	0.63 ± 0.15 a	0.0782	1.08 ± 0.19 a	1.43 ± 0.43 a	0.07 ±0.00 b	0.0000
B4	2.07 ± 0.58 a	1.95 ± 0.28 a	0.6680	0.52 ± 0.14 a	3.25 ± 0.70 b	2.27 ± 0.07 c	0.0000
Sum of dimers	7.80 ± 1.18 a	5.92 ± 0.36 a	0.1357	6.51 ± 0.33 a	10.60 ± 1.43 c	3.50 ± 0.18 b	0.0000
T	0.89± 0.36 a	1.51 ± 0.76 b	0.0119	1.54 ± 0.44 a	2.22 ± 1.14 a	0.16 ± 0.05 b	0.0121
DPm	4.15 ± 0.46 a	2.74± 0.12 b	0.0000	3.02 ± 0.30 a	4.17 ± 0.32 b	3.17 ± 0.11 a	0.0000
% G	26.12 ± 3.71 a	21.22 ± 3.49 b	0.0330	14.4 ± 2.37 a	34.24 ± 5.15 b	22.38 ± 2.85 c	0.0000

	Skin extract							
	Variety*			Vintage**				
	CS	M		2006	2007	2008		
Variable	n=15	n=15	p	n=10	n=10	n=10	p
C	0.31 ± 0.06 a	0.19 ± 0.06 b	0.0409	0.22 ± 0.13 a	0.50 ± 0.07 b	0.03 ± 0.00 c	0.0012
EC	0.25 ± 0.13 a	0.18 ± 0.07 a	0.2800	0.07 ± 0.00 a	0.55 ± 0.08 b	0.03 ± 0.00 a	0.0000
ECG	ND	ND	/	ND	ND	ND	/
Sum of monomers	0.56 ± 0.12 a	0.37 ± 0.05 b	0.0143	0.29 ± 0.13 a	1.05 ± 0.26 b	0.06 ± 0.00 a	0.0000
B1	0.06 ± 0.00 a	0.10 ± 0.06 a	0.4986	0.03 ± 0.00 a	0.14 ± 0.04 a	0.07 ± 0.00 a	0.0672
B2	ND	ND	/	ND	ND	ND	/
B3	0.58 ± 0.26 a	0.22 ± 0.03 a	0.1171	0.06 ± 0.00 a	1.05 ± 0.33 b	0.12 ± 0.00 a	0.0059
B4	ND	ND	/	ND	ND	ND	/
Sum of dimers	0.64 ± 0.28 a	0.32 ± 0.06 a	0.1329	0.09 ± 0.00 a	1.19 ± 0.37 b	0.19 ± 0.01 a	0.0056
T	0.01 ± 0.00 a	0.04 ± 0.00 b	0.0001	0.03 ± 0.00	ND	ND	0.0001
mDP	22.85 ± 1.56 a	19.42 ± 1.22 b	0.0386	23.04 ± 1.07 a	20.94 ± 1.99 a	19.4 ± 1.02 a	0.1872
% G	3.24 ± 0.29 a	1.31 ± 0.28 b	0.0000	2.06 ± 0.18 ab	2.87 ± 0.40 b	1.90 ± 0.17 a	0.0233
% P	14.99 ± 1.26 a	5.54 ± 0.63 b	0.0000	6.30 ± 0.80 a	7.41 ± 1.01 a	17.07 ± 1.18 b	0.0000

*ANOVA variety effect**ANOVA vintage effect; values with different letters within each row are significantly different (Tukey's Test, p ≤ 0.05); n=number of samples; mean values of n samples; ± standard error, ND=not detected.

In seed extracts, comparing the two effects (vintage and grape variety) vintage marks more monomer and dimmer levels together with the % G. The consecutive differences between CS and M are on the variables: C, B1, T, mDP and %G. Tab. 1 indicates that the mDP of CS (4.15) is two times higher than M (2.74). In addition, the % G of CS (26.12 %) is higher when compared to M (21.22 %).

The PCA carried out on correlation matrix of the variables C, B1, mDP and %G (Figure 1) allows CS and M discrimination according to tannin composition. The first two Principal Components (PCs) explained 71.86% of the total variance, as shown in Figure 1.

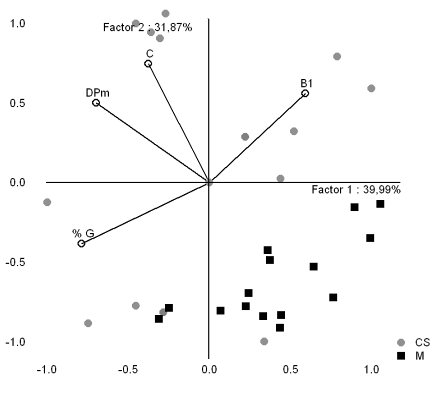

Figure 1 : PCA representation of the varieties (CS and M) and the variables (C, B1, mDP and % G) of seed extracts in the plane defined by the two first principal components.

The vintage influence is significant for all the studied variables (Tab. 1).

The PCA (Figure 2) corroborates visually that proanthocyanidin seed composition can be discriminated according to vintage.

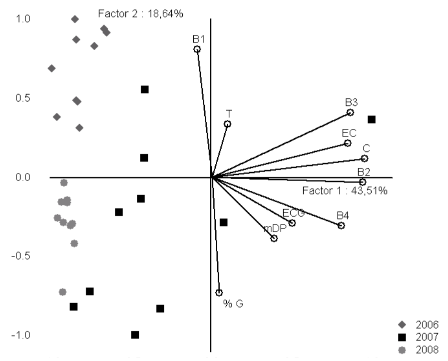

Figure 2 : PCA representation of the vintages (2006, 2007, 2008) and the variables (C, EC, ECG, B1, B2, B3, B4, T, mDP, % G) of seed extracts in the plane defined by the two first principal components.

The two first PCs, accounted for 62.15% of the total variation, separated the vintages in the light of seed proanthocyanidin composition. C, EC, ECG, B2, B3 and B4 have a positively contribution to PC1. % G and mDP are negatively heavily represented by the PC2. B1 and trimmer (T) are correlated positively with the PC2 while mDP is represented positively and negatively by the PC1 and PC2 respectively.

Regarding skin extracts, monomers and dimmers composition is influenced more by vintage than by variety (Tab. 1). Likewise, the mDP and the % G are affected more by variety (Tab. 1).

The pattern of significant differences between CS and M for the three consecutive vintages is on the variables: C, sum of monomers (C + EC), mDP, % G and % P. From the visual inspection of the PCA (Figure 3) score plot of the 30 skin extracts, it was possible to adequately discriminate the two varieties. By inspection of the eigenvectors both PCs indicates that variety discrimination of skins is influenced by the mDP, % G, % P and C. The PC1 is heavily positively correlated with the variables mDP, % G and C; the PC2 is strongly negatively correlated with % P. The M skin samples are mostly grouped on the positive side of the PC2 whereas CS skin samples are dispersed on the left part of the PC1. The CS skin extracts are characterized by a mDP (22.85), a % G (3.24 %) and a % P (14.99 %) higher than M (mDP = 19.42, % G = 1.31, % P = 5.54).

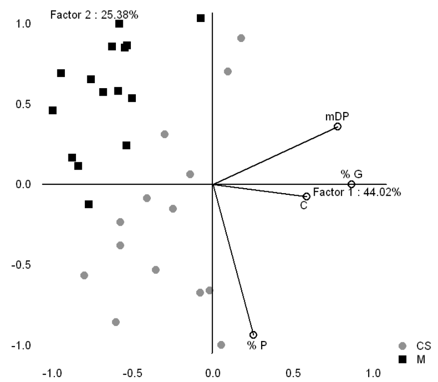

Figure 3 : PCA representation of the varieties (CS et M) and the variables (C, mDP, % G and %P) of skin extracts in the plane defined by the two first principal components.

Vintage effect was observed on the sum of monomers (C + EC), dimmers (B1 + B3), % G and %P. The PCA applied to the above variables (data not shown) shows that there is not a clear separation among vintages according to proanthocyanidin composition.

Concerning anthocyanin composition, consecutive and significant differences between CS and M for the three vintages are on Cy, Pt, Pn, Mv, acetylated and coumarylic anthocyanins (Tab. 2).

Tab. 2 : Anthocyanic composition of the skin extracts of each variety and vintage

Variables	Variety*			Vintage**			
	CS	M		2006	2007	2008	
	n=15	n=15	p	n=10	n=10	n=10	p
Dp	4.17 ± 0.26 a	4.55 ± 0.46 a	0.4376	5.40 ± 0.43 b	3.81 ± 0.41 a	3.86 ± 0.50 a	0.0168
Cy	0.84 ± 0.14 a	1.73 ± 0.21 b	0.0004	1.75 ± 0.26 b	1.18 ± 0.25 ab	0.94 ± 0.19 a	0.0206
Pt	2.58 ± 0.14 a	3.62 ± 0.28 b	0.0002	3.43± 0.22 b	3.02 ± 0.29 ab	2.84 ± 0.32 a	0.0433
Pn	3.24 ± 0.31 a	4.51 ± 0.49 b	0.0112	5.49 ±0.42 a	3.72 ± 0.54 ab	2.43 ± 0.38 b	0.0000
Mv	13.11 ± 1.07 a	10.83 ± 0.66 b	0.0260	15.42 ± 1.34 a	12.28 ± 0.70 ab	8.22 ± 0.45 b	0.0000
Sum of glucosides	23.94 ± 1.63 a	25.24 ± 1.87 a	0.4665	31.49 ± 1.80 a	24.01 ± 2.01 ab	18.29 ± 1.44 b	0.0000
Pn-acet + Mv-acet	6.99 ± 0.70 a	3.78 ± 0.35 b	0.0118	7.14 ± 1.04 a	5.03 ± 0.39 b	3.99 ± 0.59 b	0.0254
Pn-coum + Mv-coum	1.99 ± 0.16 a	3.31 ± 0.26 b	0.0000	3.24 ± 0.33 a	2.76 ± 0.28 ab	1.95± 0.26 b	0.0018

*ANOVA variety effect**ANOVA vintage effect; values with different letters within each row are significantly different (Tukey's Test, p ≤ 0.05); n=number of samples; mean values of n samples; ± standard error, ND=not detected.

Particularly, acetylated anthocyanins concentration is twice higher in CS (6.99 mg/g) than in M (3.78). M skin extracts possess almost two times greater coumarylic levels (3.31 mg/g) than that of CS (1.99 mg/g).

The discrimination of the samples based on variety was achieved; using PCA that considered the concentrations of the different anthocyanic forms in the skins extracts (Figure 4). The PC1 accounts for 56.28% of the variability and the PC2 for 24.42%.

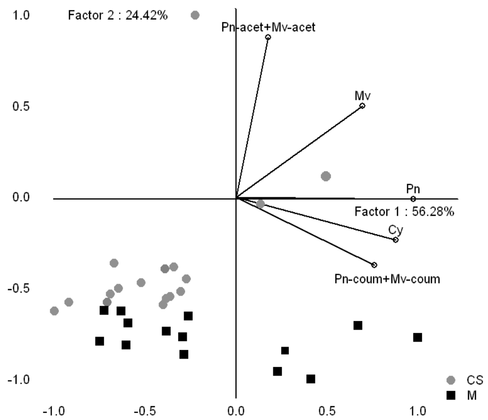

Figure 4 : PCA representation of varieties (CS and M) and the variables of skin extracts (Cy, Mv, Pn, Pn-acet+Mv-acet and Pn-coum+Mv-coum) in the plane defined by the two first principal components.

The vintage effect on anthocyanins composition is more significant than that of variety on the sum of monoglucosides whereas acetates and coumarates are more influenced by variety (Tab. 2). Figure 5 demonstrates that 2006 skin extracts have a good correlation with Pn, Mv, Dp and coumarate levels and are separated from these of 2007 and 2008. The climatic conditions of each year presented differences that could have influenced anthocyanins synthesis.

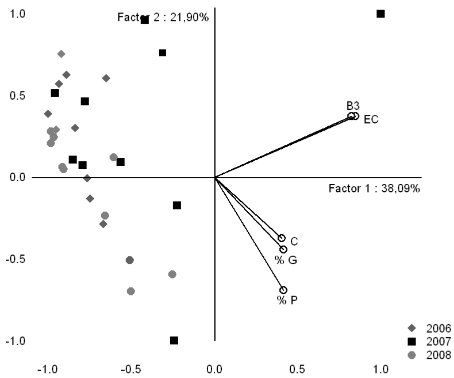

Figure 5 : PCA representation of the vintages (2006, 2007, 2008) and the variables (C, EC, B3, % G and % P) of skin extracts in the plane defined by the two first principal components.

For the three vintages, grape variety showed the same behavior pattern, it has no influence on both bitterness and astringency intensity (Tab. 3).Conversely, vintage effect is significant for both extracts and for both criteria (astringency and bitterness). I

Tab. 3 : Astringency and bitterness intensity of seed and skin extracts of each variety and vintage.

Extract origin	**Astringency intensity**						
	Variety*			**Vintage****			
	CS	**M**	**p**	**2006**	**2007**	**2008**	**p**
Seeds	4.6 ± 0.6 a	4.4 ± 0.2 a	0.3429	5.0 ± 0.5 a	4.2 ± 0.1 b	4.4 ± 0.1 ab	0.0395
Skins	4.4 ± 1.0 a	4.1 ± 0.3 a	0.1033	5.3 ± 0.4 a	4.2 ± 0.1 b	3.3 ± 0.4 c	0.0001
	Bitterness intensity						
	Variety*			**Vintage****			
	CS	**M**	**p**	**2006**	**2007**	**2008**	**p**
Seeds	3.8 ± 0.9 a	3.8 ± 0.3 a	0.5467	4.2 ± 0.7 a	4.0 ± 0.3 a	3.3 ± 0.5 b	0.0000
Skins	3.8 ± 1.2 a	3.8± 1.0 a	0.6651	5.0 ± 0.3 a	3.3 ± 0.4 b	3.1 ± 0.3 b	0.0000

*ANOVA variety effect**ANOVA vintage effect; values with different letters within each row are significantly different (Duncan's Test, p ≤ 0.05); ± standard error.

CONCLUSION.

In our work we investigated variety (CS and M) and vintage (2006, 2007 and 2008) influence on proanthocyanidin, anthocyanin composition and sensory perception of grape skin and seed extracts. Principal component analysis (PCA) models were built to identify phenolic parameters exploitable to classify varieties and vintages. In both extracts (seeds and skins) varieties were discriminated according their phenolic composition. We demonstrate for the first time in Bordeaux winegrowing region that the mDP, the % G and the % P varies in conforming with tannin source (seed or skins) and with variety. In seeds, the mDP and the %

G of CS is higher when compared to M seeds. In skins, the mDP, the % G the % P of CS is always greater when compared to M but in a different scale in relation to seeds. Regarding anthocyanic composition acetylated anthocyanins values are two times highest for CS, while M presents coumarylic anthocyanin levels higher than CS.

With reference to vintage, it had a significant impact on both phenolic composition and tannin perception. Almost all the studied variables in both extracts were more influenced by vintage than by variety. 2007 vintage presented the richest proanthocyanidin profile whereas 2006 vintage characterized by highest anthocyanic levels. Concerning tannin perception, vintage has disclosed an effect while the variety hasn't. We explore that bitterness intensity is more affected by vintage than astringency. This study, on phenolic composition, provides oenological information that can be useful to improve the quality of the wines produced.

AKNOWLEDGEMENTS. The authors gratefully thank CIVB (Conseil Interprofessionnel des Vins de Bordeaux), Ministère de La Recherche et Enseignement Supérieur for providing financial support, the Region Aquitaine for providing funds for the acquisition of the HPLC-MS(*n*)-UV material, Pr Doneche Bernard and Dr Geny Laurence for providing the grape samples and the vineyard sections to allow this research. We also thank the judges who participated in the sensory analyses

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Study of Pectin substances of grapes, for the purpose of obtaining the functional foodstuffs

Bagaturia N.,
Razmadze st. #57, Tbilisi, Georgia
GFS_company@yahoo.com
Mujiri L.,
Kazbegi ave. #55, Tbilisi, Georgia
GFS_company@yahoo.com
Begiashvili N.A.
Mosashvili st. #2, Tbilisi, Georgia
GFS_company@yahoo.com
Razmadze st. #57, Bagaturia B.N.
Tbilisi, Georgia
GFS_company@yahoo.com

Abstract

Are carried out studies on the establishment of content and quality of pectic substances in the by-products of processing grapes - crests and the refuse, for the purpose of their further use for obtaining pectincontaining functional food products.

It is established that in the grapes are contained low-methoxylized pectins, which are capable of adsorbing toxins, heavy metals, radionuclides and of deriving them from the organism. This property can be used for obtaining the products with the radio- protector properties.

Grape pectic concentrate was used for the production of nonalcoholic beverages, in baking and canning industry instead of dry pectin.

Grapes are the natural source of the nourishing and medicinal substances. The products made from the grapes have therapeutic and prophylactive properties. The pectic substances of grapes are capable to adsorb toxins, heavy metals, radionuclides and deriving them from the organism (1). Their property can be used for obtaining functional food products.

The presence in pectins of the free carboxyl groups of galacturonic acid causes their property to connect the ions of heavy metals and radionuclides in the gastrointestinal tract, consuption with the subsequent formation of the undissolved complexes (pectinates, pectates), which are not sucked and are derived from the organism.

For toxicological and hygienic reasons pectin substances were anknoledged as practically safe substance by the united committee of FAO. That is why no consuption limitations and maximum permissible doses of consuption has been esteblished.

The degree of esterification, which indicates a quantity of methoxylized carboxyl groups of polygalacturonic acid, is the basic index of quality of pectins. Industrial pectins divide into high(ly)-methoxylized and low-methoxylized. Pectins with the degree of esterification, which exceeds 50% (high(lyied-etherify), possess capability for jellification and gel formation and adapt in the canning industry for preparing of marmalade and other jellified foodstuffs.Apple and citruc' pectines belong to them. For forming the steadfast from jelly it's necessary the low index of pH (about 3,0) and the presence of sugar (2). Low-methoxylized pectins form gel in the presence of the ions of calcium or other polyvalent metals, but over wide limits pH. Specifically, low-methoxylized pectins possess the ability to form in the organism the undissolved complexes due to deme-toxification of pectin and its transformation into poly-galacturonic acid, which is connected with the specific heavy metals and the radionuclides.

Table 1

Content of polysaccharides and lignine in the second converted products of grapes (% from the empty weight of raw material)

O B J E C T	Opgeloste pectin	Protopectin	Hemicellulose A	Hemicellulose B	Cellulose	Legnin
Refuse of the grapes	2,8	3,9	13,9	7,8	21,9	32,9
Crests of the grapes	1,1	2,8	16,7	8,5	19,8	35,9
Seeds of the grapes	1,5	1,8	15,8	9,1	15,5	30,8
Trimmings of the vine	1,2	6	14,5	8,8	25	28,5
Green parts of the vine	1,3	5,4	6,6	3,9	17	21
Wood of the oak	0,8	0,9	12,9	10,1	29,8	33,4

Table 2

Influence of different extractants on the output of pectic substances from the refuse of the grapes

E x t r a c t a n t	Concentration of the acid, %	Output of pectin , %		
		I	II	III
Tartaric acid	0,5	3,54	3,01	3,03
Citric acid	0,5	2,11	1,91	2,16
Acetic acid	0,5	1,76	1,53	1,73
The oxalic acid	0,5	2,33	2,22	2,06
Oxalic acid ammonium	0,5	2,89	2,47	2,54
Sulfuric acid	0,5	5,55	5,40	5,48
Hydrochloric acid	0,5	4,32	5,41	5,10
Nitric acid	0,5	1,28	0,95	1,19

We carried out studies on the establishment of content and quality of pectic substances in the production wastes - crests and the refuse of grapes, for the purpose of their further use for receiving of pectincontaining functional food products. The industrial types of grapes of Georgia served as the subjects of the study – Kakhetian Saperavi, Rkatsiteli, Tsolikouri and Mtsvane.

The output of pectic substances depends on the form of the utilized extract. From the organic acids the best results were obtained during extracting raw material by tartaric acid; however, as citric acid is considerably cheaper in comparison with wine, further studies on the establishment of the regime parameters of the process of the extraction of pectin (fig. 1) were carried out with the use of the citric acid (1).

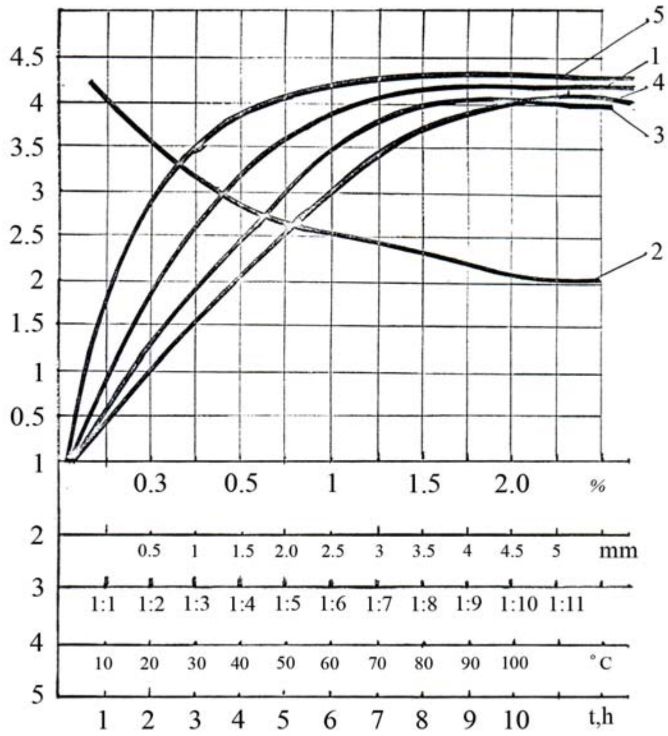

fig.1. **Extraction of pectin substances from the grapes refuse**

Data of fig.1 show that optimum conditions for the extraction of pectin are: 0,5% - citric acid, hydromodulus 1:5, the temperature 80^{0}C, the time of extracting 3,5 hours, the index of size reduction of refuse of 0,5-5mm with the humidity of refuse 8-10%.

In table. 3 are shown chemical-engineering the indices of pectin, obtained from the refuse of the grapes

Table 3

Chemical-engineering the indices of pectin, obtained from the refuse of the grapes

Concentration of the acid, %	Indices					
	General output of pectin	Ureniadi, %	The methoxy groups	Ash substances	Viscosity sec. 0,5% solution	Gel formation mm. рт. ст. 0,6% solution
0,3	3,05	82,1	10,60	4,00	150	145
0,5	3,82	85,3	8,41	3,73	172	161
0,7	4,01	89,2	6,08	3,30	169	157
1,0	4,10	91,6	4,49	2,98	150	132

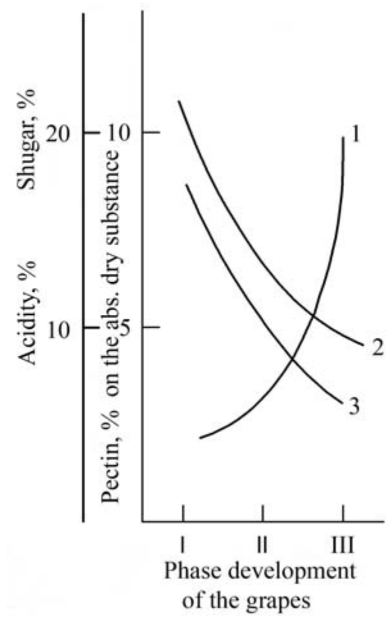

Fig.2. **dynamics is the content of pectic substances in the crests of the grapes**

Both the refuse and the crests of grapes can be used as the raw material for obtaining pectin. The percentage of crest in the cluster, depending on the degree of the maturity of grapes and ambient conditions of its growth, varies in the limits from the 1st to 8,5% and on the average composes 3,5% of the mass of cluster. With the complete maturity of berries in the majority of the types of grapes the crest is impregnated with lignine, by suberin it lignifies.

Data analysis of fig. 2 show that, in parallel with ripening of grapes, the content of pectin substances in the crests is reduced, and it comprises with the harvest of grapes on the average, depending on type, 3,8- 5,5%.

From tables. 4, we can see that according to the quality indicators pectins, obtained from the crests of the different types of grapes are close. If we judge by the degree of etherification, grape pectin should belong to low-methoxylized pectins. Such pectins can be used for radio- protection of food.

Table 4

Chemical indices of the pectic substances, obtained from the crests of different types of the grapes

Type of grapes	Methoxy groups, %	Degree of esterification, %	Content of clean pectin, %
Rkatsiteli	15,2	48,4	46,9
Tsolikouri	15,5	48,3	46,5
Saperavi	14,1	46,3	43,3
Kakheyian Green	13,6	45,1	43,7

From the data of table. 5 it is evident that the production of pectin is connected with the heavy expenses of food rectified alcohol. Additionally expenditives for producing of dry pectin is high.

Table 5

The material balance of the production of pectic powder from the grape crests

Name	Un. of measurement	Value
Source material:		
Weight	kg	300
Content of pectin	%	3,95
The end product:		
Dry pectin	kg	0,84
Humidity	%	12,3
Output from the crests	%	2,8
Ethyl alcohol:		
Initial quantity	l, abs. alcohol	212,76
Recovery		199,13
Losses	«---«	13,63
	%	

The development of science, engineering and technology of the production of pectin makes it possible to find the simpler new technological solutions by improvement and reduction of prices of its production. In particular, studies of the last years showed that it is appropriate produce not only dry pectin, which requires complex technological equipment and high power and material expenditures, but also such products as pectic extracts and the concentrates, the technologies of obtaining the which are more universal, were simple in the equipment performance and ecological.

From the grape refuse and the crests of grapes it is possible to prepare the so called pectic concentrate, so liquid preparation with the increased content of pectic substances (3-5%). For obtaining the extract damp grape refuse is washed in water with the temperature of 50-60oSC during 10 minutes (obtained extract it is used for obtaining the strong alcoholic beverage - Chacha). Then the washed refuse were flooded with the temperature of 80-90o C and they conducted hydrolysis- extracting pectic substances with the following parameters of process: relationship of refuse and water 1: 3, duration - 3 hours. In the course of the process of hydrolysis- extracting the mixture constantly was intermixed. After endurance in the hot water for 1 hour the solution (drawing) is separated from the refuse; refuse press and liquid obtained in this case is added to the basic solution. The blended the wines drawing contains little pectin - 0,3 - 0,7%. They congeal it in the vacuum- apparatus 3-4 times, they spill in the bottle and they pasteurize. pectic concentrate obtained in a described manner can be used for the production of nonalcoholic beverages, in the baking and canning industry instead of dry pectin.

The steady tendency of an increase in production and consumption of nonalcoholic beverages is observed in the entire world. Thus, the consumption of nonalcoholic beverages per year person comprises (l): in Germany - 195, including mineral waters - 80, juices - 32, the refreshing beverages - 83; in the USA - 164, Belgium - 129, Czech Republic - 110, Sweden - 55. Beverages on the basis of the juices, enriched by the pectic substances, which gives them the radio-protector and detoxicated properties, are the most promising functional products. More over, the consumers select nonalcoholic beverages with functional qualities.

Because of to their the complex- forming, gel-forming, emulsifying properties pectins also adapt in the production of confectionery, canning articles, therapeutic preparations, in the bread-baking.

In this respect obtaining of pectic extraxt for food using for therapeutic and prophylactic reason can be considered as the most interesting.

In process of developing new direction in the nourishment - production and the consumption of functional products, the role of bread in the nourishment acquire separately important significance. Bread - this is the product of daily nourishment, they eat it repeatedly during the day, and therefore it together with the high nourishing properties must have therapeutic or preventive properties, which is achieved by introduction into the formula of functional additives. The pectic substances are one of such promising additives.

It is established that the introduction of pectins in dough influences the biological, colloidal and microbiological processes. With the introduction of pectins in dough rises its initial acidity, fermentation process continues more activly (activation of fermentation process it is connected with the introduction of sugar together with pectin). Also it is observed strengthening gluten, strengthening the structural and mechanical properties of test.

Thus, the applications of pectic concentrates from crests and refuse of grapes are analogous to the application of dry pectin and make it possible not only to improve the quality of finished articles, but also to enlarge the assortment of the food of therapeutic and prophylactic designation.

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Decreasing of production of ethanol by *Saccharomyces cerevisiae* metabolism control

M. Berlot[1], M. Berovič[1]

[1]Department of Chemical and Biochemical Engineering
Faculty of Chemistry and Chemical Engineering
University of Ljubljana
Askerceva 5, 1001 Ljubljana, Slovenia
E-mail : matjaz.berlot@fkkt.uni-lj.si, marin.berovic@fkkt.uni-lj.si

ABSTRACT

Present research was focused in the development of new method for the regulation of *Saccharomyces cerevisiae* metabolism using thermal shocks on inoculum suspension of yeast cells. For time intervals 10, 20 and 30 min yeast cell suspension was exposed to the temperature of 45 °C. The most resistible cells that survived this thermal shock represented new active biomass that include also a memory effect that enables decreasing ethanol production by increasing for production of glycerol up to 100% at reduction of ethanol for 6-10 %. Present results represent a new and easy method applicable for high glycerol production also in large scale wine production.

Introduction

Due to its high specific gravity and viscosity, glycerol significantly contributes to a fullness and complexity especially in premium quality dry wines (Scanes, 1998; Nieuwoudt *et al*, 2000; Karasu Yalçin, Ozbas, 2006). Extensive studies of *Saccharomyces cerevisiae* metabolism proved that that glycerol biosynthesis of glycerol is related to yeast strain, process condition and consistence of fermentation substrate (Scanec, 1998; Remize *et al*, 1999; Karasu Yalçin, Ozbas, 2008a) 4 to 10 % available glucose is converted to glycerol (Jackson, 2008).

Under anaerobic conditions glycerol is formed in order to reoxidize NADH formed in anabolism and in the synthesis of organic acids (Nieuwoudt *et al*, 2000, Jackson, 2008, Karasu Yalçin, Ozbas, 2008b). The anaerobic conversion of glucose into ethanol by *Saccharomyces cerevisiae* is redox neutral, i.e. NAD^+ consumed initially in the Embden-Meyerhof-Parnas (EMP) pathway and is regenerated when ethanol is produced. However, when intermediates in the EMP pathway are withdrawn as precursors for synthesis of cellular material this balance is distributed since the surplus of produced NADH is not converted back into NAD^+ (Hohmann, 1998, Karasu Yalçin, Ozbas, 2005). This will eventually cause metabolism to stop unless other processes exist for the regeneration of NAD^+. During anaerobic growth of *Saccharomyces cerevisiae* NADH cannot be oxidized by oxygen but must be disposed by formation of reduced by-products like glycerol (Jackson, 2008, Oura, 1977, Karasu Yalçin, Ozbas, 2004).

In glycerol biosynthesis in wine many growth and environmental factors have been reported to influence the amount of glycerol produced as fermentation temperature, strain selection, inoculation level, sulphite concentration, sugar concentration, osmotic stress, nitrogen source and concentration, pH, aeration, grape variety and ripeness (Ribéreau-Gayon, 2000, Carrasco *et al*, 2001).

Fermentation temperature is in many technologies one of the most influential factors affecting process of various metabolite biosynthesis. Not only does temperature directly and indirectly influence yeast metabolism, but it is also one of the features over which the winemaker has the greatest control (Costenoble *et al*, 2000, Torija Jesus *et al*, 2003).

The effect of heat shock treatment in alcohol fermentation was investigated in the shochu yeast BAW-6, sake yeast K-7, wine yeast W-3, beer yeast IF01167, and whisky yeast IF02363 (Omori *et al*, 1996).

The main objective of this work was to study the extend of heat shock intervals on glycerol production, applied on cell suspension of wine yeast *Saccharomyces cerevisiae* inoculum starter culture.

Materials and Methods

Microorganism

Dry wine yeast strain (*Saccharomyces cerevisiae*, ANCHOR NT202 South Africa) was used in all of the experiments. Reactivation of 3 g of dry yeasts was performed 20 min at 30 °C in a 20 ml of water diluted (1:1) grape juice of cultivar sauvignonasse. Suspension of wine yeast exposed for 10, 20 and 30 minutes to temperature 45 ºC.

Fermentation substrate

Fermentations were carried out on a grape juice of cultivar Sauvignonasse (before Tocai Friulano) from wine growing region Goriška Brda. As bioactivator to the initial substrate 0.40 g /L Fermaid E (Danstar Ferment AG) was added .

Fermentors

All laboratory scale experiments were performed in identical standard type configuration 10 l working volume Stirred Tank Reactors (Bioengineering AG, Switzerland). In all of the experiments Bioengineering AG fermentors were equipped with : Ingold pH and redox electrodes, Industrial Lab pO_2 electrode MFG 509 and temperature control unit (Mettler Toledo). For on-line measurements SHIVA control software (BIA d.o.o., Slovenia) was applied. Fermentors head space was aerated through with the inert gas N_2 to prevent the oxidation of fermenting grape must. In all of experiments mixing of 100 rpm was applied during fermentation. Fermentor head space was continuously aerated with nitrogen.

Fermentations were performed at temperatures 18 and 22 °C. Three series of each experiment were performed. The F ratio and

Student's t test were used to determine if the samples were statistically significant at a 95% confidence level.

Analytical methods

100 ml of samples were taken every 24 hours. After filtration the major extra cellular metabolites, were determined by HPLC. The reducing sugars (glucose and fructose), glycerol, ethanol and organic acids were analysed according to validated methods proposed by BIO-RAD 1997. The samples were analysed using 300 mm × 7.8 mm Aminex HPX-87H organic acid cationic exchange column (Bio-Rad Laboratories USA). Elution was performed at 65 ºC. The mobile phase was 2 mM H_2SO_4 in bi-distilled water. The pump was operating at flow rate 0.6 ml min^{-1}. The injection volume was 20 μl ; the eluting compounds were monitored by a fixed wavelength ultraviolet (UV-VIS) detector at 210 nm. Detector was connected in series with a refractive index (RI) detector. Samples were filtered through 0,45 µm membrane. Ethanol, glycerol, glucose and fructose were detected by the RI detector. Organic acids were detected by the UV-VIS detector.

Results and Discussion

Various duration of heat shocks on *Saccharomyces cerevisiae* inoculum where yeast cells suspension was exposed to 45 °C eliminated on one side unproductive, too young or too old cells, from further fermentation process. Comparing production of biomass higher biomass was obtained at the same fermentation temperatures in experiments with applied heath shock than those in control.

Ethanol production was the most expressed at control fermentations where 104 g/L in fermentation at 22 °C and 89 g/L at 18 °C were produced. On the other side in fermentations where heath shock on inoculum was made significant differences were observed.

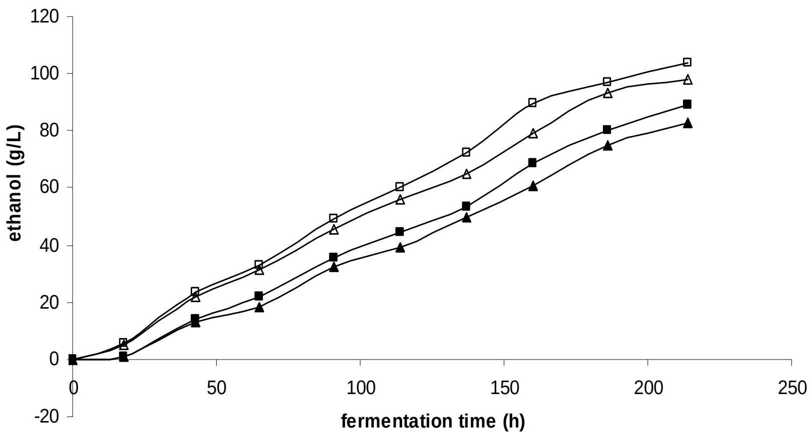

Fig. 1. Accumulation of ethanol

■ temperature 18 °C; □ temperature 18 °C - 20 minutes heat shock; ▲temperature 22 °C; Δ temperature 22 °C - 20 minutes heat shock

But it was fascinated discovery that at high temperature exposure the survival cells created a memory effect that they were exposed to high temperature, so the survived cells in further fermentation process protected themselves with expressed production of glycerol. In both control at 18 °C , 6.3 g/L and at 22 °C , 7.8 g glycerol /L was detected. The highest glycerol production was detected at 10 min heath shock and fermentation temperature 22 °C. The results in fermentation showed that the duration of the heath shock interval reasonable increase the biosynthesis of glycerol, while higher initial fermentation temperature promotes the rate of kinetic processes in biosynthesis of various organic acids.

For effective monitoring of *Saccharomyces cerevisiae* microbial activity during the fermentation *on-line* redox potential measurement was used as main monitoring parameter in all of the experiments. Through fermentation, yeast cells adjust their redox balance to the conditions in the must producing adequate amounts of ATP, maintaining favourable redox and ionic balances, and synthesize the necessary metabolic intermediates (Jackson, 2008).

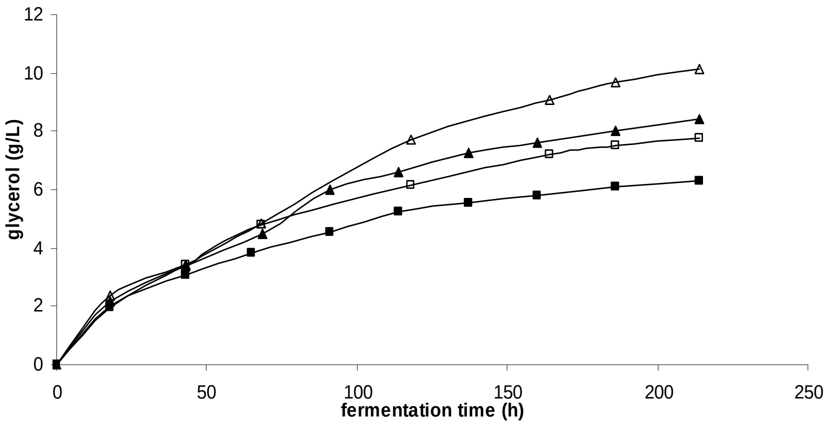

Fig. 2. Accumulation of glycerol

■ temperature 18 °C; □ temperature 18 °C - 20 minutes heat shock; ▲temperature 22 °C; Δ temperature 22 °C - 20 minutes heat shock

Conclusions

From our experiments it was found that production of glycerol is temperature dependent process. Higher fermentation temperatures initiated faster production of glycerol as the main osmoregulator and redox balancing substance. On the redox potential curve profile faster and expressive decrease was detected in the case of higher temperatures. The duration of the lag phase and the delay before the initiation of fermentation become shorter with increasing temperatures.

Application of heat shock during active fermentation process is an effective and simple method of increasing the glycerol concentration in wine. Due to its relatively high specific gravity and viscosity, glycerol may remarkably contribute to the overall sensory perception of body, as well as impression of fullness and sweetness especially in dry white wines.

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Réduction de la teneur en alcool des vins : Application sur un vin de base de Chenin en Vallée de la Loire

Pascal Poupault (1), **Philippe Cottereau** (2), **Christophe Riou** (2)
(1) IFV, 46, Avenue Gustave Eiffel – 37100 Tours – pascal.poupault@vignevin.com
Tél : 02 47 88 24 20 – Fax : 02 47 88 24 21
(2) IFV, Domaine de Donadille, 30200 Rodilhan – philippe.cottereau@vignevin.com
christophe.riou@vignevin.com

SUMMARY

The climatic conditions of these last years, probably linked to global warning climate lead to modifications in the sugar-acid balance in wine. Better conditions of maturation are detected in the Loire Valley, and lead the elaboration of wines richer in flavor but with a more important whealth in alcohol. For the base wine of Chenin, these changes lead to degrees with excessive natural alcohol, wich has consequence on good realization of the second in-bottle fermentation. An earlier harvest is not satisfactory for the aromatic quality and lead to the development of wine too acid.

Innovative techniques have been tested: reduce the sugar content of must (RTS) and wine by partial desalcoholisation. These practices have been very satisfactory, allowing more successfully second in-bottle fermentation, while controlling the final degree of the wine. The sparkling wines produced from base wines with reduced level of alcohol present a very good olfactory and gustatory balance. The physicochemical analysis of wine confirms sensory observations.

KEY WORDS : base wine, innovation, alcohol reduction

RESUME

Les conditions climatiques de ces dernières années, sans doute lié au réchauffement global du climat, entraînent des modifications dans les équilibres sucre-acide des vins. De meilleures conditions de maturations sont constatées sur la région Vallée de la Loire et entrainent l'élaboration de vins plus riches en arômes mais avec une richesse en alcool plus importante. Pour les vins de base de chenin, ces modifications conduisent à des degrés alcooliques naturels excessifs avec une conséquence sur la mauvaise maîtrise des prises de mousse. Une récolte plus précoce n'est pas satisfaisant au niveau aromatique et conduit à l'élaboration de vins trop acide.

Des techniques innovantes ont été testées sur moût : réduction de la teneur en sucre des moûts (RTS) et sur vin par une désalcoolisation partielle. Ces pratiques ont donné des résultats très satisfaisants, en permettant des prises de mousse réussies, tout en maîtrisant le degré final du vin. Les vins effervescents élaborés issus des vins de base à teneur réduite en alcool sont de très bonne qualité au niveau de leur équilibre gustatif et olfactif. Les analyses physicochimiques des vins confirment les observations organoleptiques.

MOTS CLES : Vin de base, innovation, réduction alcool

INTRODUCTION

Les politiques de qualité suivies au vignoble permettent l'élaboration de vins plus concentrés, aux arômes plus expressifs, mais souvent plus riches en alcool et parfois de manière excessive. L'évolution des conditions climatiques favorisent, y compris dans les régions septentrionales, de meilleures conditions de maturation, qui permettent d'obtenir des vins avec un profil répondant au marché. D'un point de vue qualitatif, la solution consistant à avancer la date de récolte n'est pas envisageable. Aussi, la baisse de la teneur en alcool (1)des vins a été abordée sous les deux aspects techniques proposés par les nouvelles technologies(2) : réduction de la teneur en sucres des moûts (procédé REDUX associant ultrafiltration et nanofiltration) et désalcoolisation partielle du vin (couplage Osmose Inverse et distillation)(4). Dans le cadre de l'essai mené sur Chenin, le désucrage et la désalcoolisation peuvent amener des réponses aux producteurs de vins issus de méthode traditionnelle, confrontés ces dernières années à des richesses en sucres importantes. Ces richesses en alcool des vins de base posent problème pour la réalisation de la prise de mousse. Le fait de pouvoir baisser la richesse initiale du moût ou le degré alcoolique du vin de base peut permettre une approche plus qualitative par une recherche de profil aromatique plus intéressant. Le producteur recherche un meilleur compromis entre un potentiel aromatique maximum et une richesse en alcool modérée permettant une prise de mousse dans les meilleures conditions(3).

La nouvelle OCM se traduit par une nouvelle réglementation vitivinicole où de nouvelles pratiques œnologiques sont autorisées. La désalcoolisation partielle en fait partie mais la diminution alcoolique sera limitée à -2%vol (règlement européen n° 606 du 10 juillet 2009 applicable au 1ier aout 2009). Des références ont été obtenues sur les trois derniers millésimes(5).

MATERIELS ET METHODES

La réduction de la teneur en sucre (RTS) est réalisée par l'association de 2 techniques membranaires judicieusement choisies (UF – ultrafiltration - suivie de NF – nanofiltration) (Schéma 1). C'est une alternative intéressante à la désalcoolisation des vins en permettant une réduction de la teneur en sucre des moûts de raisin. Cette technique permet de vinifier des moûts moins riches en sucres (éventuellement éviter des problèmes fermentaires) sans éliminer les arômes acquis sur les vins comme on peut le craindre avec la désalcoolisation.

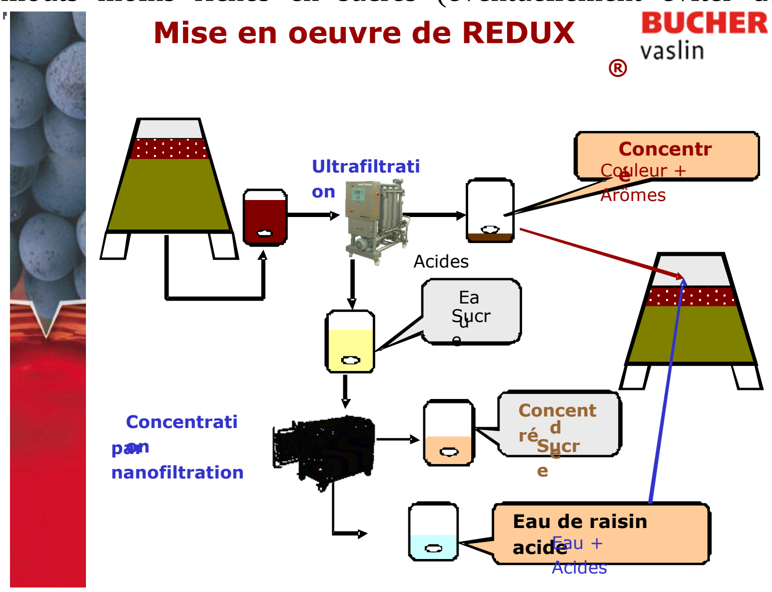


Schéma 1 : Principe de la réduction de la teneur en sucre des moûts

Le principe de la méthode repose sur une première ultrafiltration permettant d'extraire un « moût incolore » de même concentration en sucre que le moût initial. Ce « moût » est ensuite concentré par nanofiltration et le perméat récupéré, essentiellement constitué d'eau et d'acides, est réincorporé dans le moût traité. Le volume de moût concentré par nanofiltration est calculé en fonction du besoin de diminution du degré probable souhaité.

Pour l'opération de désalcoolisation (Schéma 2), le vin est traité par nanofiltration afin de séparer un perméat essentiellement constitué d'eau, d'alcool, d'acides et de minéraux, ainsi que quelques arômes. Ce perméat, représentant environ 18% du volume total pour une baisse de 2 degrés, est distillé sous vide poussé afin d'en extraire l'alcool. L'eau « native » récupérée, contenant l'eau et les éléments non volatils, est ensuite réintroduite dans le vin traité pour abaisser son degré.

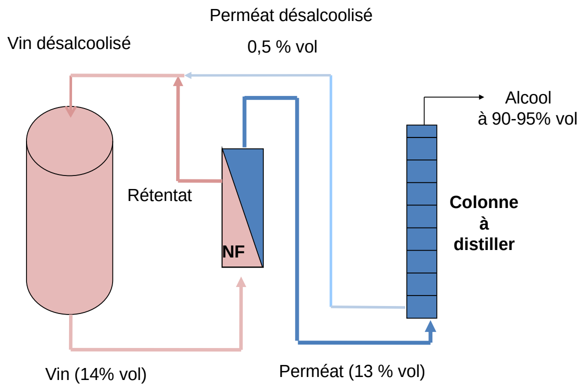


Schéma 2 : Principe de la désalcoolisation du vin

Les vinifications sont réalisées en cuve inox de 6 hl, dans des conditions identiques (maîtrise température, levurage).

Pour réaliser le lot RTS en vinification en blanc, du moût de Chenin enzymé (KZYM à 2g/hl) et débourbé est réparti en deux cuves d'une contenance de 6 hl, à raison de 550 L par cuve. Compte-tenu du volume de la cuve et de la réduction de la teneur en sucres recherchée (-1,5 à -2% en TAP), 157L (25%du volume total + 20L correspondant au volume « mort » de l'appareil) de cette fraction de moût débourbé (10 -15NTU) sont ultrafiltrés. Le perméat d'UF est concentré par NF, jusqu'à obtenir le volume voulu (concentré riche d'au moins 300 g/L de sucre). Pour chacune des dates de vendanges manuelles, un cycle de pressurage d'une durée de 3 heures est appliqué. Le sulfitage de la vendange est réalisé au pressoir, par un apport de 5 g/hL en SO_2.

Sur une partie du vin témoin de la même date de récolte que le lot RTS, la désalcoolisation est réalisée sur le vin déjà clarifié (2 soutirages), pour obtenir un vin de base « désalcoolisé » et ramené à un TAP de l'ordre de 11,5%vol. Les levains sont lancés pour l'ensemble des 8 modalités de prise de mousse. La levure choisie est la Levure Sèche Active « Actiflore Bayanus ». Deux levains différents de par l'origine du sucre sont élaborés en fonction de l'origine du sucre : un levain élaboré à base de saccharose du commerce et un autre élaboré à partir du semi-concentré issu de l'opération RTS sur moût.

L'ensemencement de chaque modalité est réalisé quand le levain descend sous une valeur de densité de 1000. L'apport du levain se fait par bouteille, par un ensemencement à 3% en volume (soit 22,5 ml de levain pour 750 ml de vin de base). Pour éviter un éventuel démarrage de Fermentation MaloLactique, chaque bouteille est additionnée de lysosyme, à raison de 250 mg/l. Pour une des modalités, la prise de mousse est réalisée avec les sucres résiduels ; pour cela, le vin est muté au froid en cours de fermentation.

RESULTATS ET DISCUSSION

Incidence de la date de récolte

L'objectif est de procéder, sur une même parcelle, à deux récoltes afin d'obtenir, pour la première date, un degré potentiel proche de celui recherché habituellement (10,5 à 11%vol) et, pour la deuxième date, un degré potentiel significativement plus élevé (12 à 13%vol).

Caractéristiques de la vendange

Une différence de maturité significative existe entre les deux dates de vendange ; elle est surtout marquée par une augmentation du taux de sucres et une baisse d'acidité pour la vendange plus tardive. Les conditions climatiques (chaleur et absence plus ou moins marquée de précipitations) ont favorisé cette concentration assez importante en sucres en une dizaine de jours et une baisse relative de l'acidité du moût.

Incidence de la technique de réduction de la teneur en sucres

Analyses des produits intermédiaires des process de désucrage appliqués

Le rétentat d'UF est réincorporé sur la cuvée initiale. Le perméat d'UF est nanofiltré et le perméat de NF est réintroduit dans la cuvée initiale. Le retentât de NF est éliminé. Le sulfitage assez important de la vendange entraîne des concentrations assez fortes de SO_2 sur les fractions réintroduites sur la cuve initiale. Ces fractions étant de petit volume par rapport au volume total, cela n'a pas d'impact sur la fermentation alcoolique (tableau 1).

Tableau 1 : Analyse des produits intermédiaires – RTS- Chenin 2008, IFV37

Modalités	Perméat UF	Concentrat UF	Concentrat NF	Perméat NF
Volume L	155	16	44	58,4
Sucres g/L	193	233	342	62
pH	3,05	3,01	3,07	2,98
AT gH_2SO_4/L	6,65	7,76	6,45	6,46
Tartrique g/L	4,66	5,61	4,74	3,64
Malique g/L	8,21	8,21	7,96	8,46
K g/L	0,92	1,15	0,95	1,12
Turbidité NTU	1	34	1	0,7
SO2 total mg/L	31	46	40	21
DO 320 sous 1cm	3,09	3,52	3,21	1,076
DO 420 sous 1cm	0,020	0,398	0,037	0,003

Photo 1 : Modules de nanofiltration

Pour l'ultrafiltration, les taux de rétention (retenu par la membrane) sont faibles à nuls pour les molécules de très faibles poids moléculaire comme le sucre, les acides tartrique ou malique, le potassium. La nanofiltration (photo 1) a des taux de rétention naturellement

beaucoup plus élevés, mais ils restent relativement faibles pour les acides et le potassium (l'acide malique passe plus facilement à travers la membrane que l'acide tartrique).
La rétention en sucre est importante (c'est l'objectif) et la teneur en sucres du Concentrat NF (concentré récupéré pour la prise de mousse) conforme aux prévisions.

Analyses des produits obtenus après le process de désalcoolisation

Le but est de désalcooliser (complètement en théorie) une partie de ce volume (26L pour un volume total de 153L de vin) : l'eau native récupérée (environ 22,5L à 0,5% éthanol) est assemblé aux 127L (153-26) de rétentat pour obtenir un vin à environ 11,6% d'éthanol. L'alcool éliminé par distillation sous vide est très concentré (3,21L à 93%). Les caractéristiques des différents produits obtenus (notamment le perméat avant distillation) confirment l'obtention du degré recherché après l'opération.

Caractéristiques des vins avant Prise de mousse

Les degrés alcooliques sont conformes aux analyses fin FA. Le vin issu de la modalité Témoin présente une acidité plus importante, ainsi que des teneurs en acide malique et tartrique plus élevée que les vins issus de la deuxième récolte .Comme en fin de FA, le vin issu de la première récolte est moins coloré (DO420).
Les opérations de désucrage (sur moût) et de désalcoolisation (sur vin) mènent à des vins très proches ; ces opérations, par rapport au vin Témoin non traité, provoquent une très légère augmentation de l'acidité totale, résultat d'une très légère modification du rapport acide malique/acide tartrique. Cela était attendu.
Les bouteilles ensemencées avec les levains et additionnées ou non de sucres sont fermées (capsules à vis) et stockées à 15°C pour la prise de mousse. Le séjour sur lattes est d'environ 6 mois. A la fin de cette période, les bouteilles sont installées sur le pupitre (l'équivalent d'un remuage automatique) et le dégorgement est réalisé juste avant l'analyse sensorielle ; il est suivi du tirage et du bouchage avec une capsule sans ajout de liqueur de tirage.

Profil sensoriel des vins de base

L'analyse de variance (Tastel) met en évidence de nombreuses différences significatives entre les vins .

Ainsi, le vin issu de la modalité « date1 » est noté plus végétal, moins fruité (et fruit moins frais), moins qualitatif au niveau des arômes, moins alcooleux, et globalement moins apprécié que les vins issus des trois autres modalités (tous les trois issus de la deuxième date).
Pour ce qui est des trois vins issus de la deuxième date de récolte : le Témoin est noté plus sucré (le « désucré » noté le moins fruité des 3). Le vin issu du désucrage sur moût est noté le plus acide des trois, le moins alcooleux des trois ; au niveau appréciation globale il est le moins bien noté des trois (non significatif).

Interprétation de l'Analyse en Composantes Principales

Le premier axe principal représente plus de 75% et est constitué de manière positive des attributs suivants : végétale, acidité, amertume, et de manière négative des attributs suivants : fruité, fraîcheur du fruit, note florale, qualité des arômes, sucrosité, gras, chaleur-alcool, équilibre en bouche, qualité globale. Le deuxième axe principal représente environ 18% et est

constitué positivement des attributs suivants : fraîcheur du fruit, et négativement des attributs suivants : intensité olfactive, longueur en bouche.
Ainsi, on peut estimer que la modalité 2 Désucrage est plutôt végétale, plus acide, amère avec plus de fraicheur du fruit ; la modalité 1 est plutôt végétale, acide, amère et une plus grande intensité olfactive. A l'opposé, la modalité 2 Désalcoolisation est plutôt fruitée (et fruit plus frais), florale, avec plus de sucrosité, de gras, de chaleur, un meilleur équilibre en bouche, une qualité aromatique et globale supérieures, la modalité 2 Témoin est plutôt fruitée, avec un fruit frais, florale, une bonne qualité aromatique et globale, une bonne intensité olfactive et longueur en bouche.

Caractéristiques des vins après Prise de mousse

Bilan analytique et aromatique des vins après prise de mousse

L'incidence de la date de récolte et des deux techniques de réduction du taux d'alcool est évaluée sur les vins après prise de mousse, par le dosage des composés fermentaires (esters éthyliques et acétates fermentaires) et des composés marqueurs d'évolution (tableau 2), en même temps que les caractéristiques physico-chimiques.
Les différences notées au niveau du degré alcoolique sont attendues et confirment la maîtrise des techniques de désucrage et désalcoolisation sur la teneur finale en alcool.
L'opération de désucrage mène à une légère acidification du moût que l'on retrouve sur le vin. L'opération de désalcoolisation partielle à un effet semblable.

Tableau 2 : caractéristiques analytiques des vins après prise de mousse – IFV 2008

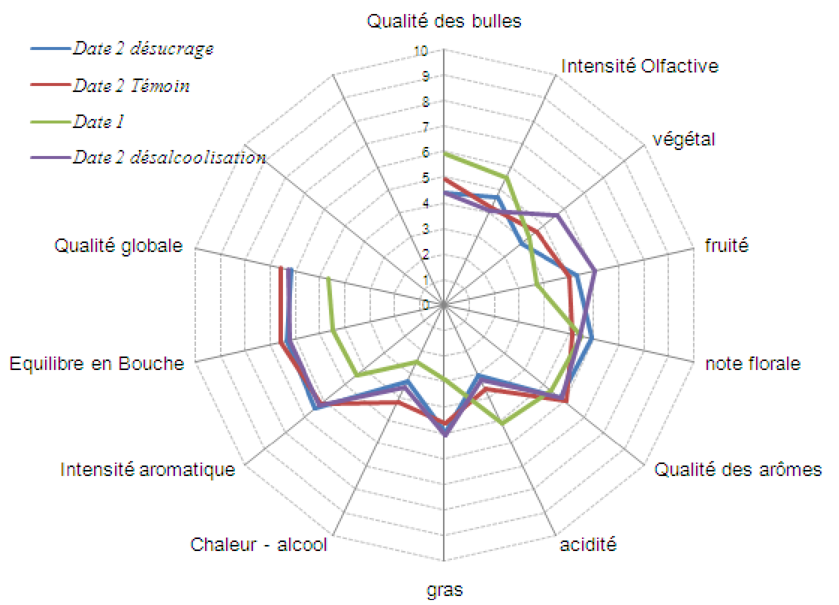
	Modalités			
	Date 1	**Date 2 Témoin**	**Date 2 issue du désucrage**	**Date 2 issue de la désalcoolisation**
Ethanol %vol.	13,07	14,62	13,12	12,89
pH	2,98	3,22	3,18	3,19
AT gH_2SO_4/L	7,34	6,49	6,59	6,57
Sucres g/L	<1	1,5	2	2
Malique g/L	8,08	7,07	7,20	7,16
Composés fermentaires en µg/L				
Hexanoate d'éthyle	440,2	1275,0	1124,0	833,3
Acétate d'hexyle	225,5	248,4	242,5	236,4
Octanoate d'éthyle	371,0	980,8	741,1	592,0
Décanoate d'éthyle	77,8	211,3	139,6	133,4
Acétate de b-phenylethyl	130,3	326,2	268,5	227,2
Acétate d'isoamyle en mg/L	1,592	1,993	1,925	1,701
Composés de vieillissement en µg èq.de 4-nona./L)				
Glycosides de composés d'arômes totaux	158	111	138	135
dont C13	94	53	67	74
Norisoprénoïdes libres en µg/L				
TDN	0,160	0,208	0,186	0,202
β-damascénone	1,889	2,309	2,055	2,369
β-ionone	0,195	0,059	0,046	0,064

D'un point de vue aromatique, la date de récolte, comme prévu, a une incidence sur le potentiel du moût que l'on retrouve sur le vin au niveau des composés fermentaires. La différence entre les deux dates de récolte D1 et D2 est significative et devrait apparaître à l'analyse sensorielle. Il apparaît que la technique du désucrage, pour ces composés, soit à l'origine d'une baisse plus importante que celle obtenue par la désalcoolisation partielle.

Pour ce qui est des glycosides, la différence la plus importante apparaît pour les C13, paradoxalement plus importants pour le vin issu de la première date. Les écarts observés au total de ces composés ne sont pas susceptibles de mener à des différences perceptibles à l'analyse sensorielle.

La composition en norisoprénoïdes libres de type C13 fait apparaître une première différence entre les deux dates de récolte, au niveau du TDN et la β-damascénone. Toutefois, cette différence – attendue – est faible, notamment pour la damascénone, exhausteur d'arômes, pour laquelle la différence entre les deux dates est à la limite de la significativité.

Il est demandé aux membres du jury de caractériser les vins effervescents au niveau olfactif, gustatif et d'évaluer la qualité de la mousse. Les données sont traitées en analyse de variance et les résultats statistiques sont présentés sur le graphique 1 ci-dessous.



Graphique 1 : Profils étoiles des prises de mousse 2008 après 10 mois sur lattes (Analyse de variance sur Tastel) déc 2009 - IFV Tours

L'analyse statistique met en évidence de façon significative les différences suivantes ;

- le vin issu de la première date de récolte (Date 1) est noté plus végétal et moins fruité, plus acide, moins alcooleux, moins intense et équilibré en bouche que les trois autres vins. Il est globalement le moins bien apprécié,
- ce même vin présente une qualité de bulles supérieure. Ce dernier critère est également apparu discriminant pour les prises de mousse issues du millésime 2007.

D'une façon non significative, le vin issu de la modalité 2ème date + désalcoolisation (Date 2 désalcoolisation) est le plus fruité et végétal.

D'une façon générale, comme pour les vins de base, la modalité issue de la 1ère date est moins appréciée au niveau gustatif surtout, à cause d'un déséquilibre, d'une plus grande acidité et d'une moindre intensité aromatique.

Après prise de mousse, les vins de base issus des autres modalités sont assez proches. Sans être significatif, la richesse en alcool du témoin semble être retrouvée par une partie du jury.

Les analyses des arômes semblent confirmer la proximité des trois produits issus de la même date de récolte, avec un impact des technologies très faible sur les caractéristiques aromatiques. La diminution de degré ne semble pas modifier de manière significative les équilibres organoleptiques en bouche.

CONCLUSION

Le procédé RTS appliqué à un moût de Chenin permet d'obtenir un vin à teneur réduite en alcool, avec un volume de « moût semi-concentré » de l'ordre de 8% par degré probable retiré. Le calcul pour déterminer le volume à traiter s'avère relativement précis ; la baisse du degré potentiel correspond aux attentes. Ce volume n'est pas perdu puisque ce sucre est réutilisé pour la prise de mousse.

La valorisation de cette fraction pour d'autres prises de mousse au sein d'une même propriété ou non peut également être envisagée.

Le procédé de couplage NF + distillation sous vide mis en œuvre pour la désalcoolisation du vin a permis de réduire le titre alcoométrique conformément aux prévisions.

D'un point de vue « qualité de la vendange », le gain de maturité se traduit par des vins fortement plus riches en arômes avec un équilibre sucrosité/acidité nettement supérieur et plus qualitatif.

L'opération de désucrage du moût entraîne une légère augmentation de l'acidité, confirmée à la dégustation du vin de base mais pas sur le vin après prise de mousse.

L'opération de désalcoolisation sur vin n'a pas entrainé de perte aromatique comme on pouvait le craindre. Le jury a même constaté un peu plus de fruit et d'équilibre, ainsi que moins d'amertume (non significatif à 5%).

Comme pour les millésimes précédents, le vin le moins apprécié est toujours celui issu de la première récolte. Les vins désucrés ou désalcoolisés effervescents sont très proches du témoin de la même date de récolte. Par contre, une meilleure qualité des bulles est constatée sur plusieurs millésimes pour le vin issu de la première date de récolte.

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SENSORY STUDY ON PARTIAL DEALCOHOLIZATION OF WINE BY OSMOTIC DISTILLATION PROCESS

M. T. Lisanti, A. Gambuti, P. Piombino, R. Pessina, L. Moio

Università degli Studi di Napoli "Federico II"
Facoltà di Agraria-Dipartimento di Scienze degli Alimenti
via T. Cappuccini 6-Avellino-Italy
mt.lisanti@hotmail.it

ABSTRACT

In recent years the demand for reduced alcohol beverages has increased, as a result of health and social concerns about the risks related to the consumption of alcohol. The aim of a dealcoholization process should be the reduction of ethanol content by preserving as much as possible the organoleptic properties of the product. In the present study, two red wines (cv. Aglianico) with different initial alcohol contents (15.46% and 13.81% v/v), have been partially dealcoholized at three levels (-2%, -3%, -5% v/v). Triangle tests have been performed in order to evaluate if dealcoholized wines differred from the untreated ones. Sensory profiles have been obtained for the studied wines by a selected and trained panel. Results show that great differences exist for a dealcoholization of 5% v/v. The most decreased olfactory notes were those of red fruits, cherry and spicy, very important for the sensory quality of Aglianico red wine. Both -5% dealcoholized wines were more astringent than the correspondent untreated ones. Slighter differences were found for the other degrees of dealcoholization.

RIASSUNTO

Negli ultimi anni la domanda di bevande a ridotto contenuto alcolico è cresciuta in seguito ad una maggiore attenzione ai rischi sociali e sanitari legati al consumo di alcol. L'obiettivo di un processo di dealcolizzazione parziale dovrebbe essere la riduzione del contenuto alcolico preservando al massimo le caratteristiche organolettiche del prodotto. Nel presente studio, due vini rossi (cv *Aglianico*) con un diverso grado alcolico di partenza (15.46% e 13.81% v/v), sono stati parzialmente dealcolizzati a tre livelli (-2%, -3%, -5% v/v). Al fine di valutare l'esistenza di differenze sensorialmente percepibili tra i vini parzialmente dealcolizzati ed il corrispondente vino di partenza, sono stati effettuati dei test triangolari. Utilizzando una giuria selezionata ed addestrata sono stati ottenuti i profili sensoriali dei vini studiati. Le maggiori differenze sensoriali sono state riscontrate per i vini dealcolizzati del 5% v/v. Le note odorose che hanno mostrato una maggiore riduzione di intensità sono risultate essere quelle di frutti rossi, ciliegia e spezie, molto importanti per la qualità sensoriale del vino Aglianico. Entrambi i vini dealcolizzati del 5% v/v hanno mostrato una maggiore astringenza rispetto ai vini non trattati. Per gli altri livelli di dealcolizzazione sono state riscontrate differenze di minore entità.

INTRODUCTION

Global warming, viticultural progress and customer demand in flavourful wines have led to the production of wines with a high alcohol content. Otherwise, in recent years the demand for reduced alcohol beverages has increased, as a result of health and social concerns about the risks related to the consumption of alcohol. Among the methods employed for removing or reducing alcohol in wines, reverse osmosis and spinning cone column are the most employed. However the application of the membrane technique called osmotic distillation, seems to be particularly promising. Wine is a complex alcoholic beverage as it contains several hundreds of organic compounds that contribute to its taste, flavor and mouthfeel. The sensory quality of a wine depends on the complex equilibrium among them. In many cases the dealcoholization could negatively affect the organoleptic quality of wine, leading in the worst cases to the unacceptability of the product. The changes in the organoleptic characteristics of wine can be due both to the reduction in ethanol content and to the losses in sensory active compounds such as volatile and polyphenolic compounds (Diban *et al*., 2008). It is well known that ethanol content affects several taste and mouthfeel sensations such as sweetness (Martin, Pangborn, 1970), bitterness (Martin, Pangborn, 1970; Fischer, Noble, 1994), sourness (Martin and Pangborn, 1970), astringency (Fontoin *et al*., 2008) and fullness (Pickering *et al*., 1998). Moreover, the volatility of some aroma compounds is reduced by the ethanol and some modifications in olfactory perception occur (Le Berre *et al*., 2007).

Currently, very few studies on the sensory modifications of wine following a partial dealcoholization treatment are available.

In the present study, two red wines (cv *Aglianico*) with different initial alcohol contents (15.37% and 13.28% v/v), were partially dealcoholized at three levels (-2%, -3%, -5% v/v). Base chemical characterization and polyphenol analyses were performed. Sensory triangle tests were performed in order to evaluate if dealcoholized wines differred from the untreated ones. Moreover, sensory profiles were obtained by a selected and trained panel.

MATERIAL AND METHODS

Wines: two red wines (cv *Aglianico*) from 2007 and 2008 harvests (TQ) were partially dealcoholized at three levels (-2%, -3%, -5% v/v) by osmotic distillation process, using a polypropylene (PP) hollow fiber membrane contactor. Tab. 1 reports the alcohol content of the studied wines, before and after partial dealcoholization.

Tab. 1 Ethanol content (% vol.) of the studied wines.

Wines	Ethanol content (% vol)	
	Aglianico 2007	Aglianico 2008
Standard	15.46	13.81
-2%	13.54	11.65
-3%	12.40	10.52
-5%	10.84	8.83

Base chemical analyses: sugars, extract, pH, total acidity, volatile acidity were determined according to the "OIV Compendium of international methods of wine and must analysis". Each analysis was performed in triplicate.

Polyphenol analysis: Total polyphenol analysis was carried out by Folin Ciocalteau colorimetric analysis as described by Singleton and Rossi (1965); Ads 420, Ads 520, Ads 620 and colour intensity (CI) were determined as described by Glories (1984); tannins content was determined according to Ribereau-Gayon & Stonestreet (1966). Each analysis was performed in triplicate.

Sensory analysis: Each partially dealcoholized wine was compared to the correspondent not dealcoholized one by triangle test. The triangle test was performed by 30 judges (16 females and 14 males), aged between 22-65. Moreover, for each wine the sensory profile was obtained by a selected and trained panel of 12 judges (5 females and 7 males), aged between 22-61. Odour descriptors were determined by panel consensus, the intensity of all the descriptors (odour and taste) was evaluated on an unstructured, 10 cm scale. Sensory profile analysis was performed in duplicate. In all sensory analyses, 30 mL of wine were served in three-digit coded, tulip-shaped, black glasses at 18°C. The wine samples were served according to a complete randomized design, water and unsalted crackers were provided to judges to rinse mouth.

Data analysis: Individual analyses of variance (AOV) were run on chemical and sensory data. Sensory profile data were also analysed by Principal Component Analysis (PCA). All statistical analyses were performed using the Statgraphics plus (5-PC) statistical packet (Manugistics Group Inc., USA).

RESULTS AND DISCUSSION

Among the existing dealcoholization techniques, the osmotic distillation presents some advantages that make it particularly promising: work temperature between 10 and 20°C (cellar room temperature), low energy consumption due to low operating pressure and the use of water as ethanol stripping agent. Moreover, it should avoid the thermal damage of volatile compounds and flavour loss (Varavuth et al., 2009). Tab. 2 reports the base chemical parameters of the analysed wines. Statistical analysis of variance showed no significant differences in each set of samples.

Tab. 2 Base chemical parameters of wines

Sample	Sugars (g/L)	Extract (g/L)	pH	TA (g/L)	VA (g/L)
Aglianico 07	3,60	35,56	3,89	5,20	0,62
Aglianico 07 -2%	4,06	35,09	3,86	5,31	0,59
Aglianico 07 -3%	3,78	35,01	3,86	5,31	0,58
Aglianico 07 -5%	3,84	35,69	3,83	5,33	0,55
Aglianico 08	2,90	32,59	3,85	5,22	0,47
Aglianico 08 -2%	2,88	32,38	3,84	5,20	0,45
Aglianico 08 -3%	2,85	33,00	3,83	5,25	0,44
Aglianico 08 -5%	2,81	33,62	3,80	5,36	0,44

(mean values n=3, no significant differences α=0.01)

Also Colorant Intensity (CI) (Fig. 1) and Ads 420-520-620 nm (Fig. 2) were not significantly different after the partial dealcoholization process.

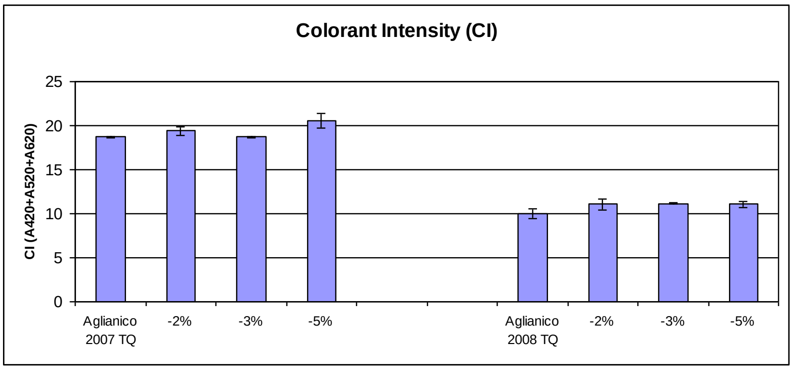

Fig. 1 Colorant Intensity (CI) of the untreated and partially delcoholized wines (n=3, no significant differences α=0.01).

Total polyphenols, expressed as Folin-Ciocalteau index, and tannins content did not show significant differences after the partial dealcoholization of both wines.

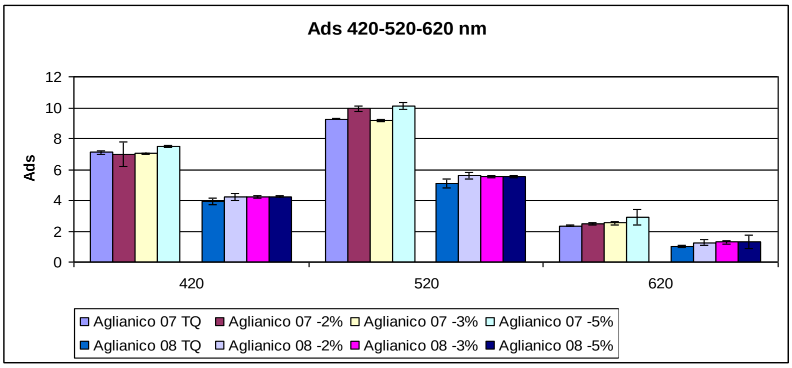

Fig. 2 Ads 420-520-620 nm of the untreated and partially delcoholized wines (n=3, no significant differences α=0.01).

As concern the existence of sensory differences among standard and partially dealcoholized wines, the results of the triangle tests (Tab. 3) were different for the two studied wines.

Tab. 3 Results of triangle test

Test	Significacy
Agl 07 TQ vs Agl 07 -2%	ns
Agl 07 TQ vs Agl 07 -3%	ns
Agl 07 TQ vs Agl 07 -5%	α =0.01
Test	**Significacy**
Agl 08 TQ vs Agl 08 -2%	ns
Agl 08 TQ vs Agl 08 -3%	α =0.05
Agl 08 TQ vs Agl 08 -5%	α =0.01

The wines dealcoholized of 2%, the actual limit for the dealcoholization of wine imposed in Europe by the Reg. (CE) 606/2009, were never perceived significantly different from the respective standard wines. On the contrary, the wines dealcoholized of 5% were in both cases perceived as significant different from the standard wines. Concerning the wines dealcoholized of 3%, for Aglianico 2007 wine there were no significant differences, while for Aglianico 2008 the dealcoholized wine was different from the standard one. Sensory profiling allowed to understand the olfactory and gustative modification due to partial dealcoholization of the two wines. Fig. 3 compares the sensory profiles of the two standard wines.

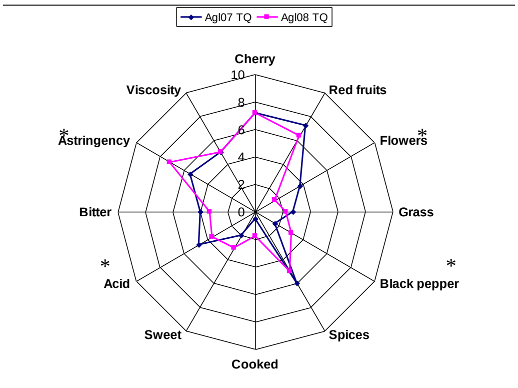

Fig.3 Sensory profiles of the two standard wines (*=descriptors significantly different α=0.05).

Aglianico 2007 was more acid and with a more intense flowers odour, while Aglianico 2008 was more astringent, as espected being more young, and with a more intense olfactory note of black pepper. Fig. 4 and 5 reports the sensory profiles of the two standard wines before and after the partial dealcoholization at three levels. For Aglianico 2007 wine (Fig. 4), the most decreased olfactory notes after dealcoholization were cherry, red fruits and flowers. Agl07 -5 presented the lowest intensity for cherry odour and an odour described as "cooked" appeared. Agl07 -5 was the most astringent, this could be due to the lowest ethanol content (Fontoin et al., 2008).

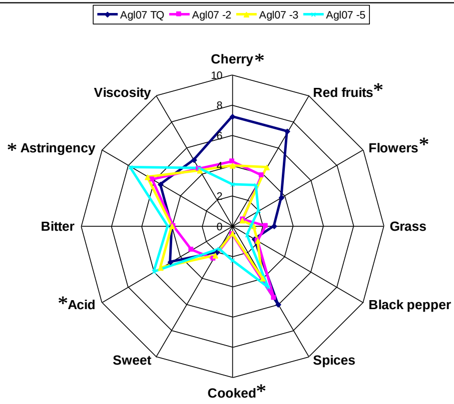

Fig. 4 Sensory profiles of Aglianico 2007 before and after partial dealcoholization (*=descriptors significantly different α=0.05).

For Aglianico 2008 the results were slight different, but with the same tendencies of Aglianico 2007.

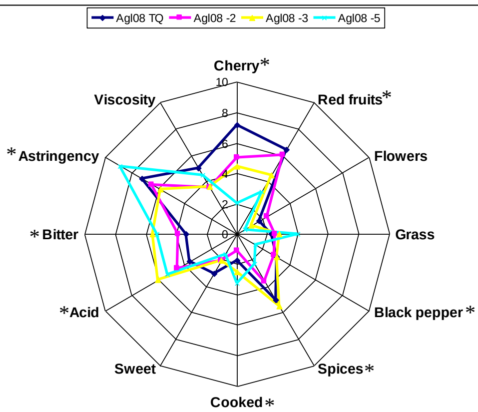

Fig. 5 Sensory profiles of Aglianico 2008 before and after partial dealcoholization (*=descriptors significantly different α=0.05).

The most reduced olfactory notes were those of cherry and red fruits, particularly in Agl08 -5. Also black pepper and spice odour were decreased and the cooked note appears after the treatment. The decreased olfactory notes are particularly important in the olfactory profile of Aglianico wine. Partial delacoholization increased the intensity of astringency in Agl08 -5, and of bitterness and acidity, in Agl 08 -3 and -5.

In order to better understand the effect of partial dealcoholization on the sensory profile of the studied wines, a Principal Component Analysis (PCA) was performed on the sensory quantitative data (Fig. 6 and 7). It is very interesting to note that the first component separated in both cases descriptors that we can consider as positive, such as fruity, floral, spicy olfactory notes along with sweetness and viscosity, and sensory characteristics that can be considered negative for red wine especially if present with a too high intensity (bitterness, acidity, astringency and the olfactory note of cooked).

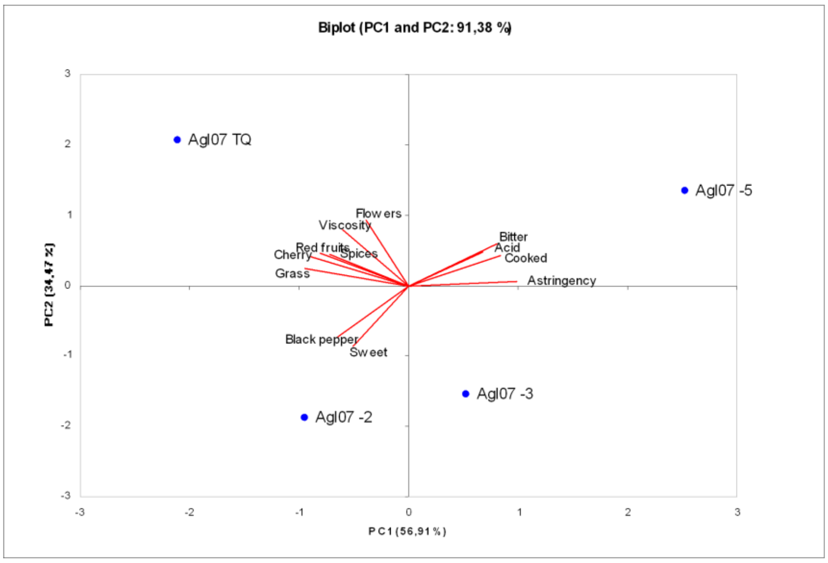

Fig. 6 Principal Component Analysis of sensory quantitative data of Aglianico 2007 standard and partially dealcoholized wines.

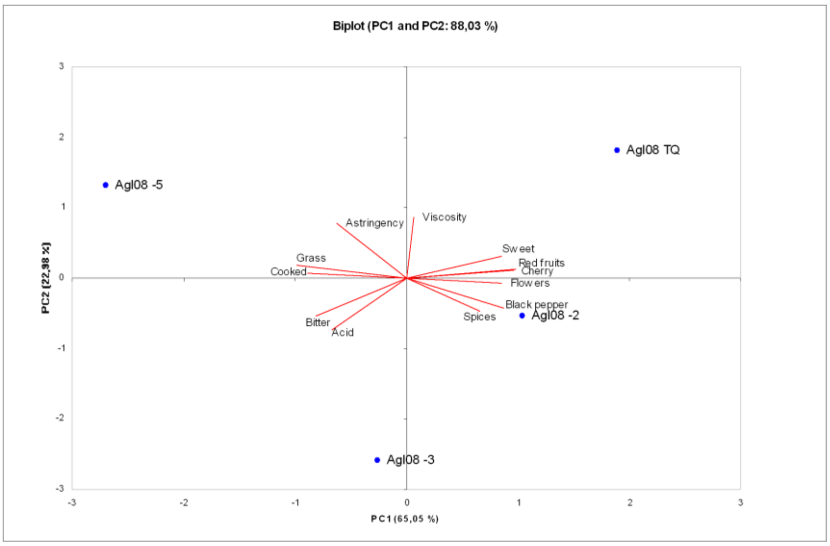

Fig. 7 Principal Component Analysis of sensory quantitative data of Aglianico 2008 standard and partially dealcoholized wines.

For both wines (Agl07 and Agl08), the correlation of the standard and partially dealcoholized wines with the variables is the same. The first component (PC1) separate standard and -2 dealcoholized wines from -3 and -5 dealcoholized ones. Standard and -2 wines were correlated to those we have defined as “positive descriptors”, in particular standard wine was always more correlated to cherry and red fruit odours and to viscosity. On the contrary, -3 and -5 dealcoholized wines were correlated to the “negative descriptors”, particularly the -5 wines that were always more astringent and with a cooked odour.

CONCLUSION

These preliminary results give a contribution to the comprehension of the effect of partial delacoholization of wine on its sensory characteristics. No significant differences in base chemical and polyphenol parameters were found, for both the studied wine. With the triangle test, -2 dealcoholized wines were never significantly distinguished from the untreated ones, while -5 dealcoholized wines were significantly different from the untreated ones in both cases. In the case of -3 dealcoholized wines, it seems that the sensory effect of partial dealcoholization depends on the standard wine. Sensory profiles showed a significant reduction of the fruity odours in all dealcoholized wines, but with an higher extent at the increasing of the dealcoholization level. The other modifications of the sensory profiles were different for the two studied wines. PCA analysis clearly showed that, with the increase of dealcoholization level, both wines became more and more different from the respective standard ones, with an increasing correlation with astringency, bitterness, acidity and cooked odour.

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GENETICALLY MODIFIED WINE YEASTS: ACTUAL SITUATION AND RISK ASSESSMENT

Manfred Grossmann[1]*, Falk Kießling[2], Julian Singer[2], Heidi Schoeman[3], Max-Bernd Schröder[1] and Christian von Wallbrunn[1]

[1]Geisenheim Research Center,
Von-Lade-Strasse 1, D-65366 Geisenheim, Germany
Email: Manfred.Grossmann@fa-gm.de
[2]Hochschule RheinMain, University of Applied Sciences Wiesbaden Rüsselsheim Geisenheim, Fachbereich Geisenheim, Von-Lade-Strasse 1, D-65366 Geisenheim, Germany
[3]The Institute for Wine Biotechnology, University of Stellenbosch
7600 Stellenbosch, South Africa

ABSTRACT

By using gene technology genetically modified (gm) wine yeasts (*Saccharomyces cerevisiae*) are now available which cover more or less all stages of the wine making process where microorganisms or commercial enzymes play a key role. Despite this availability, their usage is hampered by two important issues: firstly the legal regulations concerning the dissemination of genetically modified organisms and secondly the still existing doubts or even refuse of engineered food by the people worldwide. Only two engineered wine strains are legally on the market in the USA and Canada.

In this article, we present a short survey about existing gm wine yeast strains, the changing consumer's perception and comprehensive risk assessment studies that were performed under safety class 1 conditions in green house experiments and simulations in lab scale. The results show that also the gm yeasts persist after dissemination on winery equipment and in natural environment like other non-modified yeasts also do. The eventual problem of using multiple modified yeast strains will be discussed.

ABSTRAKT

Durch den Einsatz gentechnischer Verfahren wurden Weinhefen konstruiert, die in fast allen Bereichen der Weinbereitung eingesetzt werden können, wo Mikroorganismen oder kommerziell erhältliche Enzympräparate eine Rolle spielen. Trotz ihrer Verfügbarkeit ist der Einsatz gentechnisch modifizierter Hefen noch sehr eingeschränkt. Die Gründe hierfür liegen erstens in den zu erfüllenden gesetzlichen Vorschriften und zweitens in der immer noch existierenden Zurückhaltung der Konsumenten gegenüber Lebensmittel, die mit Hilfe der Gentechnik produziert wurden.

Der vorliegende Artikel gibt einen kurzen Überblick über die in der Literatur bereits beschriebenen modifizierten Weinhefen, den Veränderungen in der Einschätzung von Gentechnik durch den Konsumenten und umfassenden Studien über das Verhalten von gentechnisch modifizierten Weinhefen bei Freisetzungsexperimenten. Diese wurden unternommen in S1-klassifizierten Gewächshäusern und in S1 Laboren, wo Abläufe der Weinbereitung simuliert wurden. Die Ergebnisse zeigen, dass auch gentechnisch veränderte Hefen nach der Freisetzung sich auf Kellereigeräten und in der natürlichen Umgebung etablieren können. Insofern verhalten sie sich genau so wie andere nicht gentechnisch veränderte Weinhefen. Die mögliche Problematik mehrfach modifizierter Hefestämme wird diskutiert.

INTRODUCTION

Wine production and wine consumption can be addressed as early companions of human civilization. With Georgia as place of birth, wine came via the Egyptian and Roman Empires into a number of countries which are still today addicted to wine culture.

The identification of yeasts as the wine causing agents and fundamental aspects of the alcoholic fermentation are tightly linked with the name Louis Pasteur and a couple of other European chemists and biologists of that era (review by Barnett, 2000). Wine quality increased continuously since Pasteur's findings by increasing knowledge about yeast physiology and yeast genetics. Choice of wine yeast strains was early realised to have a major impact on wine quality and different flavour expressions (Kunkee and Amerine, 1970) which induced extensive yeast selection programs (for review see Fleet, 1993).

In addition to that, researchers tried to improve and combine positive attributes, mediated by different yeast strains, by using methods like hybridisation, rare-mating and spheroplast fusion. Especially hybridisation is still the mostly applied procedure to combine genetic material of yeast strains from the same species or very closely to *Saccharomyces cerevisiae* related species (Pretorius and van der Westhuizen, 1991; Querol and Ramon, 1996).

However, these classical methods lack the precision and capability of gene cloning. A continuous improvement in genetic engineering procedures took place using now a wide variety of integrating, autonomously replicating or other specialised vectors (Pretorius et al., 2003). A large amount of engineered wine yeasts which have been constructed so far (Schuller and Casal, 2005, Cebollero et al., 2007).

Table 1 displays some early as well as recent examples of genetically modified (gm) yeasts and the respective steps within the wine making process where these strains might be used for practical applications. Even physical treatments and fining of grape must or wine could be replaced by usage of engineered yeasts as well as new properties introduced..

Table 1: Effects of gm yeasts used at different steps within the wine making process

Wine aroma			
fermentation bouquet	enhancement of esterase activity (*ATF1*)	more fruitiness	Lilly et al., 2000
varietal aroma	enhancement of glycosides splitting hydrolases	more varietal character	Perez-Gonzalez et al., 1993; Ganga et al.1999
taste	enhancement of GPD1-enzyme	more glycerol, extract and full-body	Michnik et al., 1997
	malate degradation; lactate production	balanced acidity	Volschenk et al., 1997, 2001; Dequin et al., 1999
Must/wine treatment			
grape must	enhancement of pectinase activity enhancement of protease activity	improved filtration alternative for bentonite	Gonzalez-Candelas et al. 1995
young wine	enhancement of glucanase activity	improved filtration	Perez-Gonzalez et al. 1993
Wine and human health			
wine hygiene	production of bacteria inhibiting proteins	lower SO_2-requirement	Schoeman et al. 1999
health promotion	enhancement of existing enzymes and/or introduction of external enzymes	hydrolyses of bound resveratrol; formation of vitamins and unsaturated fatty acids	Gonzalez-Candelaz et al. 2000; Pretorius 2000 (review)

Despite the availability of a broad range of gm yeasts, only two strains are permitted to be used in the USA and Canada, although the yeast species *Saccharomyces cerevisiae* as such has the GRAS label which means Generally Recognized As Safe.

Gm strain ML01 (degradation of malic acid) and gm strain ECMo01 (degradation of urea) have in common that they are advertised under the aspect of improving healthiness of wine consumption as they significantly reduce the risk of the production of biogenic amines (allergenic metabolites of lactic acid bacteria) as well as of the production of ethyl carbamate (mutagenic and possibly also carcinogenic substance).

Many consumers are worried of eating gm food. While a consumer survey in Germany, done in 1994, (Menrad 1996) showed that nearly 80 % completely rejected usage of gene technology, a more detailed opinion within the population became visible in 2002, when 80 % accepted gene technology as useful tool to defeat severe human diseases (Allensbach 2002). However, yet in 2006 the rejection quota for gm food was still at 75 % (GfK-Study 2007).

The implementation of international regulations should not only cover risk assessment studies before decisions can be made allowing the marketing of GMOs but should also identity preservation systems which are claimed to be established to preserve and guarantee purity of non-GMO food (Varzakas et al. 2007).

Risk assessment studies concerning gm wine yeasts were hardly done so far. Schoeman et al. (2009) used sand columns and sand cells to demonstrate the behaviour of yeasts in a nature simulating scenario. It was shown that the gm yeast strain formed biofilms and became part of the microbial population in the sand but never dominated the yeast flora.

To broaden the view of gm yeast behaviour during the wine making process in the winery but also outside in the vineyard, we investigated the potential impact of these yeasts on natural yeast flora in a vineyard, the impact on winery flora, furthermore the behaviour of gm yeasts during waste water treatment and also in bottled wines simulating the marketing of so called unfiltered or non-filtered wines.

MATERIAL AND METHODS

Strains and culture conditions

All strains (Table 2) were cultured in (on) YEPD (agar) (1 % yeast extract, 2 % glucose, 2 % peptone adjusted to pH 6.5) or malt medium (3 % malt, adjusted to pH 6.5). In case of plates 2 % agar was added.

Table 2: Strains used in this study

Strain	Description	Reference
S.c. VIN13	commercial available wine yeast strain	Anchor Yeast, SA
S.c. VIN13-*lka*1	genetically modified strain based on VIN13 contains a gene coding for an α-amylase of *Lipomyces kononkoae*	Gundllapalli Moses et al., 2002
S.c. VIN13-*end*1	genetically modified strain based on VIN13 contains genes coding for an endo-1,4-glucanase of *Butyrivibrio fibrisolvens* and an endo-β-xylanase of *Aspergillus niger*	Strauss, 2003
S.c. S92- ML01	genetically modified strain based on S92 contains genes coding for a malate permease of *Schizosaccharomyces pombe* and a malolactic enzyme of *Oenococcus oeni*	Husnik et al., 2006

Detection of genetically modified yeast strains

<u>Phenotypic characterisation</u>

It was possible to detect the S.c. VIN13-*lka1* strain by growing on Phadebas medium plates (6.7 g DIFCO^{TM} Yeast Nitrogen Base (w/o amino acids and ammonium sulfate), 1 g glucose, 20 Phadebas pills and 20 g agar solved in 1 l A. dest. at pH 6.5 to 7). *S. cerevisiae* strains were inoculated 48 h on these plates to detect bright, clear halos around genetically modified VIN13-*lka1* strains.

Strain S.c. VIN13-*end1* was detected by growing on CMC medium plates (10 g yeast extract, 20 g glucose, 20 g peptone, 3.0 g viscose carboxymethyl cellulose and 20 g agar solved in 1 l A. dest. at pH 6.5 to 7). *S. cerevisiae* strains were cultivated 48 h on these plates. After this the plates were washed with TE buffer (150 mM Tris, 20 mM EDTA, pH 7) and a Congo Red solution (0.1 %) was used for 10 to 15 min to show bright, clear halos around the colonies in case of genetically modified yeast strains.

Genotypic detection

The genotypic detection of the different genetically modified *S. cerevisiae* strains was done by PCR. For details see Grossmann et al. (submitted)

DNA extraction

Yeast isolates were cultured in 8 ml liquid YEPD medium for 48 h. 1.5 ml samples were taken and DNA was isolated based on the protocol of Rose et al. 1990. The DNA was dissolved in 50 µl TE buffer containing 2 U RNase.

Yeast species identification

PCR amplification and RFLP analysis of the rDNA-5.8S-ITS region

To identify isolated yeast the 5.8S-ITS region was amplified in an Eppendorf Mastercycler epgradient (Eppendorf, Hamburg, Germany) using the primers ITS1 and ITS4 previously described (White et al. 1990). Amplified PCR products were digested with the restriction enzymes *Hin6*I (isoschizomer to *Cfo*I), *BsuR*I (isoschizomer to *Hae*III) and *Hinf*I (Fermentas). RFLP analysis was done after separation by electrophoresis using a 2 % agarose gel. The identification of the yeast species was performed according to the restriction patterns published by Guillamón et al. (1998), Esteve-Zarzoso et al. (1999), Granchi et al. (1999), Las Heras-Vasquez et al. (2003) and Sturm (2005).

Identification by amplification of the D1/D2 domain and sequencing

Another way to identify isolated yeast species was amplification and sequencing of the D1/D2 domain of the 26S rDNA-gene (Kurtzman & Robnett 1998). After cleaning up, the PCR product was sent to a company with a customer sequencing service (SRD, Oberursel, Germany).

Characterisation of *Saccharomyces cerevisiae* on strain level

A method to distinguish between different strains of the species *Saccharomyces cerevisiae* is the δ-PCR described by Ness et al. (1993) and Legras & Karst (2003). Using the primers δ-12 and δ-2 (it is possible to get special patterns of DNA bands for each isolated strain).

Differentiation of yeast isolates

Saccharomyces vs. Non-*Saccharomyces*

Pre-identification of yeast belonging to the genus *Saccharomyces* was performed on lysine agar selective medium (0.67 % Difco^{TM} Yeast Nitrogen Base (w/o amino acids and ammonium sulfate), 0.089 % L-lysine monohydrochloride, 5 % glucose and 1.8 % agar). Only yeast not belonging to the genus *Saccharomyces* could grow on this selective medium.

Effects of genetically modified yeast in a vineyard

Experimental vineyard

GM yeast risk experiments were done in a greenhouse under genetic safety level S1. The greenhouse was subdivided in four blocks. In each block 20 *Vitis vinifera* “Riesling” root stocks were planted in flower pots. The root stocks were treated with fertilizers and plant pesticides over the whole experimental time according to good manufacturing practice in viticulture. Typically 1.5 l of a solution with 2x10^{6} cells/ml of genetically modified yeast strains were released at several times. Block 1 was the control without any release of yeast strains. In block 2, the strains S.c. VIN13-*lka1* and S.c.VIN13-*end1* were released in several years. Only in the last year the strain S.c. S92 ML01 was released. Block 3 was also a control. The commercial wine strain S.c. VIN13 was released several times. In block 4, the genetically modified yeast S.c.VIN13-*lka1* was applied at several time points.

<u>Sampling of yeast</u>

Bark, leaves, berries from grapes and soil were used to isolate yeasts and to detect gm yeast. This material was washed with NaCl solution (0.9 % NaCl, 0.1 % Triton X-100 and diluted in decadic steps to plate aliquots on malt or YEPD plates. After two days incubation (25°C), colonies were counted and colonies were isolated for further differentiation.

<u>Enrichment cultures</u>

In some cases enrichment cultures were necessary to isolate yeasts. 250 ml Erlenmeyer flasks were prepared with 200 ml grape juice and different sample material was added directly and incubated at RT. Samples were taken to be sprayed on YEPD or malt plates for isolation.

<u>Microvinifications</u>

For spontaneous fermentation experiments, grapes of the different greenhouse blocks were harvested under sterile conditions. Grapes were pressed in sterile plastic bags by hand and 150 or 200 ml of settled must were filled in flasks for vinification at RT. Fermentation activities were shown by loss of CO_2 and weighing the flasks. Samples were taken to determine total cell counts and also to detect genetically modified yeasts.

Survival of gm yeast in bottled wine

To test the survival rate of S.c. VIN13-*lka1* in bottled wine, bottles were spiked. Defined amounts of yeast cells were added to the filled wine before closing the bottles. Filtrations were done directly, after two months and 17 months using a filtration system of Sartorius, Göttingen, Germany. Filters (pore sizes 0.45 µm and 0.2 µm) were incubated for 3 to 6 days at 25°C on malt medium and growing colonies were counted. Growing colonies were also checked for genetically modified yeast.

Simulations of a sewage plant

To do risk experiments in the field of waste water, it was necessary to construct a model system to simulate the environmental conditions of a local sewage plant under laboratory conditions. Simulations were done by taking sewage sludge of a local sewage plant (Communal sewage plant Rüdesheim, Rüdesheim, Germany). The sludge was used in a flask with a magnetic stirrer for aerobic simulation and in an OxiTop IS12 system (WTW, Weilheim, Germany) as anaerobic simulation considering the COD (chemical oxygen demand) of the respective sludge sample. In the simulations 2.2×10^7 to 1×10^8 yeast cells/ml were inoculated and incubated for five days at 25°C. In a first experiment the simulations were treated with the commercial wine yeast S.c. VIN13 later with the genetically modified yeast S.c. VIN13-*lka1*. Chloramphenicol (30 µg/ml final conc.) was used to reduce bacterial growth and to ease the isolation of yeast. Isolated yeast strains were differentiated and identified as described before.

RESULTS

Behaviour of genetically modified yeasts in vineyard experiments

<u>Effects on natural yeast flora in an artificial vineyard in greenhouse</u>

One of the topics was to test the possible influence of gm yeast on the natural yeast flora of a vineyard. It was necessary to isolate yeasts in the artificial vineyard before and after dissemination of the gm test microorganisms. In the first year of experiments the *status quo* of encountered yeast species was determined using D1/D2 domain sequencing after isolating yeasts from soil, bark, leaves and berries at several time points. In the second and third year of experiment genetically modified yeast strains and the commercial wine yeast strain S.c. VIN13 were released into different blocks of the greenhouse as described under Material and Methods. The occurrence of the natural yeast flora was tested as in the first year. Only identification of yeast species was now done by analysis of RFLP patterns of the 5.8S rRNA gene and internal transcribed spacer regions.

Over the whole period of examination, several yeast species typically for the winemaking environment could be detected. They belong to the genera of *Candida*, *Cryptococcus*, *Debaryomyces*, *Hanseniaspora*, *Kluyveromyces*, *Pichia*, *Rhodutorula*, *Saccharomyces*, *Saccharomycodes* and *Torulaspora*. Also *Yarrowia lipolytica* was detectable in samples from the greenhouse. Only minor fluctuations of the species composition were observed. In the first year *Cryptococcus species*, *Pichia anomala* and *Torulaspora delbrueckii* were not detected. In the second year *Cryptococcus species, Pichia anomala* were identified but *Kluyveromyces lactis* and *Saccharomycodes ludwigii* were absent. *Aureobasidium pullulans*, a fungi growing in some growth stages like yeast, was present, too.

In the third year *Candida lambica*, *Debaryomyces hansenii*, *Hanseniaspora uvarum* and *Pichia kluyveri* were not detectable but *Torulaspora delbrueckii* was present. Over the three years of this experiment a fluctuation range like in natural habitats was observed.

<u>Detection of genetically modified yeast strains in an artificial vineyard</u>

Over a period of three years the presence of genetically modified yeasts disseminated in different blocks of the experimental vineyard was detected. Releasing of yeast took place only in the first year. Block P1 was the control without yeast release. In block P2, S.c. VIN13-*lka1* (GMY1) and S.c. VIN13-*end1* (GMY2) were sprayed. The commercial, genetically not modified wine yeast strain S.c. VIN13 was used in block P3 also as a control. The only gm yeast released in block P4 was S.c. VIN13-*lka1*. Yeasts were isolated and identified as gmy at several time points over the three years by the protocol described above. During this period no genetically modified yeast strains could be detected in blocks P1 and P3 (control and commercial wine yeast). A detection of both liberated genetically modified yeast strains was possible during the first year in block P2 with 88 % of GMY1 and 12 % GMY2 within all isolated *Saccharomyces yeasts.* The yeast strain S.c. VIN13-*lka1* became non-detectable after winter and resulting vegetation break after the first year. Also in the third year this yeast wasn't present anymore. Only S.c. VIN13-*end1* could be found over the whole experimental period of three years in this block (8.5 % second year and 15 % third year). S.c. VIN13-*lka1* was present in the first year in block P4 (83 % of isolated *Saccharomyces*) but felt below detection level in the second year. In the third year only the other genetically modified yeast S.c. VIN13-*end1* was isolated from sample material of this block (3 % of isolated *Saccharomyces)*. This finding gave a strong evidence for cross contamination.

To point out as a result at this stage, the possibility to survive in the environment of an artificial vineyard plus the four seasons of a year depends on each single yeast strain and was not calculable a priori: one genetically modified yeast strain was always detectable in contrast to the other over the three years period.

Genetically modified yeast in fermentations and wine

<u>Influence of genetically modified yeast on fermentations</u>

The gm yeasts could be detected in different amounts depending on the yeast strain on grapes from rootstocks growing in blocks after dissemination of GMOs in the artificial vineyard. To prove that the gm yeast of this study could have an influence on vinifications, spontaneous fermentations were done with grape juice prepared from the four different blocks of the experimental vineyard. Big differences of the fermentation courses and activities could not be observed, which means that no dominance of the genetically modified yeast strains was present.

Survival of S.c. VIN13-*lka1* in bottled wines

Bottles filled with a dry white wine (Riesling) were spiked with different amounts of S.c. VIN13-*lka1* and stored for two and seventeen months at cellar temperature to test the survival of the yeasts. These wines were filtered to recover and determine the rate of surviving and genetically modified yeast. After two months an average of about 94 % surviving yeasts was detected. 17 months after spiking and bottling of the wines a survival rate of still 77 % could be observed. All isolated strains were attributed to strain S.c. VIN13-*lka1* by growth test on Phadebas medium. These results show definitely the possibility of this genetically modified strain to survive in bottled wines.

Genetically modified yeast in waste water

Survival of genetically modified yeast in sewage simulations

This part of the study was focused on the possible risks of dissemination of genetically modified yeast by waste water of wine cellars.

The simulations were inoculated with defined amounts of the non-modified commercial wine yeast S.c. VIN13. About 80 % of the *Saccharomyces* isolated by differentiation on malt and lysine and characterized by δ-PCR belonged to this strain.

Then the experiments were repeated using the genetically modified yeast S.c. VIN13-*lka1*. Amounts of 1×10^8 to 2.5×10^8 cells were inoculated. After isolating and differentiation on lysine medium in the anaerobic simulation about 99 % and in the aerobic simulation around 93 % of the yeasts were related to *Saccharomyces*. About 92 % of the yeast in the anaerobic simulation and about 85 % of the yeast under aerobic conditions showed clear halos around the colonies in a growth test on Phadebas medium (Figure 1).

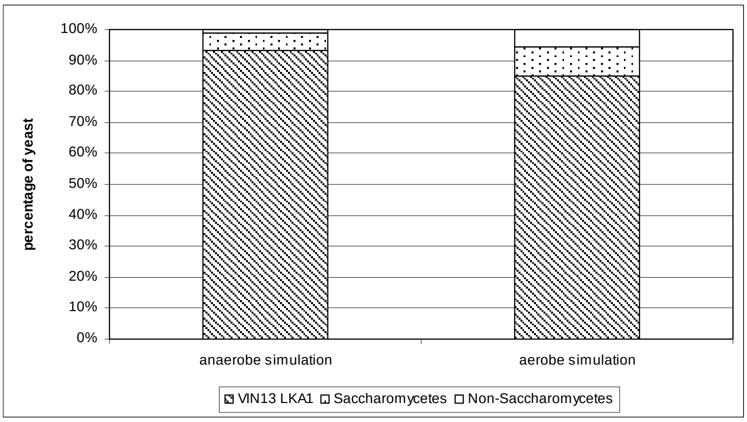

Figure 1: Percentage distribution of yeast surviving aerobic and anaerobic simulations of a sewage plant treatment. In each simulation 300 yeasts were isolated and tested phenotypically by growth on Phadebas medium and genotypically by PCR.

The results present a good demonstration for the genetically modified yeast strain which could survive in sewage plants and will be disseminated with purified effluents of the sewage plant into the environment.

DISCUSSION

Although the public perception of gm food and especially beverages is gradually becoming more positive, the overall acceptance is only given for human health related (pharmaceutical) products, i.e. for diagnosis and therapy of severe diseases. The commercialization of two gm strains in the USA and Canada since 2003 and 2006, in conjunction with differences of legal strength to demonstrate potential risks by gm products, especially between the USA and the European Union, asked for more information about the effects of long term usage of gm yeasts.

Schoeman and co-workers published in 2009 one of the first studies about the behaviour of gm yeasts in a natural but artificially constructed habitate (sandy soil). Their results clearly indicate that under these conditions gm yeasts did not interfere with other microorganisms in a way that they became dominant partners in these mini-cosms.

We elaborated over the last years test systems which demonstrate the behaviour of gm yeasts at different stages of the vinification process (vineyard, spontaneous alcoholic fermentation, cellar equipment and bottled wines).

The vineyard trials, where gm yeasts were also sprayed onto leaves and grapes to generate a “worst case scenario”, clearly showed that over the years and vintages the gm yeasts did not dominate the endogenous yeast flora. But on the other hand they also did not disappear completely but became part of the natural population. Investigations about spreading of commercial yeast strains around wineries and in vineyards came to the same results (Pretorius et al. 1999; Schuller et al. 2005). Spontaneous fermentations done with musts from grapes formerly infected with gm yeasts showed the participation of gm yeasts with the alcoholic fermentation but also no dominance.

Simulating treatments of gm yeasts containing winery effluents, revealed the same behaviour for these yeasts as for all other yeasts: reduction of cell counts but no complete elimination.

Although the tested gm yeast strains behaved more or less like other non-engineered wine yeasts, these findings should not be addressed and used as a general rule. Yeast strains which combine in their genome several enzymatic activities like pectinase, glucanase and protease activities (Rensburg et al., 1997) would be useful for the wine makers and ease the wine making process but potential side effects on grape vines or other plant material must be taken into consideration. In order to avoid the introduction of new, maybe plant pathogenic organisms precisely conducted case-by-case studies should become a prerequisite in worldwide legislation governing the usage and commercialization of gm wine yeasts.

ACKNOWLEDGEMENT

Essential parts of this project were funded by the German Ministry for Nutrition, Agriculture and Consumer Protection (Bundesministerium für Ernährung, Landwirtschaft und Verbraucherschutz, BMELV) (Project number: 01HS003). We also thank Prof. IS Pretorius and Dr. P. van Rensburg, The Institute for Wine Biotechnology, University of Stellenbosch (South Africa) for providing the gm yeast strains S. c. VIN13-lka1 and S. c. VIN13-*end*1.

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TRANSFORMATION OF WINE MATERIALS IN THE PROCESS OF SECONDARY ALCOHOLIC FERMENTATION

E. Kirtadze[(1)], G. Papunidze[(2)], G. Kvesitadze[(3)]

[(1)] S. Durmishidze Institute of Biochemistry and Biotechnology
10th km, Agmashenebeli alley, Tbilisi, Georgia
e_kirtadze@mail.ru
[(2)] S. Durmishidze Institute of Biochemistry and Biotechnology
10th km, Agmashenebeli alley, Tbilisi, Georgia
gpapunidze-44@rambler.ru
[(3)] S. Durmishidze Institute of Biochemistry and Biotechnology
10th km, Agmashenebeli alley, Tbilisi, Georgia
kvesitadze@hotmail.com

ABSTRACT

As a result of authors' 40 years of investigations by applying of radioactive (^{14}C) compounds, transformation products of ethanol, carbon dioxide, glycerin, acetaldehyde, organic acids and amino acids have been established during the process of secondary alcoholic fermentation both in the yeast cell and fermented wine. The fermentation of wine material was conducted by classical method. Yeast strain of *S.cerevisiae*-39 for secondary fermentation was isolated from one of the wineries in Eastern Georgia. The incorporation pathways of certain carbon atoms from the carbon skeletons of glycerin, ethanol, acetic acid and succinic acid into the yeast cell and wine compounds have been revealed. It has been established that modified Krebs cycle takes place under unusual conditions of secondary fermentation (CO_2 pressure, low temperature, existence of ethyl alcohol and volatile acids in the fermenting medium, etc.) during of which, reducing transformations are preferable. At the same time, all the studied compounds are partially oxidized to CO_2.

RESÜMEE

Infolge der 40-jährigen Forschung der Autoren unter Verwendung der (^{14}C) Verbindung wurden Produkte der Konvertierung des Äthanols, Kohlendioxids, Glyzerins, Äthylaldehyds, organischer und Aminosäuren während der zweiten Spiritusgärung sowohl in Hefenzellen als auch im Stillwein festegstellt. Für Nachgärung wurde in der Weinkellerei Ostgeorgiens ausgeschiedener Stamm *Saccharomyces cerevisiae*-39 verwendet. Die Gärung der Weinmaterialien erfolgte nach dem klassischen Verfahren. Es wurden Einschlußwege einzelner Kohlenstoffatome des Kohlenstoffskeletts des Glyzerins, Äthanols, der Essig- und Bernsteinsäuren in Hefen-und Weinverbindungen festgestellt. Es wurde festgestellt, dass unter extremalen Bedingungen der zweiten Spiritusgärung (CO_2, Druck, Vorhandensein des Äthylalkohols und flüchtiger Säuren im Gärungsmedium) Krebsscher modifizierte Zyklen funktioniert, in dem Reduktionskonvertionen bevorzugt werden. Alle erforschten Verbindungen oxidieren gleichzeitig während der zweiten Spiritusgärung teilweise bis CO_2.

INTRODUCTION

Secondary alcoholic fermentation being a biotechnological base for sparkling wine production essentially differs from natural alcoholic fermentation by its unusual conditions; carbon dioxide high pressure, alcoholic environment, availability of volatile acids, low temperature of fermentation and other factors affect the intensity of yeast metabolic activity. Thus, the fermenting environment and behavior of yeasts under the conditions of secondary fermentation does not correspond to the normal fermentation process and presents revelation of yeasts potential under the extreme conditions (Rose, Harrsion, 1971; Riberau-Gayon' *et al*., 2006).

MATERIALS AND METHODS

In Georgia, the amount of vine varieties suitable for production of sparkling wines equals approximately to 20. Among them, the most widespread are cultivars of *Vitis vinifera*: cv Tsitska, cv Chinuri, cv Pinot, and cv Aliogote. Wine materials obtained from the above-mentioned varieties were blended according to the following ratio: cv Tsitska – 60%, cv Chinuri – 15%, cv Pinot – 5%, cv Aliogote – 5%, 3-year aged wine (cv Chinuri) – 15%. Blended wine material was fermentation medium for yeasts. The following radioactive compounds were applied in the experiments: $1^{14}C$-glucose, $1^{14}C$-ethyl alcohol, $2^{14}C$-ethyl alcohol, $1^{14}C$-glicerine, $2^{14}C$-glicerine, $KH^{14}CO_3$, $1,2^{14}C$-acetaldehyde, $1^{14}C$-acetic acid, $2^{14}C$-acetic acid, $1,4^{14}C$-succinic acid, $2,3^{14}C$-succinic acid, $1^{14}C$-lactic acid, $1^{14}C$-malic acid, $1^{14}C$-citric acid, $1^{14}C$-glycine, $2^{14}C$-glycine, $1^{14}C$-alanine, $3^{14}C$-serine, $4^{14}C$-aspartic acid, $5^{14}C$-glutamic acid, and $1^{14}C$-lisine.

The radioactivities of ^{14}C compounds introduced into the fermentation medium were approximately equal to 23 MBq per litre. The radioactive compounds with high specific activities (25-35 MBq per ml and 2.0-3.5 MBq per mg of isotope) were applied in the experiments; therefore, pH of the fermentative medium did not vary and the amount of ^{14}C compound did not change the ranges of their normal content in wine.

The most widespread pure cultures of *Saccharomyces cerevisiae* traditionally employed in Georgia, namely Kakhuri-42, Manavi-86, Chinuri-74, and Rkatsiteli-61 were used for fermentation, qualitative and quantitative analysis of formed compounds.

Secondary alcoholic fermentation was performed by classical method under the temperature conditions – 14°C-16°C (exposure time – 15-20 days). In order to capture released CO_2, a special aerometer, gas outlet pipe of which was connected with absorption system of 30% KOH was used. The radioactivity of $^{14}CO_2$ was determined by formed $Ba^{14}CO_3$. The volatile acids were determined by the water steam distillation method. The amount of ethyl alcohol was estimated by the density of distillate. In order to obtain aminoacids of yeast proteins, protein fraction hydrolysis was carried out by 6N HCl at102-105C° for 24 hours. The fraction of free aminoacids was performed by threefold extraction of 80% ethyl alcohol for 90 minutes. The methods of chromatography and autoradiography were used to identify organic acids and aminoacids of wine and yeast aminoacids. The radioactivity of isolated fractions and identified compounds were determined on LKB scintillation spectrophotometer Rackbeta SL-30.

RESULTS AND DISCUSSION

Revelation of yeast metabolic potential, study of transformation pathways of low molecular wine compounds has an essential importance to control biotechnological processes of secondary alcoholic fermentation (Cebollero, Gonzalez, 2006).

As a result of many years research, statistically treated significant data on wine components transformation dealing with the study of main, secondary and side products of fermentation, were obtained. One of the serial experiments is presented in the work.

Tab. 1. Assimilation and conversion of ^{14}C compounds during secondary alcoholic fermentation

^{14}C-compounds	Assimilated ^{14}C-radioactivity, (%)	Distribution of radioactivity, (%)	
		yeasts	wine components
1-6^{14}C-glucose	98.0	2.5	97.5
1^{14}C-malic acid	92. 0	0.2	99.8
1^{14}C-citric acid	91. 0	0.01	99.9
1^{14}C-glycine	90. 0	16.3	83.7
3^{14}C-serine	90. 0	1.7	98.3
1^{14}C-alanine	76. 0	2.7	97.3
2^{14}C-glycine	76. 0	11.1	88.9
5^{14}C-glutamic acid	75. 0	3.9	96.1
1^{14}C-lysine	60. 0	24.8	75.2
2^{14}C-acetic acid	52. 0	3.4	96.6
1^{14}C-acetic acid	40. 0	3.3	96.7
1^{14}C-lactic acid	36. 0	2.4	97.6
4^{14}C-aspartic acid	35. 0	9.2	90.8
2,3^{14}C-siccine acid	35. 0	2.6	97.4
1,4^{14}C-siccine acid	26. 0	3.5	96.5
1,2^{14}C-acetaldehyde	23. 0	5.2	94.8
1^{14}C-glycerine	22. 0	0.3	99.7
2^{14}C-glycerine	19. 0	0.9	99.1
^{14}C-carbon dioxide	6. 0	3.2	96.8
2^{14}C-ethyl alcohol	0.7	0.5	99.5
1^{14}C-ethyl alcohol	0.6	0.4	99.6

The results of transformations have shown that the yeast cells take an active part in the metabolism of investigated compounds during the acute fermentation period, before the beginning of lysis.

In the process of secondary alcoholic fermentation, the industrial strain of *Saccharomyces cerevisiae*-39 assimilates carbon skeletons of ethanol, carbon dioxide, acetaldehyde, acetic acid, succinic acid, glycerin, and glycine. 1^{14}C-lactic acid, 1^{14}C-malic acid, 1^{14}C-citric acid, 1^{14}C-alanine, 3^{14}C-serine, 1^{14}C-lysine, 4^{14}C-aspartic acid and 5^{14}C-glutamic acid are actually involved in the metabolic processes. The results of yeast and wine analysis indicate that fermentation of major, secondary and side products are characterized by different levels of transformation (Tab. 1). In our experiments, yeasts maximally assimilate and convert glucose (98.0%), malic acid (92.0%), citric acid (91.0%), glycine (90.0%), and serine (90.0%). Ethyl alcohol (0.7%) is slightly assimilated and converted. The same data show that during the fermentation for 15-20 days, the most part of investigated metabolites transferred into the wine, in spite of yeasts short contact with wine components. At the same time, the carbon skeleton of the studied compounds was partially oxidized to carbon dioxide (Tab. 2).

Tab. 2. Formation of carbon dioxide from wine components during the process of secondary alcoholic fermentation

^{14}C-compounds	Amounts of initial substances (C^{12}+C^{14}) mg/400 ml	Radioactivities of released $^{14}C0_2$, (%)
$1^{14}C$-citric acid	168.8	65.0
$1^{14}C$-malic acid	661.0	53.0
$1^{14}C$-alanine	49.1	50.0
$1^{14}C$-glycine	13.1	38.0
1-$6^{14}C$-glucose	8892.5	25. 0
$4^{14}C$-aspartic acid	179.5	18. 0
$2^{14}C$-glycine	41.4	10. 0
$1^{14}C$-lactic acid	396.7	8. 0
$1^{14}C$-lysine	19.8	4. 0
$1^{14}C$-ethyl alcohol	42014.6	4. 0
1,$4^{14}C$-siccine acid	519.4	2. 0
$1^{14}C$-acetic acid	425.5	2.0
$5^{14}C$-glutamic acid	118.2	0. 5
$3^{14}C$-serine	134.0	0.4
1,$2^{14}C$-acetaldehyde	143.0	0.3
$2^{14}C$-acetic acid	345.0	0.2
2,$3^{14}C$-siccinic acid	540.4	0.1
$1^{14}C$-glycerine	1623.1	0.1
$2^{14}C$-ethyl alcohol	32834.3	0.1
$2^{14}C$-glycerine	1709.1	0

More than 50% of radioactivities of $1^{14}C$-cirtric acid, $1^{14}C$-malic acid, as well as $1^{14}C$-alanine assimilated by yeasts are released in the form of $^{14}CO_2$ (N. Nutsubidze et al., 2008). Different indices of radioactivity of $^{14}CO_2$ indicate the complexity of carbon skeleton oxidative conversions during transformations of $1^{14}C$- and $2^{14}C$-glycine, $1^{14}C$- and $2^{14}C$-acetic acid, $1^{14}C$- and $2^{14}C$-ethyl alcohol.

The results of chromatographic and autoradiographic analysis show that transformation products of the investigated compounds, both in yeast cells and wine components, acids and aminoacids from Krebs and glyoxalate cycles, genesis of which is associated with the conversions of relevant ketoacids are essential (Aplakov *et al.*, 2006).

The above-mentioned results allowed suggesting that yeast metabolic potential is revealed in spite of the limiting extreme factors in the process of secondary alcoholic fermentation (Kirtadze, Kvesitadze, 2008).

Autoradiographic analysis has shown that during fermentation, the main sources for succinic acid formation are carbon dioxide, 2^{14}C-ethyl alcohol, 1^{14}C- and 2^{14}C-acetic acid, 1^{14}C-malic acid, 1,2^{14}C-acetaldehyde, 1^{14}C-glycine, and 5^{14}C-glutamic acid. Carbon skeletons of succinic acid and 4^{14}C-aspartic acid are involved in the process of synthesis of malic acid. The principal sources for citric acid formation are 1,4^{14}C- and 2,3^{14}C-succinic acid and 2^{14}C-ethyl alcohol. 1^{14}C-alanine and carbon dioxide create the base for lactic acid synthesis. Glyoxal acid is yielded mainly by 1^{14}C-glycerine, 1^{14}C-acetic acid, 1^{14}C- and 2^{14}C-glycine, and 1^{14}C-lysine.

The following aminoacids – valine, leucine, and glutamic acid were identified in wine as a result of conversions of 1^{14}C- and 2^{14}C-acetic acid, 1,4^{14}C-succinic acid, 1^{14}C-malic acid, 1^{14}C-citric acid. 2^{14}C-acetic acid and 1,4^{14}C-succinic acid primarily participate in the synthesis of glycine, serine and tryptophan. Synthesis of glutamic acid is connected with 1^{14}C-acetic and 1^{14}C-citric acid conversion.

CONCLUSIONS

- The principal metabolic pathways of the compounds of different classes have been established. Partial refixation of carbon dioxide and ethyl alcohol takes place in the process of fermentation. Their carbon atoms mainly participate in the synthesis of wine organic acids and yeasts protein aminoacids.
- The role of glycerin, an important product of fermentation is revealed in the process of yeast metabolism that indicates that this triatomic carbon alcohol, similar to triose participate in the entire process of metabolism.
- The modified Krebs cycle accomplishes the transformation of large part of organic acids under the conditions of anabiosis, by oxidative transformations to α-ketoglutarate and reductive transformations from oxaloacetate to succinate.

ACKNOWLEDGMENTS

We would like to express our sincere thanks to Georgian National Science Foundation (Grant # GNSF/ST08/8-497) for financial support in arrangement of conducting experiments under the industrial conditions.

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ARE LACTIC ACID BACTERIA ABLE TO DEGRADE BIOGENIC AMINES IN WINES?

A. García-Ruiz, E. González-Rompinelli, B. Bartolomé, Pedro J. Martín-Álvarez, M.V. Moreno-Arribas *

Instituto de Fermentaciones Industriales (CSIC), Juan de la Cierva 3- 28006 Madrid, España. Tel +0034 915622900, Fax +0034 915644853. email: mvmoreno@ifi.csic.es

ABSTRACT

Nowadays, there is a growing concern about the presence of potential toxic compounds in wine, such as biogenic amines that could affect wine safety, and besides, could cause marketing troubles. Some lactic acid bacteria (LAB) isolated from cheese and other foods are able to degrade biogenic amines, through the action of amino oxidase enzymes. Because of the scarce knowledge about this theme in relation to wine microorganims, and with the final aim of avoiding the accumulation of biogenic amine during winemaking, this study evaluates the capacity of different LAB stains, isolated from wines and other related enological sources, to degrade histamine, tyramine and putrescine. A total of 50 LAB strains isolated from musts, wines and fermentation lees, and belong to the genera *Lactobacillus* and *Pediococcus* and the species *Oenococcus oeni*, have been selected for this study. The biogenic amine-degrading capacity of the strains has been evaluated by RP-HPLC in culture medium, cells free extracts and whole cells. Among the strains analysed, 25% are able to degrade histamine, 18% tyramine and 18% putrescine, although at different extend. The majority of the strains with this amino oxidase capacity belong to the *Lactobacillus* and *Pediococcus* groups, and some of them are able to simultaneously degrade more than one biogenic amine. The biogenic amine-degrading capacity of the selected LAB seems not to be associated to their amine-producing capacity, since most of the strains with amine-metabolizing capacity did not show descarboxilase activity implicated in the production of these compounds in wine.

RESUMEN

En la actualidad, existe una inquietud creciente por evitar la presencia de compuestos potencialmente tóxicos, como las aminas biógenas, que pueden afectar a la seguridad del vino, y además, causar trabas comerciales. Algunas bacterias lácticas (BAL) aisladas del queso y otros alimentos, son capaces de degradar estos compuestos, mediante la acción de enzimas amino oxidasas. Debido al escaso conocimiento sobre este tema respecto a los microorganismos del vino, y con el objetivo de evitar la acumulación de aminas biógenas durante la vinificación, este trabajo evalua la capacidad de distintas cepas de BAL aisladas de vinos y otros nichos ecológicos relacionados, de degradar histamina, tiramina y putrescina. Se han seleccionado 50 cepas de BAL aisladas de mostos, vinos y lías de fermentación, pertenecientes a los géneros *Lactobacillus* y *Pediococcus* y a la especie *Oenococcus oeni*. La capacidad de degradar aminas biógenas se ha evaluado en medio de cultivo, extractos libres de células y células enteras, mediante RP-HPLC. De las cepas ensayadas, 25% degradan histamina, 18% tiramina y 18% putrescina, aunque con diferente capacidad de degradación. La mayoría de las cepas con actividad amino oxidasa pertenecen al grupo de los *Lactobacillus* y *Pediococcus,* y algunas de ellas son capaces de

degradar simultáneamente más de una amina biógena. La capacidad de degradación de aminas biógenas por las BAL no parece asociarse con la de su producción, ya que la mayoría de las cepas con capacidad de metabolizarlas no mostraron actividad descarboxilasa implicada en la producción de estas aminas en el vino.

INTRODUCCIÓN

En el vino, la formación de aminas biógenas (i.e. histamine, tiramina y putrescina) se debe fundamentalmente a la presencia de cepas de bacterias lácticas capaces de descarboxilar los aminoácidos precursores. En trabajos realizados en nuestro laboratorio (Marcobal et al., 2006; Martín-Álvarez et al., 2006; Alcalde-Hidalgo et al., 2007) y por otros grupos de investigación (Landete et al., 2005), durante la elaboración industrial del vino, se ha comprobado que la formación de aminas biógenas tiene lugar durante al fermentación maloláctica por acción de la microbiota láctica indígena del vino. Sin embargo, también se ha comprobado en algunas vinificaciones, que durante las etapas de envejecimiento y crianza, tiene lugar un descenso en la concentración de algunas aminas biógenas que podría ser debida a la acción de enzimas amino oxidasas presentes en algunas bacterias lácticas, que mediante una reacción de degradación oxidativa, degradan las aminas biógenas para generar un aldehído, peróxido de hºidrógeno y amonio. Dependiendo del sustrato de partida, es decir de la amina biógena, estas enzimas se clasifican en diamino oxidasas (si se trata de histamina y putrescina) y monoamino oxidas (si el sustrato de partida es la tiramina).

Las enzimas amino oxidasas están presentes en organismos superiores, incluido el hombre, y de hecho estas enzimas se localizan en el tracto gastrointestinal y son las encargadas de la eliminación de aminas biógenas en el organismo, pero también se han descrito en hongos filamentosos (i.e. *Aspergillus niger*) (Frébort et al., 1999) y algunas bacterias aisladas de alimentos, como queso, embutidos y pescados (Leuschner et al., 1998; Enes-Dapkevicius et al., 2000). Debido a la falta de información sobre este tema entre los microorganismos del vino, y con el objetivo de desarrollar procedimientos que eviten la acumulación de aminas biógenas durante la vinificación, en este trabajo se ha evaluado la capacidad de distintas cepas de bacterias lácticas aisladas de vinos y otros ecosistemas relacionados, de degradar histamina, tiramina y putrescina.

MATERIALES Y MÉTODOS

Bacterias lácticas y condiciones de cultivo. La capacidad de degradar aminas biógenas (histamina, tiramina y putrescina) se ha estudiado en 85 cepas de bacterias lácticas pertenecientes a las distintas especies que pueden aislarse en mostos y vino, y que incluyen *Oenococcus oeni* (42 cepas), *Pediococcus parvulus* (7 cepas), *P. pentosaceus* (4 cepas), *L. plantarum* (6 cepas), *L. hilgardii* (9 cepas), *L. zeae* (3 cepas), *L. casei* (7 cepas), *L. paracasei* (5 cepas) y *Leuconostoc mesenteroides* (2 cepas). Estas cepas pertenecen a la Colección de bacterias lácticas del Instituto de Fermentaciones Industriales del CSIC. Adicionalmente, se han evaluado cuatro cepas de *O. oeni* previamente aisladas de preparaciones malolácticas comerciales. Las cepas de *Oenococcus oeni* se crecieron en medio para *Leuconostoc oenos* (MLO) suplementado con 100 $ml.L^{-1}$ de zumo de tomate. Las otras especies de bacterias lácticas se incubaron en el medio MRS. Todas las cepas se incubaron a 30ºC.

Determinación de la capacidad de las bacterias lácticas de degradar aminas biógenas. La capacidad de las cepas bacterianas de degradar las aminas biógenas histamina, tiramina y putrescina se ha estudiado en un medio modelo similar al propuesto previamente por Enes-Dapkevicius et al., (2000), suplementado con 0.05 g l^{-1} de cada amina biógena (dihidrocloruro de histamina , tiramina y dihidrocloruro de 1,4-diaminobutano o putrescina), separadamente a pH 5.5. Las cepas de bacterias lácticas se incubaron a 30º C por duplicado en este sistema modelo en al menos dos días diferentes, y las muestras se tomaron a tiempo 0, y después de 30-72 horas de incubación. La degradación de aminas biógenas se evaluó mediante determinación de la concentración de estos compuestos por HPLC.

Producción de aminas biógenas por las bacterias lácticas. Las cepas de bacterias lácticas se han cultivado a 30ºC en el medio MRS en el caso de *Lactobacillus* sp., *Pediococcus* y *Leuconostoc*, y en el medio MLO en el caso de *O. oeni*, en ambos casos, conteniendo 0.1 % de los correspondientes aminoácidos precursores (L-histidina, tirosina y ornitina), piridoxal-5'-fosfato y otros factores de crecimiento, previamente descritos en Moreno-Arribas et al., (2003). Además, también se ha estudiado la capacidad de las cepas bacterianas de producir aminas biógenas mediante la determinación de la concentración de aminas por HPLC, como se indica a continuación, y la detección de los genes responsables por PCR Múltiple según las condiciones descritas por Marcobal et al., (2005a).

Análisis de aminas biógenas. Las aminas biógenas se analizaron por duplicado, mediante HPLC en fase inversa (RP-HPLC) según el método descrito por Marcobal et al., (2005b), mediante una derivatización precolumna de las aminas de forma automática con el rectivo derivatizante ortoftaldialdehído (OPA) en presencia de β-mercaptoetanol. La detección de las aminas derivatizadas se realizó mediante un detector de fluorescencia (longitud de onda de excitación 340 nm y longitud de onda de emisión 425nm).

RESUTADOS Y DISCUSIÓN

Con el objetivo de evaluar qué nichos ecológicos son más atractivos para la presencia de cepas bacterianas con capacidad de degradación de aminas biógenas, en una primera fase, se ha llevado a cabo el aislamiento de bacterias lácticas a partir de distintas muestras de vinos blancos y tintos, en distintas etapas de la elaboración, especialmente estadios próximos a la fermentación maloláctica en el caso de los vinos tintos. También se partió de heces y lías de fermentación procedentes de diferentes elaboraciones, y muestras de vinos de 'sherry' durante la crianza biológica y velos de flor. La selección de estas muestras se realizó en base a que presentan una microbiota hetereogénea y abundante, y a que presentaban elevados niveles de aminas biógenas.

A partir de estas muestras, se llevaron a cabo los aislamientos de bacterias lácticas en las que se estudió la posible degradación de aminas biógenas. Para ello, se seleccionaron en una primera fase, cepas capaces de crecer en medios con histamina, tiramina y putrescina, como única fuente de nitrógeno y carbono. A continuación, se evaluó la capacidad de degradar aminas biógenas de las cepas seleccionadas en medios de cultivo específicos de crecimiento, como son el medio MRS y MLO, y en medios vínicos, en los que se determinó la influencia de distintos factores enológicos de interés (pH, % etanol y presencia de compuestos fenólicos).

Se han seleccionado un total de 85 cepas de bacterias lácticas pertenecientes a los géneros *Lactobacillus* y *Pediococcus*, y a la especie *Oenococcus oeni*, que fueron aisladas previamente de mostos, vinos y otros nichos enológicos, durante un periodo total de 8 años. En estas bacterias, se ha evaluado mediante RP-HPLC la capacidad de degradar las aminas mayoritariamente presentes en vinos (histamina, tiramina y putrescina), mediante determinación del porcentaje de desaparición de cada una de estas aminas en los medios inoculados, con respecto al medio control. De las cepas de bacterias lácticas ensayadas, un 25% mostraron capacidad de degradar histamina, mientras que la degradación de tiramina o putrescina se observó en un 18% de las cepas estudiadas. La mayoría de las cepas bacterianas con capacidad de eliminar aminas biógenas de los medios de cultivo pertenecen al grupo de los *Lactobacillus* y *Pediococcus*, especialmente *L. hilgardii*, *L. plantarum*, *L. casei*, *P. parvulus* y *P. pentosaceus*, mientras que solo se ha detectado una cepa de *O. oeni* capaz de degradar histamina, aunque con baja actividad. Entre las bacterias examinadas, se ha comprobado que algunas son capaces de degradar simultáneamente más de una amina biógena,

Con respecto al origen de las bacterias que mostraron actividad en la eliminación de aminas biógenas, no se encontró ninguna cepa bacteriana con capacidad de degradar aminas procedente de vinos blancos, mientras que a partir de las otras muestras estudiadas, se aislaron cepas potencialmente activas, con un mayor porcentaje de bacterias positivas procedentes de lías de fermentación, y especialmente, a partir de los vinos de crianza biológica, lo que sugiere que en ambos casos, se trata de nichos ecológicos muy atractivos para el crecimiento de bacterias lácticas con potencial de degradar aminas biógenas (**Figura 1**).

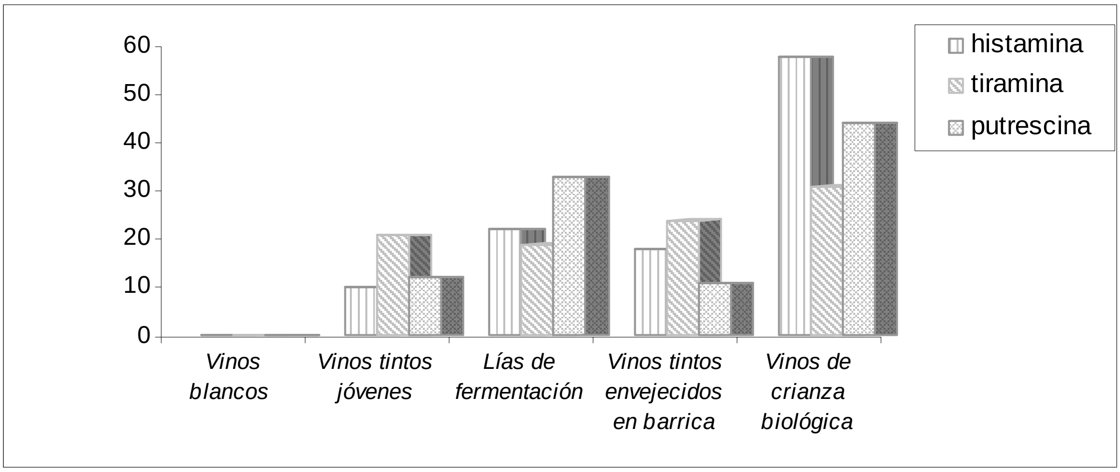

Figura 1. Porcentaje de cepas de bacterias lácticas con capacidad de degradar las aminas biógenas histamina, tiramina y putrescina según el tipo de vino del que proceden

En la **Tabla 1** se muestran de forma pormenorizada los porcentajes de degradación de histamina, tiramina y putrescina, por las bacterias lácticas más interesantes. Los resultados corresponden a valores medios de al menos 2 experimentos en días diferentes, y también los análisis cromatográficos se realizaron por duplicado. La mayoría de las cepas bacterianas con actividad amino oxidasa pertenecen al grupo de los *Lactobacillus* y *Pediococcus*, especialmente *L. hilgardii*, *L. plantarum*, *P. parvulus* y *P. pentosaceus*, mientras que solo se ha detectado una cepa de *O. oeni* capaz de degradar histamina, aunque con baja actividad. Entre las bacterias examinadas, se ha comprobado que algunas son capaces de degradar simultáneamente más de una amina biógena, como por ejemplo la cepa de *Lactobacillus casei* IFI-CA 52, que mostró capacidad de degradar las 3 aminas biógenas en estudio en elevada proporción (**Tabla 1)**.

Tabla 1. Porcentaje de degradación de las aminas biógenas histamina, tiramina y putrescina por las cepas de bacterias lácticas en medios de cultivo

	Degradación (%)*		
Cepas	**Histamina**	**Tiramina**	**Putrescina**
L. plantarum IFI-CA 26	33	n.e.	24
P. pentosaceus IFI-CA 30	10	12	49
P. parvulus IFI-CA 31	21	15	53
O. oeni IFI-CA 32	12	n.e.	16
L. hilgardii IFI-CA 41	n.e.	n.e.	20
L. casei IFI-CA 52	54	55	65
L. plantarum IFI-CA 54	23	17	24
P. pentosaceus IFI-CA 83	19	22	39
P. pentosaceus IFI-CA 86	n.e.	54	69

*Valores medios (n=3); n.e.: no se ha observado efecto

Por otro lado, también se ha estudiado la posible producción de aminas biógenas entre las cepas seleccionadas, comprobándose que la capacidad de degradación de aminas biógenas por las bacterias lácticas ensayadas, no parece que esté asociada a la de producción de estos compuestos, ya que la mayoría de las cepas con capacidad de metabolizar aminas biógenas no mostraron actividad descarboxilasa implicada en al producción de estos compuestos en el vino.

Para evaluar el comportamiento de las cepas seleccionadas durante la elaboración del vino, se realizaron a continuación distintas experiencias en medios vínicos sintéticos, a partir de la cepa de *L. casei* IFI-CA 52, que mostró ser una de las más interesantes del presente estudio. Puesto que de todas las aminas biógenas, la histamina es la que más interés despierta, debido a que es la amina que se controla en las exportaciones de vino a determinados países, se evalúo la capacidad de degradación de esta amina por la cepa bacteriana seleccionada en condiciones próximas al vino. Una vez crecida la bacteria en medios de cultivo específicos, se obtuvieron las células bacterianas, y con el fin de evaluar la funcionalidad de la enzima amino oxidasa, se procedió a la ruptura de las células y la obtención del extracto enzimático, que se incubó con histamina durante 5 horas, y se evaluó el porcentaje de degradación de la amina a lo largo del tiempo en distintos experimentos, que incluyen el efecto de distintos valores de pH, concentración de etanol y de distintos extractos y compuestos fenólicos. Los resultados obtenidos se evaluaron frente a un control, en el que el extracto enzimático se incubó con histamina a pH 7 (el óptimo para la actividad amino oxidasa), en aerobiosis y ausencia de etanol y de los otros productos ensayados. Como resultados más relevantes, cabe destacar que la actividad enzimática diamino oxidasa del extracto enzimático, se ve impedida a concentraciones de etanol próximas a las del vino y especialmente en los medios vínicos suplementados con extractos fenólicos, en los que se observó una disminución del porcentaje de degradación de histamina, mientras que no se ha observado un efecto en la actividad enzimática y en la degradación de histamina debida al pH.

CONCLUSIONES

A pesar de que hasta el momento actual, no se había descrito la capacidad de las bacterias lácticas del vino de degradar aminas biógenas, el presente trabajo muestra que ciertas cepas especialmente del grupo de los *Lactobacillus* y *Pediococcus* son capaces de reducir la concentración de histamina, tiramina y putrescina en medios de cultivo, en distinta proporción dependiendo de la cepa de bacteria láctica. La capacidad de degradación de aminas biógenas por las bacterias lácticas no parece estar asociada con la de producción de estos compuestos, ya que la mayoría de las cepas con capacidad de metabolizar histamina, tiramina y/o putrescina no mostraron actividad descarboxilasa implicada en la producción de estas aminas en el vino. Algunos factores enológicos, especialmente la proporción de etanol y la concentración y tipo de compuestos fenólicos parece interferir en la eliminación de aminas biógenas por bacterias lácticas, aunque son necesarios más estudios que evalúen el efecto real de la matriz vínica en esta propiedad, y en definitiva, que permitan valorar el potencial uso de bacterias lácticas con elevada capacidad para reducir la concentración de aminas biógenas durante la vinificación.

Agradecimientos

Este trabajo se ha realizado mediante la financiación recibida por el Ministerio de Ciencia e Innovación (Proyectos AGL2006-04514/ALI, FUN-C-FOOD Consolider Ingenio CSD2007-00063 y PET2007_0134) y la Comunidad de Madrid (Proyecto P2009/AGR-1469). AG-R agradece al CSIC el contrato JAE-predoc.

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USO DE UN NUEVO SISTEMA DE INMOVILIZACIÓN DE LEVADURAS EN VINIFICACIÓN. ESTUDIO DE SU REUTILIZACIÓN.

A. Puig[1*], E. Bertran[1], M. C. Masqué[1], M. Vilavella[1], T. García-Martínez[2], J. C. Mauricio[2], S. Mínguez[1]

[1]*INCAVI-IRTA (Institut Català de la Vinya i el Vi). Estació de Viticultura i Enologia. Plaça Àgora, 2. 08720 Vilafranca del Penedès (Barcelona). Spain. Telf: 0034938900211,* apuigpujol@gencat.cat

[2]*Departamento de Microbiología. Edificio Severo Ochoa. Universidad de Córdoba. Campus Universitario de Rabanales. 14014 Córdoba. Spain. Telf: 0034957218640,* mi1gamaj@uco.es

RESUMEN

Se ha determinado la capacidad y viabilidad fermentativa de dos cepas de *Saccharomyces cerevisiae* encapsuladas en un nuevo sistema de bioinmovilización sin soportes físico-químicos externos. La bioinmovilización se ha conseguido mediante la co-inmovilización espontánea y natural de las levaduras y un hongo filamentoso (cepa H3 de *Penicillium chrysogenum*). Las levaduras inmovilizadas en biocápsulas se han reutilizado en 11 fermentaciones sucesivas, manteniendo su potencial fermentativo durante al menos 85 días. Se ha efectuado el seguimiento cinético de las fermentaciones y se han analizado distintos parámetros enológicos para identificar posibles diferencias de producción de metabolitos en cada una de las reutilizaciones que pudieran afectar a la calidad del vino producido. La utilización sucesiva del mismo inóculo de levaduras inmovilizadas no afectó de manera significativa la velocidad en las 11 fermentaciones. Tampoco existieron diferencias remarcables en la composición final de los vinos obtenidos.

SUMMARY

The fermentative capacity and viability of two strains of *Saccharomyces cerevisiae* encapsulated in a new bioimmobilization system without physic-chemical external supports has been determined. Bioimmobilization was achieved through the spontaneous and natural co-immobilization of the yeasts and a natural filamentous fungus (*Penicillium chrysogenum* strain H3). Yeasts immobilized in biocapsules were reused in 11 successive fermentations, maintaining their fermentation potential for at least 85 days. Fermentation kinetics was recorded in each case and different parameters to identify possible differences in oenological metabolites production that can affect sensory quality of produced wine were analyzed in each of the biocapsules reuses. The consecutive use of the same immobilized yeast inoculum did not significantly affect the speed of the 11 fermentations. Also there were not remarkable differences in the final composition of obtained wines.

INTRODUCCIÓN

La inmovilización celular aplicada en diferentes aspectos de la vinificación está recibiendo un gran impulso en los últimos años por sus ventajas técnicas y económicas. Los sistemas de microorganismos inmovilizados protegen a las células contra el estrés ambiental y por ello aumentan la productividad, permitiendo un mejor control de la cinética del proceso, reducen los costes debido a la posibilidad de recuperación y reutilización (Godia *et al.*, 1991) y también influyen en el metabolismo de las levaduras y, en consecuencia, en las características organolépticas del producto final.

La mayoría de sistemas de inmovilización celular conocidos y testados son artificiales. En la industria vínica el sistema de inmovilización más utilizado ha sido la encapsulación de levaduras en alginato cálcico, compuesto orgánico que se obtiene de algas marinas. En estos casos, las levaduras no se encuentran en su estado natural y, dependiendo del daño causado por el procedimiento de inmovilización, su viabilidad puede verse alterada y su metabolismo puede sufrir desviaciones que repercuten en las características organolépticas del producto fermentado. No obstante, existen microorganismos que, espontáneamente, en determinadas condiciones, producen inmovilizaciones naturales.

Peinado, *et al*. (2005) describieron un nuevo procedimiento de inmovilización natural donde se favorece la co-inmovilización espontánea entre un hongo filamentoso de la especie *Penicillium chrysogenum* (cepa H3) y cepas de *Saccharomyces cerevisiae* en ausencia de soportes inertes externos. Mediante este procedimiento obtuvieron esferas huecas constituidas por los dos microorganismos denominadas biocápsulas de levaduras. Este formato, como otros sistemas de inmovilización celular, tienen la ventaja de que fácilmente introducen y retiran los microorganismos de los medios de cultivo después de la total o parcial biotransformación de los sustratos, reduciendo el riesgo de contaminación, acortando el tiempo del proceso y permitiendo desarrollar procesos continuos de fermentación (Groboillot *et al*., 1994).

El objetivo del trabajo que se presenta ha sido estudiar el comportamiento fermentativo a escala de laboratorio de dos cepas de *S. cerevisiae* inoculadas en formato de biocápsulas y la caracterización enológica de los vinos obtenidos cuando el mismo lote o inóculo inicial de levaduras ha sido reutilizado a lo largo de once fermentaciones sucesivas.

MATERIAL Y MÉTODOS

Microorganismos

Se utilizaron dos cepas de *S. cerevisiae*: la cepa P29 (CECT 11770) aislada en la zona vitivinícola de la D.O. Penedés, perteneciente a la colección de levaduras de INCAVI y la cepa QA23, comercializada por Lallemand BIO y seleccionada en la región de los vinos verdes por la C.V.R.V.V. y por la U.T.A.D. en Portugal.

La inmovilización de P29 y QA23 en forma de biocápsulas se efectuó en el Departamento de Microbiología de la Universidad de Córdoba según lo establecido por Peinado *et al*., 2005; Peinado *et al*., 2006 y García-Martínez *et al*., 2008.

Condiciones de fermentación

Se realizaron once fermentaciones (FA1 a FA11) para cada cepa (P29 y QA23), cada una de ellas por triplicado. Para las fermentaciones se utilizaron matraces erlenmeyer de 500 ml de capacidad con 400 ml de mosto pasteurizado de la variedad de uva blanca Parellada con un nivel de azúcar reductor inicial de 180 g/l, pH 3.25 y acidez total 7.3 g/l (expresada en ácido tartárico). Las fermentaciones tuvieron lugar a una temperatura controlada de 28ºC en una estufa con agitación a 120 rpm. El inóculo inicial en mosto en la FA1 se calculó entre 2 y 3 $x10^6$ células/ml. Cuando se alcanzaron 10,3%-10,4% (v/v) de etanol, las biocápsulas se retiraban del medio, se lavaban con agua destilada estéril en condiciones asépticas y se volvían a inocular en un nuevo recipiente con mosto.

Controles analíticos

La cinética fermentativa en cada caso se monitorizó por la cantidad de CO_2 liberado como medida de la pérdida de peso (Sablayrolles *et al*., 1987) y por el seguimiento del consumo de azúcar mediante la medida de la pérdida de grado Brix.

La pureza de la cepa implantada al final de cada fermentación alcohólica se determinó mediante el estudio del perfil de restricción de su ADN mitocondrial (mtDNA-RFLP) (Querol *et al*., 1992; Puig *et al*., 2002) para comprobar que no había habido contaminación durante el proceso.

Los parámetros químicos generales: grado alcohólico (GAV), acidez total, acidez volátil, azúcares (glucosa+fructosa) y pH se determinaron de acuerdo con métodos oficiales de la U.E. y la O.I.V. (Organización Internacional de la Viña y el Vino) (E.E.C. 1990; O.I.V., 2005). El glicerol fue analizado mediante método enzimático (Bermeyer, 1974).

Los alcoholes superiores se cuantificaron mediante cromatografía de gases (GC-FID) utilizando una columna Suprawax 280 de 30 m y diámetro interno de 0,53 μm. La Tª inicial fue de 45ºC y la Tª final de 120ºC durante 10 min con un rampa de 4,5ºC/min. La Tª del inyector fue de 220ºC y la del detector FID 260ºC.

Análisis estadístico

Los valores de los parámetros enológicos obtenidos de cada fermentación se sometieron a un análisis de la varianza (ANOVA) y una separación de medias por el método Tukey para determinar si existían diferencias significativas en las diferentes reutilizaciones. Para ello se utilizó el paquete estadístico SYSTAT 10.

RESULTADOS Y DISCUSIÓN

Seguimiento de la cinética fermentativa

La Fig. 1 muestra las cinéticas fermentativas para cada reutilización de las dos cepas de levadura según la pérdida de peso por desprendimiento de CO_2 de cada matraz. En cada caso se representa la media aritmética de los triplicados realizados. En ambas cepas existió un comportamiento similar: la primera fermentación alcohólica (FA1) se realizó más rápida (6 días) que las diez posteriores. En las siguientes, la cinética se ralentizó paulatinamente en la mayoría de los casos, observándose un retraso sucesivo a nivel de horas en su finalización. Este factor se justifica por la pérdida de viabilidad celular en el inóculo de células inmovilizadas a lo largo del tiempo. Todas las fermentaciones se consideraron acabadas a los 7 días de haberse inoculado las biocápsulas (ver concentración de azúcares en Tabla 1). Un retraso en un rango de horas en la finalización del proceso fermentativo en el transcurso de las distintas reutilizaciones puede considerarse insignificante a nivel industrial.

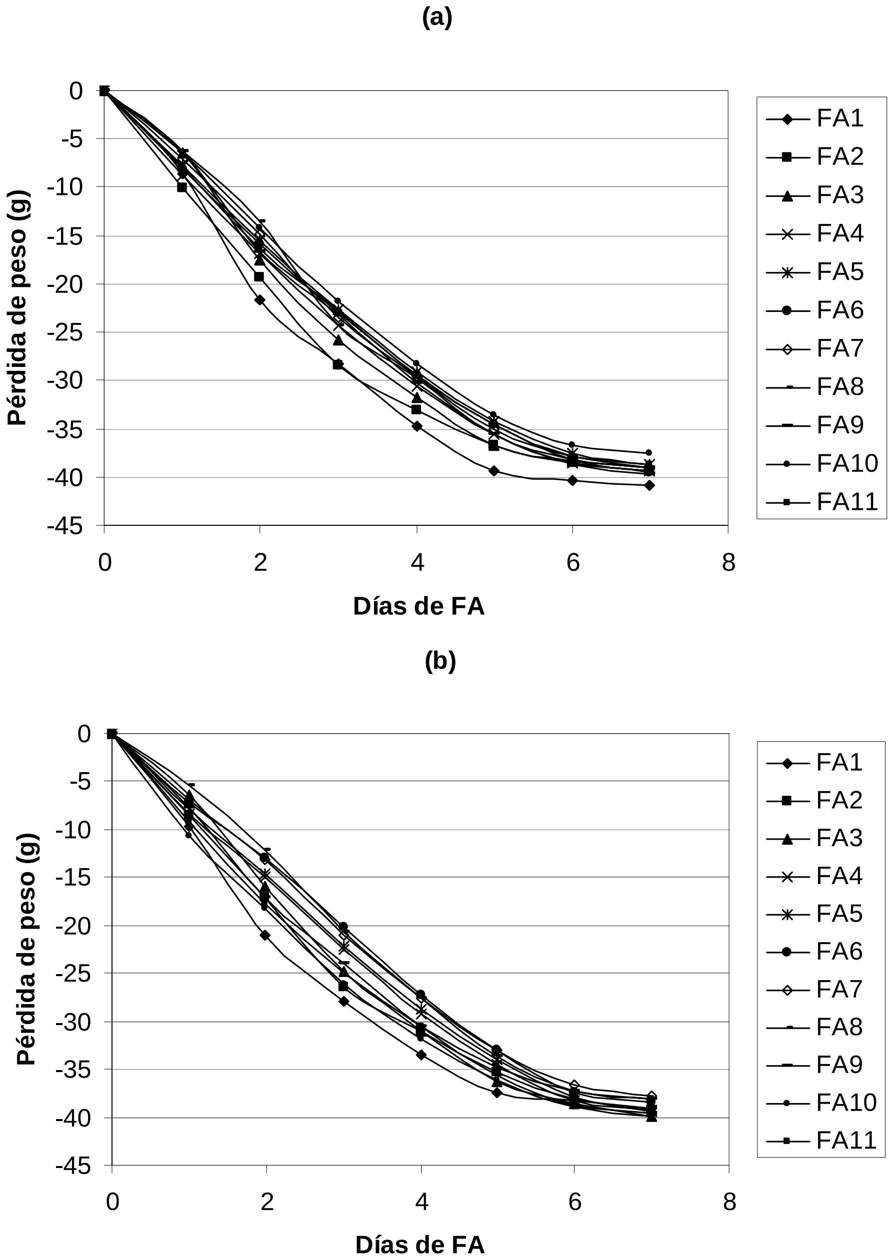


Fig. 1. Cinética de fermentación (medida de pérdida de peso por desprendimiento de CO_2 a lo largo del tiempo) del mosto inoculado con la cepa P29 (a) y QA23 (b) en 11 reutilizaciones sucesivas.

Las biocápsulas se hincharon durante la fase tumultuosa de cada fermentación debido a la emisión de CO_2 pero recuperaron su diámetro, forma y consistencia original pasada esta etapa. Esto demuestra las propiedades elásticas de esta forma de inmovilización. No se produjeron roturas ni por crecimiento de las levaduras ni por el desprendimiento de CO_2, como ocurre algunas veces cuando se utilizan otros soportes de inmovilización. El lavado con agua destilada estéril después de cada utilización no afectó el funcionamiento de las levaduras.

Aunque hubo liberación de levaduras al medio, como también ocurre cuando se utilizan inmovilizados en esferas de alginato cálcico, el inóculo de células incluidas en las biocápsulas fue suficiente para mantener la cinética fermentativa a lo largo de las 11 reutilizaciones.

Análisis de parámetros enológicos

En el sector vínico no es costumbre el mantenimiento y reutilización de un mismo inóculo de levadura en varias (o sucesivas) fermentaciones. En la industria cervecera, esta operación sí se realiza y se ha comprobado que las células se someten a considerables tensiones o estreses que afectan a su vitalidad y a la calidad del producto final (Powell *et al*., 2000; Powell *et al*., 2003). Teniendo en cuenta este hecho, aunque se ha demostrado que la viabilidad en las fermentaciones vínicas de este estudio no se vio significativamente afectada, se determinó si existían diferencias en las distintas reutilizaciones en algunos parámetros enológicos o en la producción de metabolitos importantes para la calidad organoléptica del vino. En la Tabla 1 se detallan las concentraciones de las variables enológicas clásicas determinadas en las once fermentaciones efectuadas para cada cepa.

Tabla 1. Valores de parámetros enológicos en el mosto fermentado con P29 y QA23. Los valores representan la media aritmética de las tres fermentaciones efectuadas en cada reutilización ± la desviación estándar.

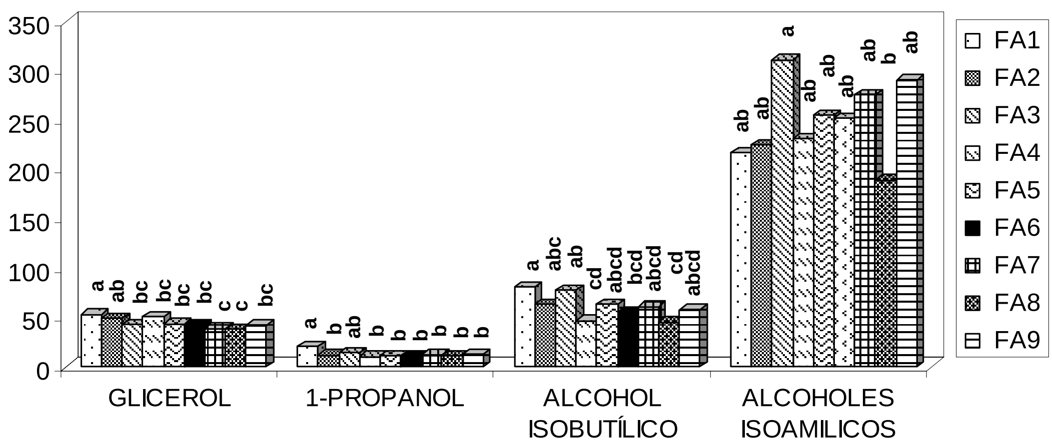
	P29					**QA23**				
Reutilizaciones	GAV (%vol)	Azúcares totales (G+F) (g/L)	pH	Ac. volátil (g/L ác. acético)	Ac. total (g/L ác. tartárico)	GAV (%vol)	Azúcares totales (G+F) (g/L)	pH	Ac. volátil (g/L ác. acético)	Ac. total (g/L ác. tartárico)
FA1	9,95±0,10	0,3±0,00	3,27±0,01	0,57±0,13	6,83±0,21	9,95±0,00	0,3±0,00	3,26±0,02	0,48±0,05	7,05±0,07
FA2	10,25±0,07	0,4±0,00	3,16±0,02	0,35±0,03	6,80±0,00	10,25±0,09	0,4±0,00	3,17±0,01	0,57±0,04	7,23±0,015
FA3	10,20±0,07	0,3±0,00	3,19±0,01	0,36±0,01	7,35±0,07	10,12±0,03	0,3±0,00	3,22±0,00	0,67±0,05	8,17±0,06
FA4	10,33±0,04	0,4±0,00	3,14±0,00	0,34±0,01	6,75±0,21	10,18±0,06	0,4±0,00	3,19±0,01	0,61±0,05	8,00±0,10
FA5	10,23±0,04	0,4±0,00	3,16±0,00	0,38±0,01	7,45±0,07	10,10±0,05	0,4±0,00	3,21±0,01	0,69±0,16	8,30±0,20
FA6	10,23±0,04	0,4±0,00	3,14±0,01	0,48±0,00	7,10±0,00	10,18±0,06	0,4±0,00	3,20±0,01	0,91±0,12	7,87±0,15
FA7	10,25±0,00	0,4±0,00	3,15±0,00	0,43±0,03	7,00±0,28	10,00±0,28	0,4±0,00	3,22±0,02	1,18±0,48	8,15±0,78
FA8	10,40±0,07	0,4±0,00	3,17±0,00	0,39±0,04	6,70±0,14	10,35±0,00	0,4±0,00	3,18±0,00	0,50±0,01	7,25±0,07
FA9	10,25±0,00	0,4±0,00	3,17±0,02	0,38±0,01	7,70±0,14	10,18±0,04	0,4±0,00	3,20±0,01	0,56±0,00	8,20±0,00
FA10	10,30±0,07	0,2±0,00	3,16±0,01	0,38±0,06	6,95±0,35	10,33±0,04	0,2±0,00	3,17±0,00	0,54±0,01	7,20±0,14
FA11	10,34±0,09	0,2±0,00	3,16±0,00	0,44±0,09	6,95±0,49	10,22±0,03	0,2±0,00	3,18±0,00	0,57±0,00	7,40±0,14

Tanto en el caso de la cepa P29 como en la QA23 se pudo observar en la primera fermentación un menor rendimiento en producción de etanol, supuestamente debido a la adaptación de las biocápsulas al medio. Esta menor concentración de etanol en la FA1 puede relacionarse con una mayor producción de glicerol (estadísticamente significativa) en ambas levaduras (ver Fig. 2). Estas diferencias a nivel de la primera reutilización no pueden atribuirse a la acción del hongo filamentoso que forma la estructura de la biocápsula ya que éste muere a los pocos días de iniciarse la fermentación alcohólica, quedando como mero soporte inerte (Peinado *et al*., 2006). La FA1 con la P29 registró un valor de acidez volátil superior al resto de reutilizaciones realizadas con la misma cepa, pero por debajo del umbral de percepción (>0,7 g/l). En el caso de la cepa QA23 se detectó un aumento de acidez volátil en las reutilizaciones 5, 6 y 7, pero asociada a una desviación estándar también más elevada de los replicados. No obstante, a partir de la FA 8, los niveles volvieron a los encontrados en las primeras fermentaciones, pudiéndose descartar una alteración del metabolismo de las biocápsulas de levadura reutilizadas.

Respecto al nivel de glicerol (Fig. 2) en los vinos producidos en las 11 reutilizaciones analizadas, existió la misma tendencia de comportamiento en ambas cepas: la mayor producción de este metabolito, con diferencias estadísticamente significativas, se detectó en la

FA1 y la FA2 (5,3 g/l y 4,7 g/l para la P29 y 5,1 g/l y 4,3 g/l para la QA23 respectivamente). En el caso de los alcoholes superiores, donde se analizaron las 9 primeras reutilizaciones, con la cepa P29 (Fig. 2a) no existieron diferencias significativas (P=0,741) en el conjunto de los alcoholes superiores (suma de 1-propanol, alcohol isobutílico y alcoholes isoamílicos), aunque sí por separado. Respecto al 1-propanol y al alcohol isobutílico, las principales diferencias en su concentración se observaron en la FA1 y FA3, algo superiores al resto, asumiéndose un periodo de adaptación al medio y mayor actividad de las levaduras. A partir de la FA4 la producción de estos compuestos se estabilizaron. En cuanto a los alcoholes isoamílicos, las diferencias significativas se encontraron entre la FA3 y la FA8, sin observarse ninguna tendencia clara a lo largo de las 9 fermentaciones sucesivas. Respecto al metabolismo de los alcoholes superiores de *S. cerevisae* QA23 (Fig. 2b), la máxima producción tuvo lugar en la FA1 y la FA3 en la mayoría de los casos, como en la P29. No obstante, con la cepa QA23 se observó un descenso en la concentración de alcoholes superiores a partir de la séptima reutilización. Esto puede significar una cierta alteración en el metabolismo de la levadura a partir de esta fermentación que podría repercutir en la calidad organoléptica del vino final.

(a)



(b)

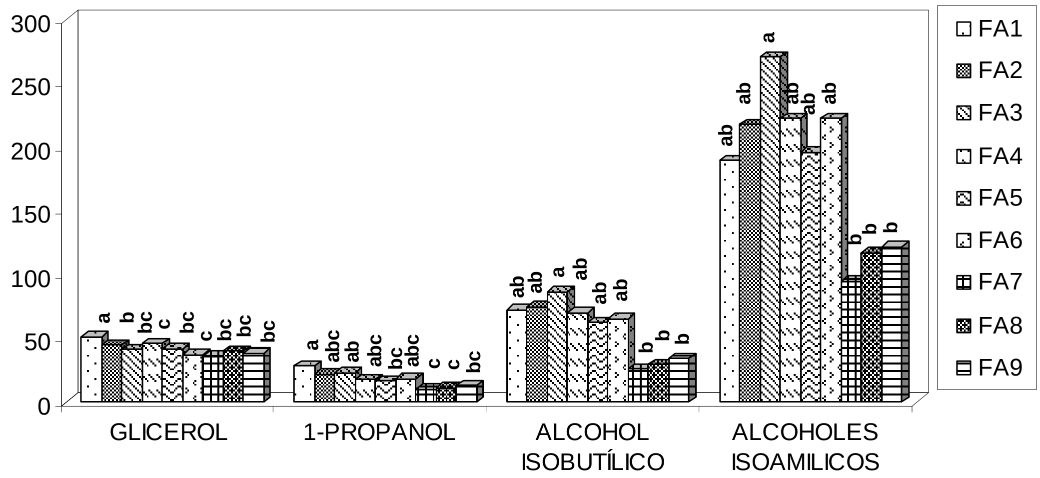


Fig. 2. Contenido en glicerol (g/L) y alcoholes superiores (mg/L) en los mostos fermentados con P29 (a) y QA23 (b): media aritmética de tres fermentaciones en cada reutilización. El valor de glicerol representado corresponde al valor real (x10). Las letras de cada columna indican la separación de medias determinada por el método Tukey.

CONCLUSIONES

Este estudio describe la aplicación de un sistema innovador de inmovilización celular para levaduras vínicas y su viabilidad para la utilización en distintos procesos fermentativos consecutivos. Se demuestra que las biocápsulas pueden reutilizarse hasta en once vinificaciones, sin perder la actividad fermentativa. Este sistema de co-inmovilización natural mantiene la viabilidad de las células de levadura al menos durante 85 días y conserva la consistencia esférica e integridad, demostrando que el hongo filamentoso actúa como un buen soporte. Los compuestos analizados en el vino se encuentran dentro de los límites de concentración descritos en la literatura y no se muestran cambios sustanciales a lo largo de las distintas reutilizaciones. Aunque son necesarios estudios adicionales con mostos de otras características y con un volumen inicial superior para poder llevar a cabo un análisis sensorial del vino, el uso de este nuevo formato de inmovilización puede permitir agilizar el proceso, reducir costes y tener una futura aplicación en procesos continuos.

AGRADECIMIENTOS

Los autores agradecen la financiación de este trabajo al Proyecto RTA2008-00056-C02-01 del Ministerio de Ciencia e Innovación (INIA).

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Influence of grape variety and the biological defense system of vineyards on grape microbiota

Cordero-Bueso, G. (1), Arroyo, T. (1), Serrano, A. (1), Tello, J. (1), Aporta, I. (1), Valero, E. (2)

(1)Departamento de Agroalimentación, Instituto Madrileño de Investigación y Desarrollo Rural Agrario y Alimentario Autovía A2, km 38,2. 28800 Alcalá de Henares, Madrid, España. teresa.arroyo@madrid.org

(2)Departamento de Biología Molecular e Ingeniería Bioquímica. Universidad Pablo de Olavide, Sevilla. Ctra. de Utrera Km 1, s/n. 41013 Sevilla, España. evalero@upo.es

SUMMARY

A three year sampling plan was designed in order to evaluate the influence of different agronomic parameters on the biodiversity of fermentative grape yeasts. Thus, two vineyards with different biological defense systems (organic and conventional), with three different grape varieties (Shiraz, Grenache and Barbera) were chosen. A total of 27 samples were collected from the two vineyards. Spontaneous fermentation occurred in 24 and 12 of the samples from the conventional and organic vineyards respectively. These results indicated a clear influence on grape associated yeast biodiversity of the phytosanitary treatment used in the vineyard.

RESUMEN

En este trabajo se ha diseñado un plan de muestreo en dos viñedos de la Comunidad de Madrid (convencional y ecológico) con tres variedades de vid diferentes (Syrah, Garnacha y Barbera) durante tres años, para evaluar la influencia de distintos parámetros agronómicos sobre la biodiversidad de levaduras fermentativas de la uva. De las 27 muestras tomadas en ambos viñedos, 24 fermentaron espontáneamente en el ecológico, frente a las 12 del convencional lo que indica la incidencia de los tratamientos fitosanitarios sobre la microbiota de levaduras asociada al viñedo.

1. INTRODUCTION

The study of the biodiversity of yeasts in order to obtain more information on the communities present on grape berries and their influence on the wine making process is a major challenge in wine microbiology. Grapes are a primary source of natural yeasts in wine production. The composition and properties of different grape varieties has been extensively investigated (Chavan *et al*., 2009; Francesca *et al*., 2010). Thus, wine quality is influenced, partially, by the composition of the grape juice and by the microorganisms present in the fermentation process (Callejón *et al*., 2010). The grape microbiota can be either beneficial or detrimental to the quality of wine and wine products. Consequently, for winemakers it is important to have more information about the yeast communities present on grapes in order to produce high quality wines with representative characteristics.

Biochemical interaction between yeasts and musts derived from the varieties of *Vitis vinifera* is paramount. Species of the genera *Candida*, *Hanseniaspora*, *Hansenula*, *Issatchenkia*, *Kluyveromyces*, *Metschnicowia*, *Pichia*, *Saccharomyces*, *Torulaspora* and

Zygosaccharomyces are known to be present on the surface of grape berries. The population density and the diversity of indigenous yeasts on the grape berries are intrinsically linked to numerous factors, such as climatic conditions, the geographical location of the vineyard, the ripeness of the grape berries, the age of the vineyard, the type of soil, the grape variety, the application of antifungal products and the harvesting technique (Pretorius, 2000; Valero *et al*., 2005; Chavan *et al*., 2009). However, there is insufficient quantitative data available to establish general conclusions on the influence of these factors. Because wine-makers recognize that some indigenous yeast species may enhance or damage the quality of wine, deeper knowledge of the effects of these factors on the yeast ecology of grapes is required.

The use of selected strains of *Saccharomyces cerevisiae* has improved the fermentative processes and the quality of wines, but its continual use has led to the colonization and consequent elimination of the native microorganisms present in the wineries. Hence, because the vineyard may be the main reservoir of native yeasts of oenological interest, it is necessary to preserve, and even encourage the presence of fermentative species in it. In fact, the use of native yeast strains is preferable since they are better acclimatised to the environmental conditions and assure the maintenance of the typical sensory properties of the wines of a given region.

Consumer concern about the quality of food and beverages, particularly regarding how, when and where these products are produced is on the increase. The effects and consequences on the environment of the farming system used are also a cause of concern. Organic food is likely to contain lower residues of agricultural chemicals than nonorganic food. Studies typically find that, in comparison with conventional farming, organic farming results in a greater abundance of species across a number of different areas (Callejón *et al*., 2010).

Thus, a three year sampling plan in the vineyards of the Madrid region of Spain was designed in order to evaluate the yeast populations present. Three grapes varieties (Shiraz, Garnacha y Barbera) grown in two vineyards with different biological defense systems (conventional and organic) were chosen in order to obtain precise information on these agronomic parameters. This work shows the results obtained taking into account the biological defense system of the vineyard.

2. MATERIALS AND METHODS

This study was carried out from 2006 to 2008 in two vineyards (organic and conventional) located in the Madrid (Spain) winegrowing region, with three different grapevine varieties (*Vitis vinifera* L.*)*: Shiraz, Grenache and Barbera. The grapes were cultivated in both vineyards in bare soil by tillage and on vertical trellises facing in the direction of the gradient with Guyot pruning. Irrigation was performed through a drip system.

Approximately 2 Kg of grapes were harvested and squeezed by hand in aseptic conditions. The musts obtained were fermented at 20ºC for the time needed in order to reduce the weight of the must by 70 g L^{-1}, corresponding to the consumption of about two thirds of the sugar content. Ten-fold dilutions of the resulting fermented must were spread on plates with YPD medium and incubated for 24 – 48 hours. After that, 30 colonies were randomly selected from each fermentation. Yeast DNA was extracted using a commercial kit. The isolates were analyzed by PCR of ITS of 5.8 S ribosomal region of DNA. After that, RFLP of the ITS fragment was carried out with three restriction enzymes (*HaeIII*, *CfoI* and *HinfI*) (Esteve-Zarzoso *et al*., 1999; Fernández-Espinar *et al*., 2001; Sabaté *et al*., 2002). Strains identified as *S. cerevisiae* were genotyped by microsatellite multiplex PCR (Vaudano & García-Moruno, 2008). Fragment differentiation and allele size determination was performed by single capillary automatic electrophoresis in ABI 3130 Genetic Analyzer.

3. RESULTS AND DISCUSSION

The aim of this work is the study and evaluation of the influence of the grape variety and the biological defense system on yeasts associated with the vineyard. To study the influence of different grape berries on the microbiota associated, three different varieties were chosen: Shiraz, Grenache and Barbera. They were collected from two vineyards with different biological defense systems (conventional and organic).

In order to get more detailed data, this study was carried out over a period of 3 consecutive years (2006-2008). Every year, 18 grape samples were collected, 3 for each biological defense system and for each grape variety (54 samples in total). Thus, 13, 14, and 9 spontaneous fermentations occurred in samples for the 2006, 2007 and 2008 samples, respectively. A total of 1080 colonies were isolated from these fermentations, 81% corresponding to non-*Saccharomyces* and 19% to *Saccharomyces* strains. The information on the fermentations and the distribution of the yeast strains isolated are shown in Table 1.

Table 1. General data of the fermentation process and yeasts strains isolated in the 3 years

	Vintage		
	2006	2007	2008
Samples	18	18	18
Spontaneous Fermentations	13	14	9
Isolates of *Saccharomyces*	30	108	66
Total of *Saccharomyces* and percentage (%)		204 (19%)	
Isolates of non-*Saccharomyces*	360	312	204
Total of non-*Saccharomyces* and percentage (%)		876 (81%)	
Total of isolates	390	420	270
		1080	

The PCR and RFLP analysis of the isolates obtained from the fermentations (Esteve-Zarzoso *et al*., 1999; Fernández-Espinar *et al*., 2000) showed an important variation of the size of the fragments for the different species. 9 species were identified, corresponding to *Candida sorbosa* (anamorphic *Issatchenkia occidentalis*), *Candida stellata*, *Hanseniaspora guilliermondii*, *Kluyveromyces thermotolerans*, *Metschnicowia pulcherrima*, *Pichia anomala*, *Pichia toletana*, *Saccharomyces cerevisiae* and *Torulaspora delbrueckii*. *K. thermotolerans*, *S. cer*evisiae, *H. guilliermondii* and *C. stellata* were the most abundant species, representing 32.69%, 18.89%, 18.43% and 15.93% respectively. *P. anomala*, *T. delbrueckii and M. pulcherrima* were present in lower percentages: 6.48%, 4.35% and 2.78%. *P. toletana* and *C. sorbosa* were occasionally found (0.37% and 0.09%). This data is shown in Table 2.

Table 2. Species identified in 2006, 2007 and 2008. Size of the amplified products (AP) and the restriction fragments of the species obtained with three different endonucleases (*HaeIII, CfoI and HinfI*). (Esteve-Zarzoso *et al*., 1999; Fernández-Espinar *et al*., 2000)

Species	AP (bp)*	Restriction fragments size (bp)			Total of isolates	% of isolates
		HaeIII	*CfoI*	*HinfI*		
Metschnicowia pulcherrima	400	280 + 95	210 + 80	190	30	2,78
Candida stellata	500	490	210+115+70	230+230	172	15,93
Candida sorbosa	600	600	560	315	1	0,09
Pichia anomala	630	620	550	310+310	70	6,48
Kluyveromyces thermotolerans	700	300+210+85	305+280	355	353	32,69
Pichia toletana	700	600	625	375	4	0,37
Hanseniaspora guilliermondii	775	775	340+320+105	360+200+160	199	18,43
Torulaspora delbrueckii	800	750	320+210+140+100	410+375	47	4,35
Saccharomyces cerevisiae	850	325+250+185+150	375+325+150	375+365+110	204	18,89

**AP= 5.8S-ITS amplified product size*

The 204 strains identified as *S. cerevisiae* were genotyped by Microsatellite Multiplex PCR analysis (Vaudano and García-Moruno, 2008) using SC8132X, YOR267C and SCPTSY7 primers. Nine different electrophoretic patterns (A, B, C, D, E, F, G, H, I) were obtained. The allele size obtained by single capillary automatic electrophoresis and their repeatability are indicated in Table 3.

Table 3. Patterns obtained from 204 *Saccharomyces* cerevisiae strains analyzed by microsatellite multiplex PCR, and their frequency..

Genotype	Number of *S. cerevisiae*	Allele size (bp)					
		SCPTSY7-1	SCPTSY7-2	SC8132X-1	SC8132X-2	YOR267C-1	YOR267C-2
A	55	292	292	212	310	308	389
B	35	269	269	193	193	421	421
C	31	261	312	155	212	389	389
D	18	271	271	206	206	389	389
E	21	280	280	209	209	389	389
F	28	280	280	209	209	407	407
G	14	261	261	212	212	389	389
H	1	261	269	193	212	389	421
I	1	286	286	181	181	389	389

3.1. Biological defense system

3 samples were collected from each vine variety (Shiraz, Grenache and Barbera) in each biological defense system (organic and conventional vineyard), totaling 18 samples for each year (2006, 2007 and 2008). In the organic vineyard, spontaneous fermentations occurred in 8 and 7 samples of Shiraz and Grenache, respectively, while in Barbera they occurred in all of the samples. In the conventional vineyard, spontaneous fermentations occurred in 4, 3 and 5 musts from Shiraz, Grenache and Barbera grape varieties respectively. As a result, 1080 yeast strains were isolated, 30 colonies for each fermented must. Thus, 876 non-*Saccharomyces* were isolated, 62% for the organic and 38% for the conventional vineyard. On the other hand, 204 *Saccharomyces* were obtained, 87% for the organic and 13% for the conventional vineyard (Table 4).

Table 4. Distribution of the global data obtained by biological defense system (organic and conventional vineyard) and variety (Shiraz, Grenache and Barbera) in 2006, 2007 and 2008.

Biological defence system	Organic Vineyard			Conventional Vineyard		
Vine variety	Shiraz	Grenache	Barbera	Shiraz	Grenache	Barbera
Samples	9	9	9	9	9	9
Spontaneous fermentations	8	7	9	4	3	5
Isolates of *Saccharomyces* and percentage (%)	30 (17%)	36 (20%)	112 (63%)	26 (100%)	0 (0%)	0 (0%)
Total of *Saccharomyces* and percentage (%)		178 (87%)			26 (13%)	
Isolates of non-*Saccharomyces* and percentage (%)	209 (39%)	174 (32%)	158 (29%)	95 (28%)	90 (27%)	150 (45%)
Total of non-*Saccharomyces* and percentage (%)		541 (62%)			335 (38%)	
Total of isolates				1080		

3.1.1. Organic vineyard

In Shiraz musts, *K. thermotolerans* (50.21%) was the most abundant species, followed by S. cerevisiae (12.55%), *C. stellata* (12.55%), *M. pulcherrima* (12.55%) and *H. guilliermondii* (12.13%). In Grenache musts, five species were found: *H. guilliermondii*, *K. thermotolerans*, *P. anomala*, *S. cerevisiae* and *C. stellata* (see Table 5 for percentages). In Barbera musts, the number of *S. cerevisiae* strains was very high (41.48%). Regarding the non-*Saccharomyces* strains isolated, *H. guilliermondii* was the most abundant species (29.63%). Other species found were *K. thermotolerans*, *C. stellata* and a minor percentage of *T. delbrueckii* (Table 5).

Nine of the 10 genotypes obtained (B, C, D, E, F, G, H, I and J) were found within the *Saccharomyces cerevisiae* analyzed. This demonstrates the great biodiversity of fermentative strains in the organic vineyard.

3.1.2. Conventional vineyard

A predominance of non-*Saccharomyces* strains were found in the isolates in the conventional vineyard. Of the species isolated in Shiraz musts *K. thermotolerans* was the most abundant (48.76%), followed by *P. anomala*, and, to a lesser extent, *P. toletana*, *C. sorbosa* and *T. delbrueckii* (Table 5). For the musts obtained from the Grenache variety, only two species, *K. thermotolerans* (the most abundant) and *H. guilliermondii* were found. In the Barbera musts the isolated strains were principally *C. stellata* followed by *T. delbrueckii* and *K. thermotolerans*. The *S. cerevisiae* strains were poorly isolated, being found only in the Shiraz musts (21.49%) (Table 5).

A unique genotype (A) was found within the 26 isolations of *Saccharomyces* analyzed in Shiraz must.

Table 5. Distribution of the yeast species (number and percentage) isolated from musts obtained from different grape varieties harvested from the conventional vineyard in 2006, 2007 and 2008.

Species	Organic vineyard						Conventional vineyard					
	Shiraz		Grenache		Barbera		Shiraz		Grenache		Barbera	
	Isolates	(%)	Isolates	(%)	Isolates	(%)	Isolates	(%)	Isolates	(%)	Isolates	(%)
S.cerevisiae	30	12.55	36	17.14	112	41.48	26	21,49	0	0	0	0
C.sorbosa	0	0	0	0	0	0	1	0,83	0	0	0	0
C.stellata	30	12.55	32	15.24	30	11.11	0	0	0	0	80	53.33
H.guilliermondii	29	12.13	60	28.57	80	29.63	0	0	30	33.33	0	0
K. thermotolerans	120	50.21	42	20	42	15.56	59	48,76	60	66.67	30	20
M.pulcherrima	30	12.55	0	0	0	0	0	0	0	0	0	0
P.anomala	0	0	40	19.05	0	0	30	24,79	0	0	0	0
P.toletana	0	0	0	0	0	0	4	3,31	0	0	0	0
T. delbrueckii	0	0	0	0	6	2.22	1	0,83	0	0	40	26.67

The species *K. thermotolerans* was the predominant non-*Saccharomyces* species found in the musts obtained from both vineyards. It is a species commonly found in hot and dry areas, such as the Madrid region. *H. guilliermondii* was the second most frequent species of non-*Saccharomyces* found. The species *C. stellata*, *T. delbrueckii* and *P. anomala* were also found in both biological defense systems. Regarding *T. delbrueckii*, it was more commonly isolated in the musts obtained from the grapes harvested from the conventional vineyard. This species has a strong fermentative capacity, being alcohol-tolerant (until 10% v/v) and resistant to antiseptics (Francesca *et al.*, 2010). The proportion of other species was relatively low. *M. pulcherrima* was found only in the Shiraz musts obtained from the organic vineyard grapes. A very low percentage (<5%) of *C. sorbosa* and *P. toletana* was isolated from the Shiraz musts obtained from the conventional vineyard. To our knowledge, it is the first time that *C. sorbosa* and *P. toletana* have been described in vineyards of the Madrid region of Spain.

There are differences in the distribution of the yeast populations within the biological defense system,. An significant quantity of non-*Saccharomyces* yeasts and *Saccharomyces* strains were isolated in musts from the organic vineyard in comparison with the isolates from the conventional vineyard. With regard to the yeast biodiversity, seven different species were isolated in the conventional, and five species were found in the organic vineyard. But if one takes into account the number of *S. cerevisiae* genotypes identified, the biodiversity was greater in the organic vineyard. In this study, the results showed that the phytosanitary treatment affected the grape microbiota negatively, reducing the number of yeasts isolated and the biodiversity.

3.2. Grape Variety

The amount and distribution of the 1080 yeast strains identified in this study differed among the vine varieties. Nine different yeast species were found in the Shiraz grape variety, while five were isolated for both Grenache and Barbera. *K. thermotolerans* was the predominant species isolated for the Shiraz and Grenache musts, representing 49.58% and 34% respectively. It was the third most abundant (17.14%) in the Barbera must. An important percentage of *H. guilliermondii* and *C. stellata* was found in all the varieties studied. *P. anomala* was isolated from Shiraz (8.31%) and Grenache musts (13.33%), but it was not found in the Barbera must. *T. delbrueckii* was detected in all of the vine variety musts except Grenache. A very low percentage (<10%) of species such as *C. sorbosa*, *M. pulcherrima* and *P. toletana* were only found in the musts obtained from Shiraz grapes. Our results show the presence of *S. cerevisiae* species in all of the different musts studied: Shiraz (15.79%), Grenache (12%) and Barbera (27.38%) (Table 6).

Table 6. Distribution of yeast species (number and percentage (%)) isolated from musts obtained from three different grape varieties over the three years.

Species	Grape variety					
	Shiraz		Grenache		Barbera	
	Isolates	(%)	Isolates	(%)	Isolates	(%)
S. cerevisiae	57	15.79	36	12	112	26.67
C.sorbosa	1	0.28	0	0	0	0
C.stellata	30	8.31	32	10.67	110	26.19
H.guilliermondii	29	8.03	90	30	80	19.05
K. thermotolerans	179	49.58	102	34	72	17.14
M. pulcherrima	30	8.31	0	0	0	0
P.anomala	30	8.31	40	13.33	0	0
P.toletana	4	1.11	0	0	0	0
T.delbrueckii	1	0.28	0	0	46	10.95

Regarding the different genotypes of *S. cerevisiae* identified by Microsatellite Multiplex PCR, the Barbera grapevine was the most important reservoir of fermentative yeast strains (*S. cerevisiae*), having six different genotypes (A, B, C, D, E and G), followed by the four genotypes found in the Grenache grapevine (F, H, I and J). In the Shiraz grapevine variety only three genotypes were identified (A, C and D), two in the organic (C and D) and a unique ecotype (A) in the conventional vineyard.

The composition and properties of the grapes differ depending on the vine variety. Varietal factors such as thickness of the grape skins can play an important role in the yeast microbiota present on grapes (Li *et al*., 2010). Non-*Saccharomyces* yeasts were the most abundant species found in all the varieties (Shiraz, Grenache and Barbera) harvested from the organic vineyard. Therefore, the amount of *Saccharomyces* was higher in comparison with those isolated in the conventional vineyard.

With regard to non-*Saccharomyces* yeasts, the greatest number and variety of isolated species were found in the Shiraz variety. This could be due to the high resistance of this variety to the most common vineyard diseases. The number of isolates of *Saccharomyces* in the Barbera variety was higher compared to those found in Grenache or Shiraz. Moreover, the Barbera grapevine was found to have the greatest quantity of *Saccharomyces* genotypes. The different genotypes could influence the final quality of the wine, for example as a result of their capacity to form aroma. This capacity depends not only on the yeast species but also on the particular strain of this species. The different genotypes found will be the basis of further investigation, focusing on their oenological characterization.

CONCLUSIONS

This study showed the significant influence of the grape variety and the biological defense system on the yeast microbiota associated with the vineyard. These results showed that the phytosanitary treatments affected the grape microbiota negatively, reducing the number of yeasts isolated and their biodiversity. Thus, from an agronomic point of view, the organic biological defense system is the best option in terms of the vineyard being a rich natural reservoir of yeasts of oenological interest .

Non-*Saccharomyces* yeast strains were the most abundant in both kind of vineyards and in all the different varieties studied (Shiraz, Grenache and Barbera). Nevertheless, the organic vineyard was found to be a more important reservoir of *Saccharomyces* strains than the conventional vineyard. The Barbera grapevine was the most appropriate variety in terms of the number and biodiversity of *Saccharomyces* yeast strains.

To our knowledge, this is the first time that *C. sorbosa* and *P. toletana* have been found in vineyards of the Madrid winegrowing region of Spain.

ACKNOWLEDGEMENTS

This work was supported by the Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria (INIA), and the Ministerio de Ciencia e Innovación, Spain (projects RM 2006-00012-00-00 and RM2007-00008-C02-00**).** J.T. thanks the Instituto Madrileño de Investigación y Desarrollo Rural, Agrario y Alimentario for his grant.

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ULTRAVIOLET LIGHT (UV-C) IRRADIATION AS AN ALTERNATIVE TECHNOLOGY FOR THE CONTROL OF MICROORGANISMS IN GRAPE JUICE AND WINE

M. Lorenzini, F. Fracchetti, V. Bolla, E. Stefanelli, F. Rossi, S. Torriani*

DiSTeMeV, University of Verona,
Via della Pieve 70, 37029 San Floriano (Verona), Italy;
*sandra.torriani@univr.it

ABSTRACT

Modern consumer demand for grape juice and wine free of chemical preservatives makes finding commercially viable alternatives to sulphur dioxide (SO_2), since it could cause health troubles in sensitive subjects. Moreover, under EU law, it is mandatory to label any wine with more than 10 mg/L SO_2 as containing sulphites. Thus, it is urgent to conceive alternative control methods, like the treatment based on light irradiation in the ultraviolet wavelength interval, which has been already used to inactivate the microorganisms present in water, fruit juices, liquid foods and also grape juice and wine. This study reports the microbiological and physico-chemical data obtained from industrial-scale trials carried out by the SurePure UV-C technology (Milnerton, South Africa) in some wineries. Two different dosages of UV-C were employed on diverse types of grape juice and wine, both white and red. The technology applied was proven to be effective to assure microbiological stability and could exert a strong impact in winemaking industry by eliminating or reducing the use of SO_2 in the near future.

RIASSUNTO

L'esigenza di trattamenti alternativi all'impiego di anidride solforosa (SO_2) per ridurre la contaminazione microbica del mosto d'uva e nel vino sta assumendo un'importanza sempre maggiore, in quanto l'SO_2 può rappresentare un problema per la salute dei consumatori. Nei paesi europei è obbligatorio riportare nelle etichette alimentari la presenza di solfiti quando la concentrazione supera 10 mg/L. Diventa pertanto necessario attuare forme di controllo alternative, come l'irraggiamento nelle lunghezze d'onda dell'ultravioletto (UV-C), già applicato con successo per inattivare microrganismi in acqua, succhi di frutta, alimenti liquidi e anche in mosto e vino. In questo lavoro sono riportati i risultati microbiologici e chimico-fisici di alcune sperimentazioni condotte in cantina con la tecnologia UV-C sviluppata da SurePure Ltd (Milnerton, Sud Africa), utilizzando due dosaggi di raggi UV-C su diversi tipi di mosti, vini bianchi e rossi. La tecnologia applicata si è dimostrata efficace per assicurare la stabilità microbiologica e nel prossimo futuro potrebbe avere un forte impatto nell'industria enologica eliminando o riducendo l'uso della SO_2.

INTRODUCTION

Winemaking is a well-known fermentation process, where yeasts and lactic acid bacteria (LAB) play a key role in determining the safety and quality of the final product (Ribéreau-Gayon *et al.*, 2006). Prominent in this process are *Saccharomyces* species (predominantly *S. cerevisiae*), which dominate the alcoholic fermentation (AF), and the lactic acid bacteria (LAB, mainly *Oenococcus oeni*), which carry out the malolactic fermentation (MLF).

Together with yeasts and LAB with positive roles in AF and MLF, the spontaneous microflora of grape juice and wine can include spoilage microorganisms that are unwanted at any particular place and time in the winemaking process. Therefore, the same microorganism can be both beneficial and detrimental. For example, growth of *S. cerevisiae* is required during the AF, but not in bottled wine (Mills *et al.*, 2008). Many other species of yeasts (Loureiro, Malfeito-Ferreira, 2003) can spoil wine at every stage of the winemaking process. One of the mostly feared is *Dekkera bruxellensis*, as it can persist throughout the process producing off-flavors (Woolfit *et al.*, 2007). Also several species of the genus *Pichia* can depreciate wine by producing undesirable compounds at the initial stages of AF. In particular *P. membranaefaciens* produces films at the surfaces exposed to air (Mora, Rossello, 1992; Lopes *et al.*, 2009).

Also diverse groups of bacteria can spoil wine at different steps of the winemaking process. Acetic acid bacteria (AAB) are strict aerobes well adapted to various sugar and ethanol rich environments, therefore they can colonize grape juice and wine in presence of oxygen increasing the volatile acidity up to undesirable levels (Bartowsky, Henschke, 2008). Growth of LAB strains with negative traits, such as production of biogenic amines (Lonvaud-Funel, 2001), ropy polysaccharides, and off-flavors (Lonvaud-Funel, 1999; Bartowsky, 2009), can also impair the safety and quality of wine.

Since the spectrum of grape juice and wine spoilage microorganisms (both bacteria and yeasts) is wide, and they can lower the quality of the products at every stage of the winemaking process, the most effective methods to stabilize microbiologically grape juice and wine are mainly based on chemical or physical principles.

The traditional procedure is represented by the addition of sulphur dioxide (SO_2), that acts as both an antimicrobial agent and an antioxidant (Romano, Suzzi, 1993). Though this well-established practice is considered almost unavoidable in winemaking, it is known that an excess of SO_2 ingestion causes health troubles in sensitive subjects, such as headaches, intolerances and allergies. For this reason, the European Community law imposes to report on the label the phrase “containing sulphites” if the concentration of SO_2 in wine is higher than 10 mg/L. Besides health law and trade-related concerns, the modern consumer is oriented toward healthy products free of chemical preservatives. Thus, it is urgent to conceive alternative control methods that eliminate the need for these compounds.

Several physical principle-based technologies have been developed to this purpose, such as light irradiation in the ultraviolet wavelength interval (UV-C), ultrahigh pressure treatments, high power ultrasounds and pulsed electric fields (Bartowsky, 2009). Among these techniques, UV-C treatment represents undoubtedly a promising technology. Indeed this approach has been successfully applied to inactivate microorganisms present in water, fruit juices, and liquid foods (Koutchma, 2009). As determined so far, the UV-C irradiation is effective in reducing spoilage microorganisms, e.g. *D. bruxellensis*, *Lactobacillus plantarum*, *Pediococcus acidilactici*, *S. cerevisiae*, *O. oeni*, *Acetobacter aceti*, when artificially inoculated at high level in grape juice and wine batches (Fredericks *et al.*, 2009).

In this research, industrial-scale trials were carried out by using the SurePure UV-C system (Milnerton, South Africa) to treat grape juice and wine produced in several wineries of Northern Italy. The aim of these trials was to evaluate the effect of this UV system on the microbiological and physico-chemical characteristics of the authentic samples.

MATERIALS AND METHODS

UV-C system inactivation experiments

Experiments were performed in five wineries located in three regions of Northern Italy (Friuli Venezia Giulia, Piemonte and Veneto) using the UV-C reactor system designed and manufactured by SurePure (Milnerton). A volume of 5000 L was placed into the holding tank (2-3°C for grape juice and 10-15°C for red and white wines) of the UV-C treatment unit. The reactor contains 40 UV-C (254 nm) germicidal lamps in series, and exploits an advanced turbulent flow system to optimize the penetration of UV-C and improve microbial inactivation. Grape juice and wine batches were circulated at a constant flow rate of 4000 L/h by means of an eccentric screw pump. The UV-C dosage varied in relation with the liquid medium turbidity. In particular, dosages of about 1000 J/L were applied to white grape juice (Moscato and Prosecco) and white wine (Soave), while dosages of about 2000 J/L were applied to red wines (Barolo, Syrah and Valpolicella).

An in-line sampler was used to collect aseptically the samples (50 mL) from the flow stream without stopping the treatment process, every fifteen minutes.

Microbiological analysis

Enumerations and isolation of microorganisms. Samples of grape juice and wine were transported to the laboratory in a cooler with ice packs. For enumeration of total yeast, aerobic bacteria and LAB, samples were spread on Wallerstein laboratory nutrient agar (WL, Fluka, Milan, Italy), WL Differential (WLD, Fluka) and MRS agar (Fluka) supplemented with fructose 0.5% w/v (Fluka), malic acid 0.1% w/v (Fluka), 1 mL/L tween 80 (Fluka), respectively. Microbial counts were done on serial dilutions and on 47 mm membrane discs of 0.45 μm pore size (Sartorius, Florence, Italy) used to filter 10 mL red wine or 50 mL white wine. WL and WLD were incubated aerobically at 27°C for five days; MRS was incubated under anoxic conditions using the Anaerocult A system (Merck, Darmstadt, Germany) at 27°C for seven days.

To identified the microbial contaminants, 24 colonies were randomly picked from different agar plates with different samples, and purified by streaking on the same enumeration media.

Identification of isolates. Genomic DNA of representative isolates was extracted with standard procedures. The divergent D1/D2 domain of the 26S rRNA gene of yeasts was PCR amplified by using the NL1 and NL4 primer pair, as described previously (Kurtzman, Robnett, 1998). For bacteria, the V3 variable region of the 16S rRNA gene was amplified by the universal HDA primer pair in the conditions described by Walter *et al.* (2000). The amplification products were separated by electrophoresis on agarose gel (1.5%) stained with 0.5 μg/mL ethidium bromide, and purified using the NucleoSpin Extract II kit (Macherey-Nagel, Germany) according to the manufacturer's instructions. The reverse primers NL4 and HDA2 were used for sequencing the DNA fragments from yeasts and bacteria, respectively, by the Big Dye Terminator v3.1 Cycle Sequencing method at BMR Genomics (Padua, Italy). The newly obtained sequences were aligned by the BLAST tool with the GenBank data library at the National Center for Biotechnology Information (NCBI) for identification of the isolates.

Chemical analysis

The grape-juice and wine samples were analyzed at a certified centre (Enocentro, Verona, Italy), following official methods and procedures (OIV, 2009). The evolution of the samples was monitored by taking into account the following parameters: pH, total acidity (expressed in g/L of tartaric acid), concentration of alcohol, reducing sugars (g/L), glycerin (g/L), and color. For color analysis the optical density was determined at 420, 520 and 620 nm, to calculate color intensity.

RESULTS AND DISCUSSION

Effect of UV-C treatment on the yeast population

Tab. 1 and 2 summarized the results of the microbiological analysis carried out before, during and after the UV-C treatment.

The Moscato and Prosecco grape juices from the two wineries contained high levels of total yeasts, with counts of 10^7 and $5{\cdot}10^6$ CFU/mL, respectively. Yeasts isolated from these samples were identified as *Saccharomyces bayanus*. After UV-C treatment a remarkable reduction (6-7 log) of the yeast population was observed for these samples: indeed only few viable cells were detected in 50 mL of grape juice. Therefore, the UV-C treatment was proven to be extremely effective in decreasing the level of *S. bayanus*.

The three batches of red wine, Barolo, Valpolicella and Syrah, had a initial yeasts load of $2{\cdot}10^3$, $6{\cdot}10^2$ and ~ 10 CFU/mL, respectively. Only after a long UV-C treatments the desirable reduction of the contamination was obtained (Tab. 1). This can be explained either with higher resistance of the specific yeast contaminants or with the presence of substances interfering with the transmission of the UV-C radiation through the sample.

Identification of selected isolates from these red wines revealed the presence of a heterogeneous initial microflora. Indeed, isolates were ascribed to three different yeast species, i.e. *S. cerevisiae, Pichia galeiformis/membranafaciens* and *Zygosaccharomyces bailii*. *P. galeiformis/membranafaciens* was found both before and after the treatment, thus it could be assumed that this yeast is quite resistant to UV-C. *P. membranafaciens* and the sibling species *P. galeiformis* (Kurtzman, Fell, 1998) are oxidative yeasts usually isolated from grapes, grape juices, and wines (Mora, Rossello, 1992). Their growth during winemaking increases the levels of acetic acid and esters and, consequently, the production of off-flavors. Moreover, this yeast can produce thick films at the surface of wines exposed to air. This trait is associated to the production of exopolysaccharides and that could protect them from UV-C rays. *Zygosaccharomyces bailii* was isolated from the Syrah wine only before the treatment, thus suggesting that the UV-C radiation can inactivate this yeast which is a common wine contaminant and a deteriorating agent (Fugelsang, 1996).

Effect of UV-C treatment on the bacterial population

Bacterial loads in the grape juice and wine samples before UV-C irradiation were of the order 10^2 - 10^3 CFU/mL in most cases. Higher values were found in the wines Soave and Valpolicella, that showed anaerobic counts of the order 10^4 CFU/mL. Of these, the Soave wine was not contaminated by aerobic bacteria (Tab. 2). In all the analyzed samples aerobic bacteria exhibited a 3 log diminution after the treatment, while the LAB showed an even more marked reduction in the samples Soave and Valpolicella.

Most of the bacteria isolated before the UV-C treatment belonged to AAB genera, i.e. *Acetobacter*, *Gluconacetobacter* and *Gluconobacter*, with the unique exception of an isolate from Prosecco grape juice with 100% sequence homology of the 16S rRNA gene V3 region to *Pediococcus parvulus/damnosus/inopinatus*. The latter was no more found in the UV irradiated samples among microorganisms concentrated from a 50 mL volume of grape juice indicating high effectiveness of this treatment to prevent the ropy defect.

After the treatment, AAB isolates could be obtained only by concentrating cells from 10 or 50 mL of sample. Those identified belonged to the genera *Acetobacter* and *Gluconobacter*. Therefore, the AAB genera isolated in this study are the same indicated as the most frequently associated to grapes and wines (Bartowsky *et al*., 2008). Moreover, the species univocally identified in the treated Moscato grape juice and Syrah wine, i.e. *A. aceti* and *A. pasteurianus*, are those primarily implicated in the spoilage of wine during storage in barrels or in bottle (Bartowsky *et al*., 2003). Nevertheless, the strong reduction in number of AAB after UV-C irradiation can be considered a noticeable advantage of this treatment over SO_2 addition, since the latter has an effect on AAB only at the highest concentration used (Du Toit *et al*., 2005).

From the UV-C treated sample of Moscato grape juice, three different LAB isolates were obtained. These isolates were identified as *L. casei/paracasei/rhamnosus*, *L. paracasei*, and *O. oeni*, respectively. Considering the relatively low initial level of anaerobes in this sample and the remarkable reduction of LAB in the more heavily contaminated wines, the existence of LAB strains or species particularly resistant to UV-C irradiation can be hypothesized.

Effect of UV-C treatment on the chemical characteristics

The UV-C processing did not change the temperature of the products during the treatment. Chemical and physical parameters of the samples were determined before and after UV-C treatment. The initial composition of the grape juices and wines in terms of alcohol, reducing sugars and glycerine content, total acidity, pH and color was similar to that reported in literature for these types of products.

After UV irradiation no changes were observed for these parameters in all the samples, including the red wines, which were subjected to higher UV-C dosage.

It can be mentioned that the Moscato grape juice batches maintained stable sugar (155-158 g/L) and alcohol (2.1-2.4 g/L) contents for up to 20 days, as a consequence of the strong reduction of the yeast population that delayed spontaneous fermentation.

CONCLUSIONS AND PERSPECTIVES

The application of the UV-C treatment to different grape juice and wine samples in five diverse wineries assured the desired decrease of viable microorganisms down to levels below one CFU/mL. The samples were stabilized enough to avoid spoilage during the winemaking process. To our knowledge, these are the first trials of UV-C treatments carried on directly in the wineries in which the natural contaminants of grape juice and wine were evaluated. The outcome of the new sanitization procedure was clearly successful.

The time required to lower the content of culturable microorganisms to less than one CFU/mL varied according to the characteristics of the samples. More in detail, red wines needed longer treatments, probably because of the higher concentration of substances interfering with the transmission of the UV-C rays through the sample.

The species *P. galeiformis/membranaefaciens*, isolated after the treatment, have never been studied in dedicated trials (Fredericks *et al.*, 2009). In the next future the attention could be focused specifically on the resistance of this species, in order to determine the time necessary for an effective treatment. The possibility that some LAB species or strains can be highly resistant to UV-C irradiation should be also better investigated. Moreover, further investigations are needed to examine the growth and spoiling capacity of the surviving AAB during storage.

The UV-C treatment did not to alter the color and chemical parameters of grape juice and wine, even in the case of prolonged treatments.

The usage of UV-C treatment needs aware staff and requires clean equipments and facilities to keep must and wine stable during storage in barrels and in bottles. Indeed, after the treatment, the samples could be newly contaminated by spoilage microorganisms in poorly sanitized containers.

The promising results reported here demonstrated that physical methods, such as UV-C, an incoming technology already commercially available, could exert a highly positive impact in the winemaking industry by eliminating or reducing the use of SO_2 in the near future.

ACKNOWLEDGEMENTS

We are grateful to the wineries that performed the experiments, and to the oenologists and technicians for their valuable assistance and support.

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Tab. 1. Inactivation of microbial population in grape juice and wine by UV-C treatment.

Product	**Enumeration** (CFU/mL)					
	Before UV-C treatment			**After UV-C treatment**		
	Yeasts	*Aerobic bacteria*	*LAB*	*Yeasts*	*Aerobic bacteria*	*LAB*
Moscato grape juice	$5 \cdot 10^6$	$2 \cdot 10^2$	$1 \cdot 10^2$	n.d. (45)	n.d. (45)	n.d. (45)
Prosecco grape juice	$1 \cdot 10^7$	$4 \cdot 10^3$	$5 \cdot 10^3$	n.d. (180)	n.d. (120)	n.d. (120)
Soave wine	~ 10	~ 10	$1 \cdot 10^4$	n.d (40)	n.d (40)	n.d. (40)
Barolo wine	$2 \cdot 10^3$	$6 \cdot 10^3$	$3 \cdot 10^3$	~ 10 (360)	~ 10 (360)	n.d. (120)
Syrah wine	~ 10	~ 10	~ 10	n.d. (120)	n.d. (120)	n.d. (120)
Valpolicella wine	$6 \cdot 10^2$	$1 \cdot 10^3$	$2 \cdot 10^4$	n.d. (160)	n.d. (30)	n.d. (60)

n.d.: less than 1 CFU/mL
( ): minutes of treatment necessary to lower the microbial counts down to n.d.

Tab. 2. Identity of selected contaminants determined by genetic marker sequencing.

Product	**Isolate**	
	Before UV-C treatment	**After UV-C treatment**
Moscato grape juice	Yeasts: *Saccharomyces bayanus*	Yeasts: *Saccharomyces bayanus* Bacteria: *Acetobacter aceti* *Lactobacillus casei /paracasei/rhamnosus* *Lactobacillus paracasei* *Oenococcus oeni*
Prosecco grape juice	Yeasts: *Saccharomyces bayanus* Bacteria: *Acetobacter senegalensis /aceti/tropicalis/indonesiensis* *Pediococcus parvulus/P. damnosus*	Yeasts: *Saccharomyces bayanus*
Barolo wine	Yeasts: *Saccharomyces cerevisiae* Bacteria: *Acetobacter aceti* *Acetobacter senegalensis /pasteurianus/aceti* *Gluconobacter albidus /sphaericus/cerinus/oxidans* *Gluconobacter japonicum/frateurii*	Yeasts: *Saccharomyces cerevisiae*
Syrah wine	Yeasts: *Pichia galeiformis/membranaefaciens* *Saccharomyces cerevisiae* *Zygosaccharomyces bailii*	Yeasts: *Pichia galeiformis/membranaefaciens* *Saccharomyces cerevisiae* Bacteria: *Gluconobacter oxidans*
Valpolicella wine	Yeasts: *Saccharomyces cerevisiae*	Yeasts: *Saccharomyces cerevisiae*

Méthodes d'analyse en Œnologie. Perspectives dans l'avenir

Curvelo-Garcia A.S. [(1)], Catarino S. [(1)], P. Barros [(2)]

(1) Instituto Nacional de Recursos Biológicos, I.P. – INIA (Dois Portos), Portugal
curvelo.garcia@inrb.pt;sofia.catarino@inrb.pt
(2) Instituto dos Vinhos do Douro e Porto, I.P. (Porto), Portugal
pbarros@ivdp.pt

RESUME

Après l'analyse de l'évolution récente des méthodes d'analyse en Œnologie et des méthodologies de contrôle de la qualité des vins et des autres produits d'origine viticole, on fait une prévision des perspectives dans l'avenir des laboratoires d'Œnologie.
L'automatisation de l'analyse. L'évolution des équipements depuis 1970: l'analyse à flux continu segmenté, l'analyse à flux continu non segmenté, l'analyse séquentielle, la technique infrarouge à transformée de Fourier (IRTF). L'augmentation du nombre des paramètres et de la vitesse de l'analyse, le traitement immédiat des données obtenues, l'analyse en ensemble des divers paramètres. L'avenir: la technique IRTF élargie à un très élevé nombre de paramètres.
Le rôle de l'analyse sensorielle.
L'augmentation de la qualité des laboratoires. La validation des méthodes d'analyse. L'harmonisation des pratiques des laboratoires. La détermination des caractéristiques des méthodes d'analyse. Les avancements dans l'interprétation des résultats analytiques. Les études collaboratives internationales. L'avenir: l'intensification et la généralisation universelle de ces pratiques, nommément les études collaboratives internationales.
L'évolution des équipements d'instrumentation analytique. L'avenir: l'augmentation de la sensibilité pour la détermination de quelques espèces chimiques et l'augmentation des possibilités de la spéciation de quelques analyses. Les incidences dans la définition des limites.
L'importance du contrôle des pratiques œnologiques, selon le *Code International des Pratiques Œnologiques* et le *Codex Œnologique International*. L'avenir: l'intensification et la généralisation universelle du contrôle de l'application correcte des pratiques œnologiques, des adjuvants et des auxiliaires technologiques

SUMMARY

After analyzing the recent developments of analytical methods in Enology and methodologies for quality control of wines and other viticultural products, we make a forecast of future prospects in the laboratories of Enology.
The automation of the analysis. The evolution of equipments since 1970: segmented flow analysis, unsegmented flow analysis, sequential analysis, Fourier transformed of infrared technology (FTIR). The increase in the number of parameters and throughput of analysis, the immediate processing of data obtained, the simultaneous analysis of various parameters. The future: FTIR technique extended to a very high number of parameters.

The role of the sensorial analysis.
Increasing the laboratories quality. The validation of analytical methods. The harmonization of laboratory practices. The determination of the analytical methods characteristics. The advancements in the interpretation of analytical results. The international collaborative studies. The future: the increase and universal generalisation of these practices, namely the international collaborative studies.
The development of analytical instrumentation equipments. The future: increasing of sensitivity for the determination of some chemical species. The study of the physicochemical forms under chemical species is present in wines (its speciation). The implications in defining the limits.
The importance of control of enological practices, according to the *International Code of Enological Practices* and the *International Enological Codex*. The future: the widespread monitorization of proper implementation of enological practices, processing aids and additives.

L'AUTOMATISATION DE L'ANALYSE

Depuis 1970, on a observé une énorme évolution des équipements disponibles pour l'automatisation de l'analyse, normalement après leur application dans des autres domaines : l'analyse à flux continu segmenté, l'analyse à flux continu non segmenté, l'analyse séquentielle, la technique infrarouge à transformée de Fourier (IRTF).

Pendant les années, on a observé aussi une augmentation croissante du nombre des paramètres et de la vitesse de l'analyse, du traitement immédiat des données obtenues, de l'analyse en ensemble des divers paramètres, surtout après les plus récentes évolutions des équipements pour la technique infrarouge à transformée de Fourier (IRTF).

Pour l'avenir, et en considérant ces plus récentes évolutions, ont peut prévoir la technique IRTF comme technique d'excellence et élargie à un très plus élevé nombre de paramètres, avec des cadences encore bien supérieures. Cette technique sera sûrement la technique à adopter génériquement. Elle sera aussi clairement élargie à divers autres produits d'origine viticole, comme les moûts, les jus de raisin, les eaux-de-vie, les alcools, …

L'AUGMENTATION DE LA QUALITÉ DES LABORATOIRES

On a observé, pendant les dernières années, un intense et continu améliorement des méthodologies pour la validation des méthodes d'analyse et pour la détermination des caractéristiques des méthodes d’analyse employées par les laboratoires de contrôle œnologique, particulièrement la validation des méthodes usuelles relativement aux méthodes de référence (OIV, 1998 ; OIV, 1999a ; OIV,1999b). On a observé aussi l’affirmation des limites de détection et de quantification des méthodes comme base pour la définition des limites d’occurrence de quelques espèces chimiques.

Ainsi, on peut prévoir l’élargissement de tous ces aspects et aussi de l’harmonisation des pratiques des laboratoires dans tout le monde OIV, 2002).

On devra aussi assister aux divers avancements dans l’interprétation des résultats analytiques, et à l’intensification et la généralisation universelle des études collaboratives internationales, indispensables pour la reconnaissance internationale des méthodes et encore à la généralisation de l’utilisation des matériaux de référence.

En ce qui concerne les matériaux de référence certifiés (MRC), il est important noter l’amélioration de l’offre actuelle de ces matériaux pour les produits d’origine viticole. En effet, actuellement, il y a encore quelques difficultés pour obtenir MRC pour quelques espèces chimiques ou para mètres analytiques.

De même il devra être considéré le cas de l'analyse sensorielle, à travers l'adoption de méthodologies universelles et de la mise en place de procédures pour contrôler les performances individuelles des évaluateurs, en tout équivalents à celles adoptées dans les méthodes instrumentales d’analyse.

L’ÉVOLUTION DES ÉQUIPEMENTS D’INSTRUMENTATION ANALYTIQUE

L’évolution des équipements d’instrumentation analytique sera permanente, ce que devra certainement déterminer l’augmentation de la sensibilité pour la détermination de quelques espèces chimiques et l’augmentation des possibilités de la spéciation de quelques analyses. Ainsi, les possibilités de l’analyse pour caractériser les produits, pour protéger la sécurité alimentaire et même pour déceler quelques fraudes seront bien augmentées.

Par exemple, dans l’OIV, les limites maximales admissibles pour quelques éléments minéraux sont définies pour la teneur total, mais la toxicité de l’élément est dépendante de leur forme chimique. Ainsi, le développement des études de spéciation pourra conduire à une nouvelle définition de quelques limites maximales admissibles

Les connaissances sur la distribution d'un déterminé analyte sur ses différentes formes chimiques, permettra encore comprendre son influence sur les caractéristiques sensorielles, en optimisant en conséquence les techniques de production.

Il sera certainement les cas des métaux, des différentes familles de composés phénoliques et de composés nitrogenés, des composés de l'arôme, de la discrimination des isotopes, etc.

Cependant on tire l'attention sur un important aspect : l'augmentation de la sensibilité des méthodes analytiques ne devra être la base pour la définition des limites pour l'occurrence des différentes espèces chimiques : ces définitions devront toujours avoir comme base seulement des raisons d'ordre qualitatif et toxicologique, comme l'OIV a déjà bien référé (OIV, 1994).

L'IMPORTANCE DU CONTRÔLE DES PRATIQUES ŒNOLOGIQUES

Pour le contrôle de la correcte application des pratiques œnologiques, il est chaque fois plus importante suivre les prescriptions inscrites dans le *Code International des Pratiques Œnologiques* et le *Codex Œnologique International*. Dans l'avenir on peut prévoir l'intensification et la généralisation universelle du contrôle de l'application correcte des pratiques œnologiques, des adjuvants et des auxiliaires technologiques, selon ces textes de référence internationale.

Mais il faut faire noter que, actuellement, il y a beaucoup de carences dans les prescriptions de ces différentes pratiques, donc la nécessité de faire la distinction entre les informations et les avis sur l'application des pratiques (comme un *Code de Bonnes Pratiques Œnologiques*) et les prescriptions obligatoires, nécessaires pour assurer la sécurité alimentaire des produits el la loyauté de la compétitivité (Curvelo-Garcia, 2006 ; Curvelo-Garcia, 2008).

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SENSITIVE LYSOZYME TESTING IN RED AND WHITE WINE USING THE RIDASCREEN®FAST LYSOZYME ELISA

Lacorn M., Gößwein C., Haas-Lauterbach S., Immer U.
R-Biopharm AG
An der Neuen Bergstr. 17, 64297 Darmstadt, Germany
m.lacorn@r-biopharm.de

ABSTRACT

Lysozyme from egg white has been used to control the malolactic fermentation during wine production. In recent years, reasonable suspicion has emerged that residues of this protein can remain in the wine and this could exert an allergic reaction in hypersensitized consumers. According to directive 2007/68/EC, all wines labelled after December 31st 2010 must declare if allergens including egg and therefore lysozyme were used during production. Therefore, methods for the detection of this protein are needed. The detection limit should be as low as possible since no limit is given in the directive. For sample preparation, the wine is diluted in extraction buffer, heated for 10 min at 60°C and centrifuged. The polyclonal antibodies used in the sandwich-ELISA RIDASCREEN®FAST Lysozyme allow the measurement of red and white wines and revealed no matrix interferences. The resulting limit of quantification is 0.05 mg/L. Using a wine spiked with lysozyme intra-assay and inter-assay coefficients of variation were in the acceptable range of less than 10%. The ELISA is therefore suitable for the simple and reliable detection of lysozyme in white and also red wine at the very low mg/L-level.

ABSTRACT

Lysozym aus Eiklar wird zur Kontrolle der malolaktischen Gärung während der Weinbereitung eingesetzt. In den letzten Jahren hat sich der Verdacht erhärtet, dass der Einsatz von Lysozym zu Rückständen in Wein führen kann und sei sensibilisierten Verbrauchern zu allergischen Reaktionen führen könnte. Laut Direktive 2007/68/EC muss auf allen nach dem 31.12.2010 etikettierten Weinen die Verwendung von Ei und seinen Bestandteilen deklariert werden. Daraus ergibt sich die Notwendigkeit, Lysozym sicher und schnell zu detektieren. Da keine Grenzwerte formuliert sind, sollte der Nachweis mit hoher Empfindlichkeit erfolgen. Für die Probenvorbereitung wird der Wein 1:20 in Extraktionspuffer verdünnt und nach 10-minütiger Erhitzung bei 60°C anschließend zentrifugiert. Die polyklonalen Antikörper des Sandwich ELISA RIDASCREEN®FAST Lysozyme erlauben den Einsatz von Rot- und Weissweinen und zeigen keine Matrixeffekte. Die Bestimmungsgrenze liegt mit 0.05 mg/L sehr niedrig und die intra- und inter-assay Variationskoeffizienten liegen unterhalb 10%. Das ELISA System ist daher sehr gut geeignet, Lysozym in Weiss- aber auch Rotwein in kurzer Zeit sicher und kostengünstig zu bestimmen.

INTRODUCTION

Bacteria in grape must and wine originate from the vineyard, grapes, and winery processing equipment, and can survive under appropriate conditions during wine making procedures. They are responsible for undesirable effects such as negative sensory characteristics or an uncontrolled malo-lactic fermentation (Bartowsky, 2009).

The addition of special strains of yeast for alcoholic fermentation in excess compared to spoilage bacteria only led to a nearly clean fermentation. Sulphur dioxide is commonly used to control the unwanted effects of these bacteria but the applicable amount is limited due to a worldwide legislation and is sometimes not sufficient to prevent the above mentioned effects (Lopez et al., 2009).

As an alternative, lysozyme was introduced to control Gram-positive bacteria as lactic acid bacteria. The enzyme is isolated from egg-white and is commercially used in cheese and wine. The enzymatic activity leads to a damage of bacterial cell walls by catalyzing hydrolysis of 1,4-beta-linkages between N-acetylmuramic acid and N-acetyl-D-glucosamine residues in a peptidoglycan and between N-acetyl-D-glucosamine residues in chitodextrins (Strynadka and James, 1991). It has little activity against Gram-negative bacteria (bacteriostatic not bactericidal) and no activity against eukaryotic cells (Hughey and Johnson, 1987).

Addition of lysozyme at different stages during wine making led to the following results. (1) The malo-lactic fermentation in red wines was delayed; (2) Longer maceration and microoxygenation to improve color stabilization, including polyphenol extraction are possible; (3) Increase of volatile acidity during stagnant alcoholic fermentation was prevented; (4) Malo-lactic fermentation in white wines was delayed or inhibited; (5) Histamine production was reduced during storage; (6) In combination, sulphur dioxide concentrations could be lowered; (7) Higher activity in white wines than in red wines due to polyphenol content of red wines; (8) Removal from musts by filtration up to 90% (cited or described in Lopez et al., 2009; Delfini et al., 2004; Gerbaux et al., 1997).

In recent years, reasonable suspicion has emerged that residues of this protein can remain in wine and this could exert an allergic reaction in hypersensitized consumers. During normal wine making procedures, added lysozyme is only completely removed by bentonite (Marchal et al., 2002). Addition of lysozyme to wine without bentonite only led to a reduction of 20 up to 40 % maybe due to interaction with tannins (Marchal et al., 2000).

Weber et al. (2009) performed skin prick tests (SPT) with lysozyme solutions, lysozyme-containing wines, and wines, which were treated with bentonite after lysozyme application. Due to the very low number of patients (n=3) the results showed that 2 of 3 patients reacted on lysozyme in wine (not bentonite treated) at a lysozyme concentration of greater 250 mg/L. After bentonite treatment there were no specific reactions in the SPT and even a concentration step of 100 led to no lysozyme specific reaction. Additionally, Kirschner et al. (2009) used comparable samples and found positive reactions (4 out of 5 patients) only on the pure fining agent lysozyme. The performance of double-blind, placebo-controlled food challenges revealed no reaction with bentonite-fined lysozyme-containing wine samples (Kirschner et al., 2009). Minimum eliciting doses for lysozyme are –until today and to our best knowledge- not published.

Nevertheless, according to the EU-directive 2007/68/EC, all wines labelled after May 31st 2009 must declare if allergens including egg (and therefore lysozyme) were used during production. An extension of the term until Dec 31st 2010 was decided by the Standing Committee on the Food Chain and Animal Health of the European Union. Therefore, a method for the detection of lysozyme in wine is needed. The detection limit should be as low as possible since no limit is given in the directive and for security reasons of the patients. HPLC-methods are available and suitable for confirmation but are not suitable for exact measurement, because precipitated lysozyme is not covered by the method (Tirelli and De Noni, 2007). Therefore it was the aim of the study to set up a simple ELISA system which detects the total lysozyme in wine and which is therefore able to screen for negative and positive samples with a high analytical throughput.

MATERIAL AND METHODS

Wines and spiking material

Commercial available white wines and red wines from different regional origins and cultivars are used for determination of background and for spiking experiments. As spiking materials the following substances were used: A pure lysozyme preparation (Sigma-Aldrich, Taufkirchen, Germany) and two lysozyme containing commercial matrials for the practical use (BactiCare, Erbslöh, Geisenheim, Germany and LallZyme Lyso®, Lallemand, Rexdale, Canada).

Sample preparation for ELISA

One mL of wine is diluted with 19 mL pre-heated extraction buffer, which is provided with the described RIDASCREEN® test kit. The extract is heated for 10 min at 60°C and centrifuged for 10 min at 2500 g. After filtration, 100 µL of these extracts are used in the sandwich ELISA. In case of values above 1.35 mg/L, the extracted samples were diluted.

ELISA for detection of lysozyme

For quantification of lysozyme, the RIDASCREEN® FAST Lysozyme (R 6412; R-Biopharm, Darmstadt, Germany) sandwich ELISA is used. The assay is calibrated with a commercial available lysozyme standard ranging from 2.5 up to 67.5 µg/L. For testing, 100 µL standard or sample solutions were pipetted to the wells, incubated for 10 min, and washed three times. Next, 100 µL enzyme-antibody conjugate is added, incubated for 10 min followed by three washing steps before pipetting 100 µL substrate/chromogen solution to each well. The colour development is stopped after 10 min with 100 µL stop solution and absorbance is measured at 450 nm.

RESULTS AND DISCUSSION

ELISA quality parameters

The limit of detection (LOD) for the lysozyme determination in wine was calculated from measurements of 10 lysozyme-free white wines and 10 lysozyme-free red wines. The mean optical densities (OD) plus the threefold standard deviation was in both cases near the OD of the zero standard. Considering the dilution factor of 20, the estimated limit of quantification is 0.05 mg/L.

The intra-assay coefficient of variation (CV; repeatability) was determined by measuring the analyte in a spiked sample in one assay run (n=4). A wine spiked with 5 mg/L revealed a CV of 2.6%. Using the same sample in duplicate, the inter-assay CVs (reproducibility) was 7.1% (n=3).
Spiking white wine with lysozyme at levels of 1 and 5 mg/L resulted in recoveries of 105 and 111%, respectively. For red wine spiked at the same levels the recoveries were 108 and 101%, respectively.

The commercially available lysozyme preparations (with unknown lysozyme concentrations) show parallel calibration curves, indicating that their antibody reactivity is comparable to pure lysozyme and the method is suitable for the determination of the commercial products.

Spiking experiments at high levels of 500 mg lysozyme per liter of wine revealed a precipitate in red wine. This was described before and was the reason for low recoveries of lysozyme from red wine determined by HPLC (Tirelli and De Noni, 2007). In the case of the determination by the described ELISA system, the suspended precipitate is re-dissolved by the extraction buffer and the treatment at 60°C. The recovery was quantitative.

CONCLUSIONS

The described assay is suitable for high throughput analysis of lysozyme in red and white wine. Due to the very low detection limit, only traces of lysozyme could be measured. Therefore the method is perfectly suited for screening purposes to determine lysozyme-free wines.

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NEW APPROACH FOR IDENTIFICATION OF RED GRAPE VARIETIES BY FINGERPRINT OF WINE ANTHOCYANIN SPECTRUM

N. Shakulashvili [1], T. Chavchanidze [1], N. Ksovreli [2]

[1]"Wine Laboratory" Ltd,
Road Didi Digomi - Gldani, 4/60, Tbilisi, 0131, Georgia
n.schaku@gmx.de

[1]"Wine Laboratory" Ltd,
Road Didi Digomi - Gldani, 4/60, Tbilisi, 0131, Georgia
tamtachav@yahoo.com

[2]JSC "Kindzmarauli Corporation"
Tchavtchavadze 55, Kvareli, Georgia
nksovreli@hotmail.com

ABSTRACT

HPLC-method has been applied to obtain anthocyanic profiles of various red wines produced from various grape cultivars. Ratio of peak areas of separate anthocyans were evaluated. Amount of anthocyan compounds depends on many factors, but constancy of above mentioned ratios are strongly predetermined by genetic factors. Certain ratios can be estimated as constant and typical for concrete grape variety. 140 analyzed wine samples were commercially available bottled wines of different harvest years from different regions of Georgia produced by different wine-makers from grape variety *Saperavi* and other Georgian varieties, as well as some home-made young and aged wines and also several imported wine samples from non-Georgian varieties. 122 of samples were classified and distributed in several groups accordingly with combination of constancy of certain anthocyans' ratio. Each group corresponds to one variety.

HPLC-Verfahren wurde fuer das Erreichen von Anthocyanprofile verschiedener Rotweine verwendet, die aus verschiedenen Rebsorten hergestellt wurden. Die Relation der Peakflaechen einzelner Anthocyanen wurde abgeschaetzt. Das Anthocyangehalt haengt von mehreren Parameter ab, aber die o.g. Relationen streng von genetischen Faktoren verursacht sind. Bestimmte Relationen kann man als konstant und typisch fuer konkrete Rebsorte definieren. Analysiert wurden 140 sowohl kommerziell zugaengliche Flaschenweine verschiedener Jahrgaenge aus verschiedenen Regionen und Kellereien Georgiens, als auch junge und gealterte Hausweine, hergestellt aus *Saperavi* und anderen georgischen Rebsorten und auch die nicht aus georgischen Rebsorten hergestellten importierten Flaschenweine. 122 Proben wurden klassifiziert und in einigen Gruppen entspraechend den Kombinationen von Konstanz der Relationen von Anthocyanen eingeordnet. Jede Gruppe entspricht einer konkreten Rebsorte.

INTRODUCTION

The examination of authenticity of grape varieties is the important aim of wine controlling. Among other known methods evaluation of anthocyan compositions in red wines is the important approach for the identification of red grape varieties. The anthocyans are important quality parameter of red grapes, due to their ability to give red colour to wines by the extraction of anthocyans from grape skin into the wine during maceration and winemaking.

Nine major anthocyans are identified in red wine, which are more responsible for colour and other wine properties due to anthocyans. There are - monoglucosides of delphynidin (De-3-gl), cyaniding (Cy-3-gl), petunidin (Pt-3-gl), peonidin (Pe-3-gl) and malvidin (Mv-3-gl), as well as acylated and coumarylated glucosides of peonidin (Pe-3-acgl, Pe-3-cugl) and malvidin (Mv-3-acgl, Mv-3cugl).

The anthocyans are genetically predetermined in red grape cultivars and are typical for certain grape variety. Although the absolute amount of antocyans in wine depends on wether conditions, vintage, vineyard treatment, vinification technique, all these variables have small effect to composition of anthocyans in wine [1] and thereby the relative proportions of individual anthocyans are typical for concrete grape variety [2,3]. On the other hand, some authors correspond, that oxidation, hydrolysis, condensation and other reactions occur from beginning of wine production and also ageing cause decrease of anthocyans in wine [4-7].

Despite of this, relations between the anthocyans can be used for characterisation of grape cultivars and recently some authors correspond about several approaches for identification of varieties through relative proportions (ratio) of individual anthocyans [8,9]. Main approach is the ratio between acylated and coumarilated monoglucosides of peonidin and malvidin. But only one variable is not enough for reliable identification and classification because of overlapping of ratios for several varieties. As the additional variable for identification and classification shikimic acid [10], sum of acylated anthocyans [11,12], shikimic acid and flavonols - myricetin and quercetin [13] have been applied. The further possibility is the multivariate data analysis of individual anthocyans [14-16]. Application of discriminant analysis allows to distribute unknown sample to appropriate groups of varieties [14].

The aim of our work was selection of such ratios of certain anthocyanins which are constant (so-called variables) and in combination can be used for characterisation of many red grape varieties with high level of confidence.

MATERIALS AND METHODS

Apparatus

Knauer (Berlin, Germany) system with gradient pump WellChrom K-1001; DAD detector WellChrom UV-Vis K-2501 with 190 - 740 nm range; Software – ChromGate V.3.1.

Reagents

Formic acid (98%) (Merck, Germany), acetonitrile – HPLC grade (Merck, Germany);

HPLC conditions

Column – LiChroCart 250-4 with LiChrospher 100 RP 18 (5 µm) (Merck, Darmstadt, Germany); Operating temperature - 50°C; Wavelength – 518 nm; Injection volume – 20 µl; Flow rate – 0.8 ml/min; Solvent A – water/formic acid/acetonitrile 87:10:3 (v/v/v); Solvent B – water/formic acid/acetonitrile 40:10:50 (v/v/v);

Gradient program

Time (min)	**solvent A % (v/v)**	**solvent B % (v/v)**
0	94	6
1	94	6
15	92	8
18	75	25
21	70	30
24	60	40
35	50	50
37	20	80
44	20	80
46	94	6
57	94	6

Wine samples

The test material used were commercially available bottled wines of different harvest years from different regions of Georgia produced by different wine-makers, as well as some home-made young and aged wines vinified from grape cv *Saperavi*, including several clones of cv *Saperavi* and other Georgian grapes – cv *Usakhelauri*, cv *Alexandrouli*, cv *Aladasturi* and cv *Ockhanuri Sapere.* One group of investigated wines was Georgian wine "Khvanchkara", which is produced from two Georgian grapes cv *Alexandrouli* and cv *Mujuretuli*. Some wines were of unknown origin. Several imported wine samples from non-Georgian varieties were analyzed too. Those were: cv *Merlot* (from France), cv *Blauer Spaetburgunder* (from Germany), cv *Cabernet Sauvignon* (from Chile) and cv *Shiraz* (from Chile)

DISCUSSION

HPLC-method have been used to achieve the fingerprints of anthocyan profiles in wine samples. Gradient program of prescribed by O.I.V. basic [17] method was changed significantly to achieve baseline separation of Petunidin-3-glucoside and Malvidin-3,5-diglucoside if this last is presented in wine and to avoid wrong peak quantification in case if Mv-3,5-gl peak is overlapped with Pt-3-gl peak. Other reason for gradient changing was achieving of better washing and equilibration of column for the next analysis. Changed method was validated and tested in an international inter-laboratory study. Ratios of individual anthocyanin components was calculated by dividing of peak areas expressed in AUs. For this, peak areas of nine major anthocyanins were summed up and set as 100%. Peak areas of unidentified compounds have not been taken into account. 140 wine samples have been analyzed. Among them were wines of known and unknown origin.

Taken into account, that biosynthesis of anthocyans is genetically predetermined and also that relative ratios of anthocyan content is weakly influenced by vinification technique, main idea of authors of this work was to select such ratios of individual anthocyans, which are constant and this constancy is typical only for concrete grape variety.

All possible variations of ratios of peak areas of separate anthocyans have been evaluated. Regularity was found in three cases. One of them is well known and recently substantially described by many authors - ratio of acylated anthocyans to cumarylated ones. The second regularity was observed with ratio of Pt-3-gl to De-3-gl. The third regularity was the ratio of Mv-3-gl to sum of De-3-gl, Cy-3-gl, Pt-3-gl and Pe-3-gl. These observed regularities (or constancy) can be determined as variables for the identification of grape cultivars. Applying three variables simultaneously, certainty of correct identification of grape cultivars grows significantly up and correspondingly - probability of mistake becomes very negligible.

Majority of analyzed wines of different names, of different type, of different place of origin, of different geographical location, of different yield and different wine-makers were produced from mostly popular and widely distributed in Georgia wine grape cv *Saperavi*. Obtained results as numerical data were divided to several groups accordingly to combinations of abovementioned 3 variables. Finally 106 samples have been divided to 10 groups with different number of samples. In contrast to conventional ratio $R_{ac/cum}$ we used in our work $R_{cum/ac}$ to make 3D image more perceptible. 10 groups just only for one cv *Saperavi* indicate to different clones of this grape variety. Thus, our approach is appropriate for the identification of not only different grape varieties, but also different clones of them too. Statistical data are given in Tab. 1. Among these 106 samples several wines were of unknown origin, but they were distributed in groups in accordance to combinations of proper variables.

Tab. 1. Statistical data for the Ratio of Pt-3-gl to De-3-gl (**$R_{Pt/De}$**), Mv-3-gl to sum of De-3-gl, Cy-3-gl, Pt-3-gl and Pe-3-gl (**$R_{Mv/DeCyPtPe}$**) and ratio of cumarylated anthocyans to acylated ones (**$R_{cum/ac}$**) in 10 groups of cv *Saperavi* clones**.** Abbreviations S1 - S10 designate 10 clones of cv *Saperavi*.

Ratio \ **Cultivar**		**S1**	**S2**	**S3**	**S4**	**S5**	**S6**	**S7**	**S8**	**S9**	**S10**
Number of samples		26	23	14	10	9	3	6	7	3	5
$R_{Pt/De}$	**Min**	1.18	1.04	1.03	1.13	1.28	0.98	1.09	1.21	0.94	1.32
	Max	1.36	1.18	1.16	1.25	1.40	1.09	1.19	1.35	0.97	1.42
	Mean	1.30	1.08	1.10	1.18	1.34	1.04	1.16	1.27	0.95	1.37
$R_{Mv/DeCyPtPe}$	**Min**	2.42	1.67	1.75	2.02	2.69	1.44	1.80	2.10	1.56	3.18
	Max	2.61	1.82	1.91	2.15	2.80	1.51	1.94	2.22	1.61	3.30
	Mean	2.52	1.75	1.81	2.11	2.73	1.47	1.87	2.16	1.58	3.23
$R_{cum/ac}$	**Min**	1.10	1.39	1.09	0.84	0.84	1.98	0.71	1.22	1.23	0.75
	Max	1.25	1.54	1.24	0.99	0.98	2.04	0.80	1.36	1.32	0.85
	Mean	1.18	1.47	1.17	0.89	0.90	2.01	0.75	1.28	1.28	0.81

Despite of the objective, that some groups with 3 or 5 samples aren't certain from statistical point of view, oenological practice indicates that vineyards from which mentioned wines were produced, belongs to cv *Saperavi*.

Some wines produced from other Georgian authentic grape varieties such as cv *Usakhelauri* (1 sample), cv *Alexandrouli* (1 sample), cv *Aladasturi* (1 sample) and cv *Ockhanuri Sapere* (2 samples – from the same vineyard but different yields) were analyzed. All of them show different combinations in comparison with cv *Saperavi* clones. Combinations of both samples of cv *Ockhanuri Sapere* are the same within specific defined limits. Additionally, 4 imported wines produced from grape varieties - cv *Merlot* (from France), cv *Blauer Spaetburgunder* (from Germany), cv *Cabernet Sauvignon* (from Chile) and cv *Shiraz* (from Chile) were analyzed. Obtained combinations of variables were different in all cases. Statistical data are given in Tab. 2.

Tab. 2. $\mathbf{R_{Pt/De}}$, $\mathbf{R_{Mv/DeCyPtPe}}$ and $\mathbf{R_{cum/ac}}$ ratios for other varieties analyzed in our work.

Varieties	$\mathbf{R_{Pt/De}}$	$\mathbf{R_{Mv/DeCyPtPe}}$	$\mathbf{R_{cum/ac}}$
Alexandrouli	0.97	1.33	3.27
Aladasturi	1.48	5.04	0.67
Usakhelauri	1.20	2.94	0.68
Ockhanuri Sapere	1.22	1.64	0.42
Ockhanuri Sapere	1.19	1.70	0.39
Merlot	0.86	1.60	0.40
Blauer Spaetburgunder	2.09	2.07	-
Cabernet Sauvignon	1.06	3.30	0.28
Shiraz	1.85	6.67	0.27

Separate group is combined from 7 samples of Georgian wine "Khvanchkara", which is produced from two Georgian grapes cv *Alexandrouli* and cv *Mujuretuli*. There is not prescribed by the legislation ratio of these two cultivars for winemaking, but many wine cellars keep to the traditionally established ratio of 70:30 v/v, that is why the combination of variables typical for this ratio can be partly applied for identification of this wine.

The combinations of three variables have been selected in our work. If we'll draw three-dimensional model from all obtained data (variables) for all samples of all varieties, we'll receive the image given at figure 1.

As see from Fig.1, separate plane (2D) projections can't give any information about different groups of varieties because the dispersion of groups in accordance to typical variables doesn't allow to recognize these groups separately in whole matrix of variables. The more varieties, the homogeneously is the matrix. But 3D image gives full impression regarding individual groups of varieties, because combinations of variables for each cultivar built separate three-dimensional groups. They are well distinguished from each other like galactics in cosmos Each group corresponds to one concrete grape variety or clone. Thus, 3D method is suitable for classification of grape varieties. Discrete points those significantly stand out against the groups correspond to wine sorts for which only one sample of wine has been analyzed.

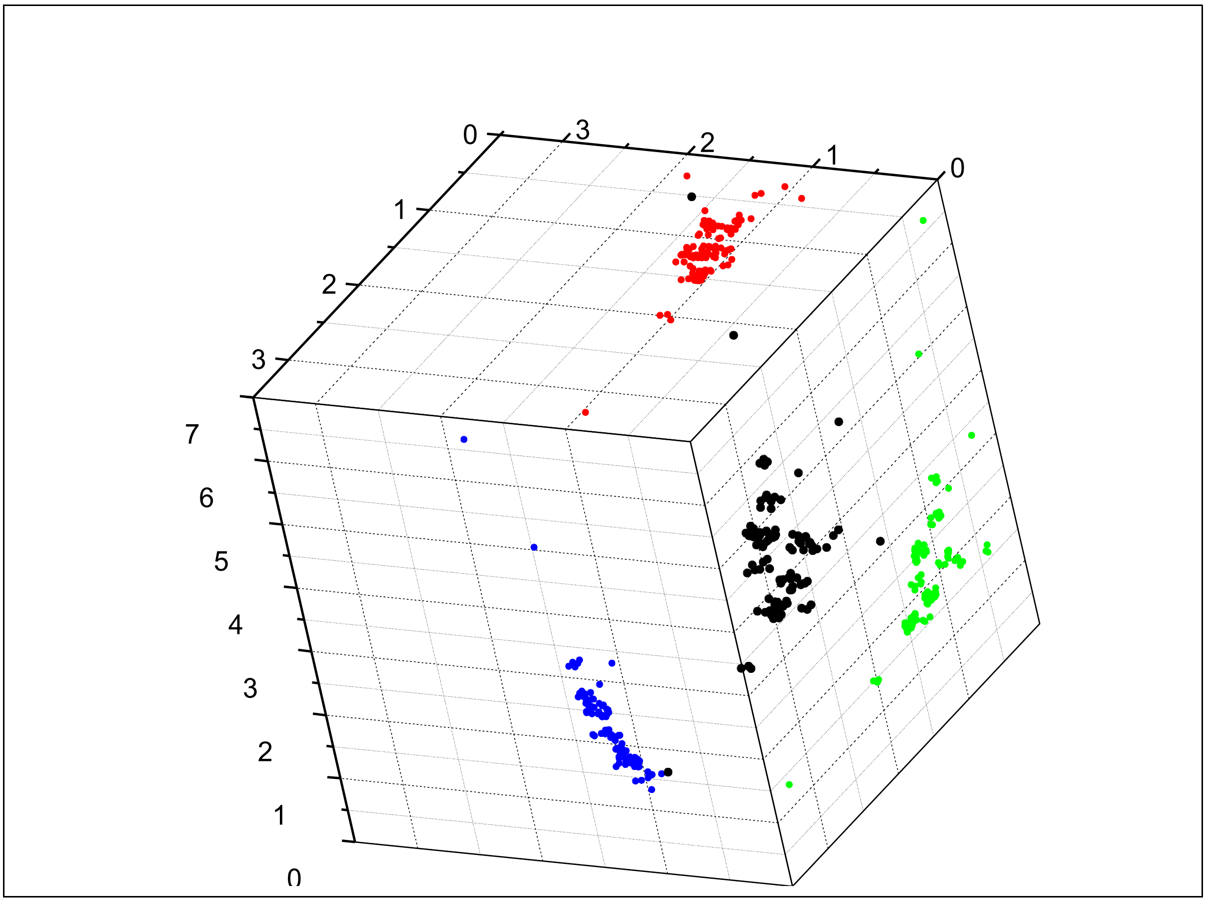

Fig. 1 3D-image for all combinations of variables of all samples.

In the end it must be noted, that our approach is effective for "pure" wines, which means for wines produced from one definite grape variety.

CONCLUSIONS

Thus combinations of three variables - Ratio of Pt-3-gl to De-3-gl (**$R_{Pt/De}$**), ratio of Mv-3-gl to sum of De-3-gl, Cy-3-gl, Pt-3-gl and Pe-3-gl (**$R_{Mv/DeCyPtPe}$**) and ratio of cumarylated anthocyans to acylated ones (**$R_{cum/ac}$**) are typical for each grape variety. Moreover, different clones of grape varieties have their own typical combinations of these variables.

122 samples from 140 were distributed in several groups accordingly with combination of constancy of certain anthocyanins' ratios. 18 samples could not be classified. Obviously they belong to wines produced from several different cultivars or clones.

Created from obtained data three-dimensional model allows distribute in space and by this determine and classify groups from different grape varieties.

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COMPOSES SOUFRES VOLATILS DANS LE VIN : UNE METHODE DE DOSAGE ORIGINALE - APPLICATION A DES VINS ELABORES AVEC UNE LEVURE PEU PRODUCTRICE DE H_2S

Vincent FARINES[a], Cloé SALMON[b], Alain BLAISE[a]*

[a] Centre de Formation et de Recherche en Œnologie - UMR 1083 « Sciences pour l'Œnologie » - Université Montpellier I - UFR des Sciences Pharmaceutiques et Biologiques.
15 Avenue Charles Flahault, BP 14491, 34093 MONTPELLIER Cedex 5, France.
Tel +33(0) 467 548 670 ; Fax +33(0) 467 548 686 ; e-mail* vincent.farines@univ-montp1.fr

[b]AGROVIN France, distribué par Devèze Biotech Oenologie
4 rue Evariste Galois – Z.I. La Deveze, 34500 BEZIERS, France.
Tel/Fax +33(0) 467 940 262 ; e-mail: agrovinfrance@agrovin.com

RESUME

L'apparition de caractères olfactifs de « réduction » dans un vin est liée à la présence de composés soufrés volatils légers. Au cours de la fermentation alcoolique plusieurs facteurs peuvent influencer l'apparition de ces composés : la teneur en azote assimilable des moûts et la souche de levure sont primordiaux. Dans un premier temps, ce travail propose une méthode innovante d'analyse des composés soufrés légers dans le vin. Dans un deuxième temps, une étude menée sur le moût et le vin de deux cépages considère les effets d'une nouvelle souche de levure et de la nutrition azotée sur la genèse des composés soufrés légers de réduction dans le vin. En complément d'une gestion réfléchie de l'azote assimilable, l'utilisation d'une nouvelle souche de levure comportant différentes variations au niveau des gènes codant pour le complexe protéique sulfite réductase permet de diminuer les caractères réducteurs du vin avec une augmentation de la détection des composés aromatiques positifs. Par ailleurs, la technique de l'espace de tête statique combinée à l'analyse en GC-PFPD constitue une méthode très sensible, rapide et sans solvants pour l'analyse des composés soufrés volatils dans le vin. L'absence d'étape de pré-concentration des échantillons constitue un avantage à l'égard de la faible stabilité chimique des espèces à analyser.

ABSTRACT

The appearance of "reduced" type olfactory characters in wine is linked to the presence of light volatile sulphur compounds. During alcoholic fermentation several factors may influence the development of these compounds: the concentration of fermentable nitrogen in must and the yeast strain are of primary importance. The first part of this report presents an innovative analytical method for the determination of light sulphur compounds in wine, whereas the second part presents the application of this method in a study on the effects of a new yeast strain and nitrogen nutrition on the generation of light sulphur compounds in wine.

In conjunction with rational management of fermentable nitrogen, the use of a new yeast strain with different variations in the genes coding for the protein complex sulphite reductase, has enabled the 'reduced' diminished character of wine to be diminished, with a concomitant increase in the detection of positive aromatic compounds. In addition, static headspace combined with GC-PFPD analysis has proved to be a very sensitive solvent-less technique for the analysis of volatile sulphur compounds in wine. The fact that a pre-concentration step is not required represents an advantage as regards the low chemical stability of these analytes.

INTRODUCTION

La présence dans les vins de composés soufrés volatils (CSV) tels que l'hydrogène sulfuré, le méthanethiol ou l'éthanethiol contribue à déprécier leurs profils aromatiques et gustatifs [Goniak, Noble, 1987], [Rauhut, Sponholz, 1992]. Les perceptions de ces composés sont associées aux descripteurs d'œuf pourri, de choux ou encore d'oignon rassemblées sous le terme de « réduit » rapporté dans les jurys de dégustations [Ribéreau-Gayon *et al.*, 1998].
La volatilité intrinsèque des CSV fait de la chromatographie en phase gazeuse une technique de choix pour effectuer l'analyse de ces espèces moléculaires, le choix du mode de détection allant de la simple ionisation de flamme (FID) à la spectrométrie de masse (MS) [Mestres *et al.*, 2000]. Cependant, des techniques de détection plus spécifiques et très sensibles telles que la photométrie de flamme (FPD) ou la chemiluminescence (SCD) sont utilisables. La détection en photométrie de flamme pulsée (PFPD) est une technique alternative plus sensible que le FPD [Tsachaki, 2006]. Les concentrations des CSV dans les vins sont faibles et justifient des étapes de pré-concentration et/ou d'isolation en amont de l'analyse en chromatographie. Plusieurs stratégies d'échantillonnage sont disponibles : l'extraction liquide-liquide [Lavigne-Delcroix *et al.*, 1996], l'espace de tête statique [Lavigne *et al.*, 1993], [Park et al., 1994], [Mestres *et al.*, 1997], l'injection de large volume [Rauhut *et al.*, 1998], l'espace de tête dynamique [Leppanen *et al.*, 1980] et plus récemment, la micro-extraction en phase solide (SPME) [Mestres *et al.*, 1999a], [Fang, Qian, 2005], [Fedrizzi *et al.*, 2007], [López *et al.*, 2007]. Par ailleurs, l'analyse des CSV du vin constitue un défi analytique au regard de leur faible stabilité chimique. Ces composés sont éminemment sensibles à l'oxydation, à la lumière ou à la présence de métaux à l'état de traces [Wardencki, 1998]. Cette faible stabilité chimique implique des pertes d'analyte au cours des étapes diverses d'extraction et d'analyse [Haberhauer-Troyer *et al.*, 1999], [Lestremau *et al.*, 2004]. En conséquence, ce travail propose un retour sur la technique de l'espace de tête statique couplée dans notre expérimentation à l'analyse en GC/PFPD. A cause de l'absence d'étape de concentration, il est reproché à la technique de l'espace de tête statique un seuil de détection trop élevé, mais un dosage direct peut être considéré comme un avantage déterminant au regard de la faible stabilité chimique des espèces à analyser. D'autre part, la sélectivité et la haute sensibilité permises par le PFPD peuvent suppléer à l'absence de pré-concentration. Ainsi, la première partie de ce travail s'attachera au développement d'une technique innovante pour le dosage des CSV des vins. La méthode propose l'injection de petits volumes d'espace de tête gazeux en équilibre avec la matrice pour l'analyse des CSV en chromatographie gazeuse capillaire, couplée à la détection en PFPD.
L'H_2S est un sous-produit naturel du métabolisme levurien qui le forme notamment par réduction des sulfates, des sulfites et du soufre élémentaire au cours de la synthèse des acides aminés. Si, en cours de fermentation alcoolique, cette voie métabolique se déroule en présence de suffisamment d'azote, l'ion HS^-, dont dérive l'H_2S, est piégé par l'O-acétyl sérine et l'O-acétyl homosérine pour donner naissance aux deux acides aminés, méthionine et cystéine. En revanche, en cas de carence azotée, l'H_2S libre est stocké dans la cellule levurienne et libéré dans le moût en fermentation [Swiegers, Pretorius, 2007]. La clé de voûte de cette séquence métabolique résulte dans le transport des sulfates, du moût vers la levure, par la sulfate perméase. Ces sulfates sont alors transformés en sulfites puis en H_2S par le biais d'enzymes ATP-sulfurylase et sulfite-réductase. C'est en modifiant le gène (par technique non OGM) de cette dernière enzyme, que l'Institut de Recherche Vitivinicole Australien (AWRI) a développé une levure dont le métabolisme produit moins d'H_2S, moins de cystéine et moins de méthionine, même dans un moût appauvri en azote [Sutherland *et al.*, 2003]. Ainsi, la deuxième partie de ce travail propose d'évaluer l'utilisation de cette souche ,

Maurivin Platinum® (Agrovin France, distributeur), en lien avec des apports en azote assimilable variables sur moûts, sur les profils analytiques en CSV de vins blancs et rosés.

MATERIEL ET METHODES

1. Vinifications

Les moûts blancs de Picpoul et ceux de Grenache rosés sont séparés, après débourbage enzymatique, en trois lots homogènes de 330 HL. Ces lots se différencient par le choix de la souche de levure LSA ensemencée et par les apports d'activateur réalisés en cours de fermentation alcoolique (et donc par la teneur en azote facilement assimilable).

- Lot 1 : LSA témoin. A densité initiale – 20 points et – 40 points, ajout de 15 g/HL d'Actimax Ferm®, activateur complet à base de thiamine, sulfate d'ammonium et écorces de levures.
- Lot 2 : LSA Maurivin Platinum®. A densité initiale – 20 points et – 40 points, on ajoute 15 g/HL d'Actimax Ferm®.
- Lot 3 : LSA Maurivin Platinum®. A densité initiale – 20 points, on ajoute 20 g/HL d'Actimax Ferm® et à – 40 points, 30 g/HL d'Actipasa Plus®, activateur riche en sulfate d'ammonium et en thiamine.

Les doses d'activateur employées respectent les doses maximales autorisées en thiamine. Une dose de 30 g/HL d'Actimax Ferm® concède au moût 45 mg/L d'azote facilement assimilable (AFA), tandis que 30 g/HL d'Actipasa Plus® en libèrent 62 mg/L.
Tous les ajouts d'activateur sont réalisés avec une aération. Tous les vins sont traités, au cours de la fermentation alcoolique, à 20 g/HL de bentonite. En fin de fermentation alcoolique, les vins sont sulfités à 4g/HL puis soutirés.

2. Dosage des composés soufrés volatils

Les différents composés étudiés sont : l'hydrogène sulfuré (H_2S), le méthanethiol (MeSH), l'éthanethiol (EtSH), le diméthylsulfure (DMS), le diéthylsulfure (DES), le diméthyldisulfure (DMDS), et le diéthyldisulfure (DEDS). L'éthylméthylsulfure (EtSMe) est utilisé comme étalon interne (EI).
Pour préserver l'intégrité des composés soufrés, toutes les solutions standard sont préparées et stockées en flacons scellés protégés de la lumière, dans de l'éthanol désoxygéné à – 30 °C. A partir de solutions individuelles (≈ 1500 mg/l), une solution standard globale contenant tous les composés en mélange est préparée (gamme : 0.8 – 1.5 mg/l).
Les solutions pour la gamme d'étalonnage sont obtenues de la façon suivante :

- 3 g de NaCl avec différentes quantités de vin synthétique (9.925 à 9.700 ml) sont placés dans un flacon de 20 ml destiné à l'extraction en espace de tête : ce mélange est désoxygéné par bullage d'azote et le flacon est immédiatement scellé,
- l'addition de solution globale de standards (25 à 250 µl) est alors effectuée dans le flacon à travers le septum,
- 50 µl d'une solution d'EI (7 mg/l) sont ajoutés à chaque flacon.

La variation d'éthanol (volume et %) apportée par l'addition de standard et de l'étalon interne est corrigée en utilisant plusieurs vins synthétiques. Au final, chaque flacon contient 10 ml de vin synthétique avec 12 % d'éthanol, 3.5 g/l d'acide tartrique à pH 3.5.
Les échantillons sont traités sur un passeur automatique équipé d'une seringue de gaz de 1 ml. L'extraction en espace de tête est effectuée à une température de 65 °C pendant 40 minutes avec agitation à 250 tours/minute (8s oui, 2s arrêt). A l'issu de l'incubation, 250 µl de l'espace de tête sont prélevés par la seringue maintenue à 70 °C, et injectés dans le chromatographe (250 µl/s) équipé d'un détecteur PFPD. La température de l'injecteur

(splitless) est de 250 °C. Une colonne DB-WAXetr (30 m × 0.32 mm D.I., 1.0 µm d'épaisseur de film) est utilisée avec de l'helium comme gaz vecteur (1,2 ml/min). La programmation de température est la suivante : 35 °C pendant 3 min, 10 °C/min jusqu'à 100 °C et 20 °C/min jusqu'à 220 °C (5 min).

RESULTATS et DISCUSSION

Un chromatogramme type obtenu pour les composés soufrés (20 à 30 µg/l) traités suivant les conditions optimales d'incubation est présenté dans la figure 1.

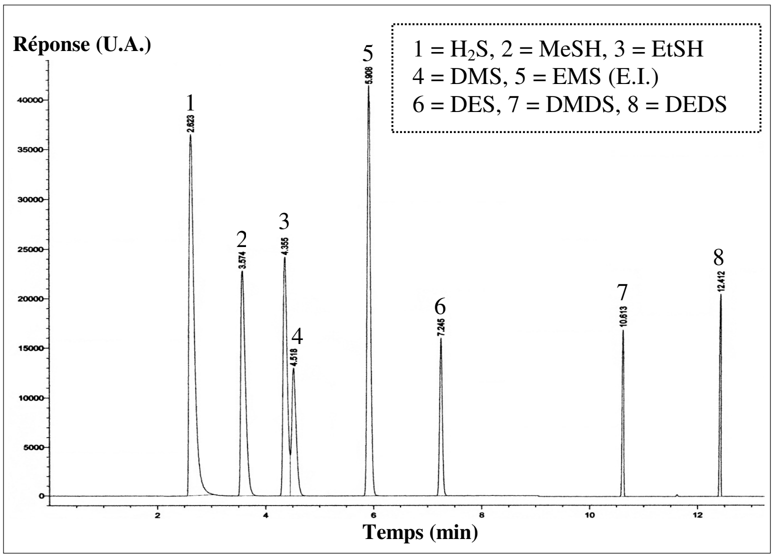

Figure 1: Chromatogramme de composés soufrés en vin synthétique

L'éthanol, par son effet solvant, peut jouer un rôle prépondérant sur l'extraction des composés depuis la phase liquide [Mestres et al., 1998], [Mestres et al., 1999b], [Fedrizzi *et al.*, 2007]. L'influence du TAV entre 10 et 20 % sur le dosage des composés soufrés par la méthode proposée a donc été étudiée sur des solutions de vins synthétiques : H_2S 30 µg/l, MeSH 25 µg/l, EtSH 20 µg/l, DMS 20 µg/l, DES 15 µg/l, DMDS 20 µg/l et DEDS 25 µg/l. Comme cela est montré sur la figure 2, l'augmentation du TAV entraîne une forte diminution de tous les composés soufrés détectés par le PFPD.

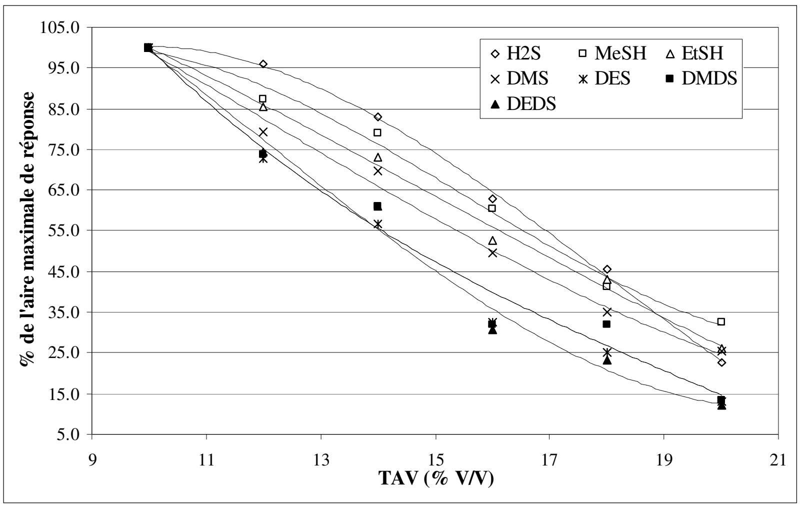

Figure 2 : Influence du TAV sur l'aire des pics de composés soufrés

L'aire des pics des composés décroît de façon quasi linéaire (R^2 = 0.9560 to 0.9970) à mesure que le TAV augmente. Les utilisateurs de la SPME justifient cet effet par les phénomènes de compétitions à l'adsorption sur la fibre entre l'éthanol et les composés d'intérêt [Lopez *et al.*, 2007]. Dans notre cas et étant donné qu'aucune fibre n'est utilisée, l'explication se situe plus en amont dans le procédé d'extraction. En fait, la distribution des analytes entre la phase liquide et la phase gazeuse est régie par leurs coefficients de partage. Les composés soufrés sont plus solubles en phase organique qu'en phase aqueuse : lors de l'augmentation du TAV, le coefficient de partage des composés est augmenté et leur sensibilité pour l'espace de tête est ainsi diminuée [Kolb, Ettre, 2006]. Ce problème peut être facilement résolu en ramenant le TAV du vin synthétique utilisé pour la calibration à celui du vin à analyser.

Ainsi, le Tab. 1 présente les données de calibration obtenues avec deux vins synthétiques de TAV différents correspondant aux vins blancs et aux vins rosés de l'expérimentation en chais (*cf* Tab. 3).

Tab. 1 : Données de calibrations sur vins synthétiques de différents TAV

		Vin synthétique, TAV 13.1 % (Vin blanc)			Vin synthétique, TAV 12.8 % (Vin rosé)		
Composé	Gamme (µg/l)	Pente	Ord. origine	R^2	Pente	Ord. origine	R^2
H_2S	0 – 36.5	14943.3	6534.3	0.9982	15363.3	6717.9	0.9985
MeSH	0 – 32.8	10293.6	3581.7	0.9987	10544.7	3669.1	0.9964
EtSH	0 – 25.4	10568.4	4575.2	0.9953	10842.3	4693.8	0.9942
DMS	0 – 24.3	9910.5	4962.6	0.9965	10174.1	5094.6	0.9989
DES	0 – 20.7	7303.9	4018.6	0.9943	7544.7	4151.0	0.9984
DMDS	0 – 26.5	4082.4	3285.5	0.9939	4211.2	3389.1	0.9918

La méthode proposée donne des réponses linéaires dans les gammes de concentration explorées. La pente la plus élevée est obtenue avec l'H_2S. Les pentes pour le MeSH, l'EtSH et le DMS sont comparables. Pour les composés moins volatils (DES, DMDS et DEDS), les pentes diminuent avec l'augmentation des points d'ébullition. Le DEDS présente une pente très faible et une ordonnée à l'origine très élevée malgré un coefficient de corrélation satisfaisant (données non montrées) : ce composé a été exclu de l'étude par la suite.

A partir des données de calibrations obtenues précédemment, les trois vins blancs et les trois vins rosés étudiés ont été soumis à la méthode de dosage des composés soufrés (10 ml de vin, 3 g de NaCl). Les résultats sont donnés dans le Tab. 2.

Tab. 2 : Dosage des composés soufrés volatils dans les vins

Composé	**Vins blancs**			**Vins rosés**		
	LB1	**LB2**	**LB3**	**LR1**	**LR2**	**LR3**
H_2S	2,1	ND	ND	< 1,3	ND	ND
MeSH	0,5	ND	ND	< 0,4	ND	ND
EtSH	< 0,5	ND	ND	< 0,5	ND	ND
DMS	3,7	2,2	ND	2,9	< 0,8	ND
DES	ND	ND	ND	ND	ND	ND
DMDS	2,5	0,6	ND	1,1	ND	ND

LB : Lot Blanc, **LR** : Lot Rosé, **ND** : non détectable, **<** : présent mais inférieur au seuil de détection mentionné

La teneur en H_2S dépasse le seuil de perception (0.8 µg/l) dans le lot témoin blanc (LB1). Il est intéressant de noter que pendant la fermentation, ce lot a du être aéré à la densité de 1060 car il présentait une odeur de réduction. L'H_2S est aussi présent dans le lot témoin rosé (LR1) mais a un niveau inférieur à la limite de détection de la méthode. Dans les lots traités avec la levure sélectionnée (LB2, LB3, LR2 et LR3), l'H_2S n'est pas détectable ce qui correspond donc bien aux attentes de cette souche. La sulfite reductase est un hétérotétramère composé de deux sous unités (α et β) codées par les gènes MET10 et MET5. L'enzyme, une hemoflavoprotéine, lie les cofacteurs flavine adenine dinucleotide, flavine mononucleotide et siroheme. Des sélections successives ont pris en compte les caractères du gène MET10 de telle sorte que la sous-unité α ne peut plus lier les co-facteurs mais peut toujours former le complexe protéique hétérotétramère avec la sous unité β. La sous-unité α rendue non fonctionnelle permet de réduire la proportion de sulfite reductase active dans la levure et ainsi, de réduire la formation d'H_2S. En outre, la production de méthionine et de cystéine par la levure se trouve aussi diminuée car elle est liée à l'H_2S intracellulaire [Sutherland *et al.*, 2003].

L'EtSH, quant à lui, est susceptible d'être généré par action directe de l'H_2S sur l'éthanol [Rauhut, 1993], [Landaud *et al.*, 2008]. Lié à la quantité d'H_2S dans le vin, ce composé soufré, dont le seuil de perception est de 0.1 µg/l, est effectivement présent dans les lots témoins et absents des autres lots.

Parmi les voies potentielles de sa formation, le MeSH peut provenir de la dégradation de la L-méthionine. Celle-ci est oxydée en méthional qui est à son tour dégradé en acrylaldéhyde et en MeSH qui est alors le précurseur de thioesters et disulfures organiques (DMDS et DES) [Vermeulen, 2005]. Dans les lots témoin, le MeSH est présent à un niveau supérieur à son seuil de perception (0.3 µg/l) dans le vin blanc et à une teneur inférieure dans le vin rosé. Ce composé soufré est absent des autres lots traités avec la levure sélectionnée qui produit moins de méthionine précurseur du MeSH.

Tandis que le DES n'est présent dans aucun vin, le DMDS est représenté dans les lots témoins à une teneur qui reste toutefois inférieure à son seuil de perception (2.5 µg/l). Ce composé est quasi-absent des lots traités avec la levure sélectionnée.

Enfin, l'origine du DMS n'est pas certaine mais ce composé peut être formé par les levures à partir de la cysteine, de la cystine ou du glutathion ou encore par la réduction du diméthyle sulfoxyde catalysée par une diméthyle sulfoxyde réductase et de la dégradation de la S-méthylméthionine [Ségurel *et al.*, 2005]. Cette réductase est d'autant plus active chez *S. cerevisiae* que cette dernière fermente en conditions de carence azotée. Dans notre étude, on ne peut pas évoquer de carence nutritive avérée (*cf* Tab. 3).

Tab. 3 : Analyses des moûts et des vins

Paramètre	**Moût***		**Après F.A.**					
	Blanc	**Rosé**	**LB1**	**LB2**	**LB3**	**LR1**	**LR2**	**LR3**
TAV (% vol.)	-	-	13.08	13.11	13.13	12.90	12.86	12.75
pH	3.32	3.39	3.22	3.24	3.24	3.26	3.25	3.23
Sucres réducteurs (g/L)	213	198	1.7	1.3	1.6	0.9	1.0	2.4
Acidité Totale (g H_2SO_4/L)	3.78	2.82	4.29	4.43	4.45	3.70	3.74	3.74
Acidité Volatile (g H_2SO_4/L)	0.03	0.13	0.30	0.41	0.43	0.29	0.32	0.29
SO_2 libre (mg/L)	-	-	17	16	16	16	20	20
SO_2 total (mg/L)	-	-	86	97	98	63	69	70
Durée de F.A. (jours)	-	-	16	19	18	12	13	13
N total (mg/L)	315.0	332.5	217.0	262.5	259.0	199.5	175.0	252.0
N assimilable (mg/L)	158.2	156.8	58.8	86.8	74.2	70.0	75.6	74.2
*Potentiel Redox (mV)***	- 51	- 59	- 165	- 105	- 101	- 135	- 138	- 138

LB : Lot Blanc, **LR** : Lot Rosé, * : avant ajout d'activateurs, ** : Electrode de référence Ag/AgCl.

On peut toutefois souligner que les lots 3 des vins ont fermenté avec plus d'azote assimilable que les deux autres lots et donc en conditions fermentaires plus favorables pour la levure. En effet, si l'on compare les lots 2 et 3 qui ne diffèrent que par la quantité d'activateurs apportés, la teneur en DMS est plus élevée dans les lots 2 que les lots 3.

Les 6 vins finis ont été soumis à un collège de trente dégustateurs confirmés. Au delà de l'ensemble des paramètres organoleptiques analysés (figure 3), il est intéressant de constater que les vins témoins sont systématiquement et significativement plus réduits. Ces vins sont qualifiés de « fermés » sans être pour autant qualifiés par les descripteurs classiques « d'œuf pourri, de croupi, d'oignon, de coing ou encore d'asperge », généralement corrélés à la présence de composés soufrés. Dans notre expérimentation, les teneurs en composés soufrés sont limites au seuil de détection intrinsèque de chaque composé. Il est donc vraisemblable que les composés dans leur ensemble participent au caractère réduit des vins sans les marquer par des défauts organoleptiques pénalisants.

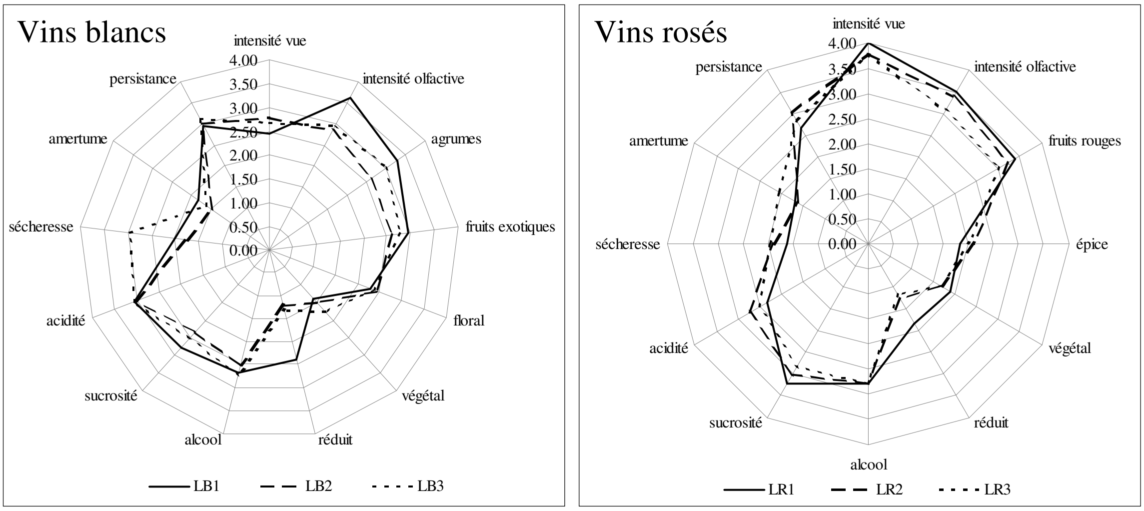

Figure 3 : Analyse sensorielle des vins finis

L'association entre les « goûts de réduit » et la présence de produits soufrés est justifiable par les systèmes redox des thiols-disulfures qui se caractérisent par des valeurs de potentiel normal E'_0 particulièrement basses ($- 270 \leq E'_0 \leq - 220$ mV), par rapport aux valeurs potentiels redox des vins ($+ 220 \leq E_h \leq + 450$ mV). On comprend ainsi que l'apparition de thiols dans certains vins et de la perception du caractère réduit suppose un potentiel d'oxydo-reduction abaissé [Ribéreau-Gayon *et al.*, 1998]. Les potentiels redox des vins mesurés fin F.A. montrent d'une part que le potentiel du vin blanc témoin (- 165 mV) est bien plus bas que celui des 2 autres lots (≈ - 100 mV) et que d'autre part, dans les vins rosés, les potentiels redox sont équivalents (≈ - 135 mV). Ainsi, de façon globale, le caractère réduit est plus prononcé dans le vin blanc témoin que dans le vin rosé témoin.

CONCLUSION

L'espace de tête statique combiné avec l'analyse en chromatographie phase gazeuse avec un détecteur PFPD constitue un outil efficace pour doser les composés soufrés volatils des vins. La méthode proposée est rapide, sans solvants et ne fait pas appel à l'utilisation des fibres de SPME avec leurs contraintes : choix de la fibre, phénomènes de saturation, compétition entre les composés à l'adsorption, coût… L'influence de la teneur en alcool sur le dosage a été pris en compte et l'utilisation, pour la calibration, d'un vin synthétique dont le TAV est ajusté à celui du vin à analyser permet d'obtenir des résultats très satisfaisants.

D'autre part, les résultats obtenus dans notre expérimentation en chais confirment d'une part l'incidence du choix de la levure et d'autre part celle de l'apport en azote assimilable sur la genèse des composés soufrés volatils dans le vin. Ces deux facteurs, sans oublier une réflexion sur la nutrition azotée en amont au vignoble, restent encore aujourd'hui les deux leviers du vinificateur pour s'affranchir de la présence des composés soufrés volatils indésirables dans le vin.

REMERCIEMENTS

A Mademoiselle Virginie BERTHUIT (Œnologue) et Monsieur Cyril PAYON (Directeur et Œnologue) de la Cave de l'Ormarine (Hérault, 34), lieu de ces expérimentations.

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LA TENSIÓN INHERENTE A LA RELACIÓN ENTRE LOS DERECHOS DE PROPIEDAD INTELECTUAL Y LAS NORMAS TÉCNICAS: ALTERNATIVAS A LAS ORGANIZACIONES DE NORMALIZACIÓN EN EL SECTOR VITIVINÍCOLA

Fabíola Wüst Zibetti[1], Kelly Lissandra Bruch[2]

[1]Faculdade de Direito, Universidade de São Paulo
São Paulo, SP, Brasil
fwzibetti@gmail.com; fabiola.zibetti@usp.br

[2] Universidade Federal do Rio Grande do Sul e Universidade Luterana do Brasil
Porto Alegre, RS, Brasil
kellybruch@gmail.com; kellybruch@ulbra.br

ABSTRACT

Currently, in an increasingly interconnected world, with the aim of providing better compatibility, interchangeability and interoperability of products and processes, and to ensure standards of quality, performance and safety to protect health and environment, international technical standardization has assumed a larger importance in many different sectors. However, although it is recognized the importance of these standards, in many cases its adoption is hampered by the fact of including technologies protected by intellectual property rights. The tension inherent in the relationship between intellectual property and technical standards, in some situations, creates a large disincentive on the adoption of standards, and negative effects on trade, technology transfer and particularly sustainable development. Based on this situation, the present article introduces the issues emerging from this tension and, based on the experience of international standardization organizations and some countries, presents alternatives and measures that could be taken by the standardization organizations in the wine sector.

RESUMEN

Actualmente, en un mundo cada vez más interconectado, con el objetivo de proporcionar mayor compatibilidad, intercambialidad e interoperabilidad de productos y procesos, así como para garantizar padrones de cualidad, desempeño y seguridad, para la protección de la salud y del medio ambiente, las normas técnicas internacionales han asumido un papel de grande relevancia en los más diversos sectores. Sin embargo, aunque se reconozca la importancia de esas normas, en muchos casos su adopción es perjudicada por el hecho de incluir tecnologías protegidas por derechos de propiedad intelectual. La tensión inherente a la relación entre propiedad intelectual y normas técnicas en ciertos casos genera un crecente desincentivo a la adopción de las normas y produce efectos negativos al comercio, a la transferencia de tecnología y especialmente al desarrollo sostenible. Frente a ese escenario, el presente artículo tiene como propósito introducir la problemática que emerge de esa tensión y, con base en la experiencia de organizaciones internacionales de normalización y de algunos países, presentar medidas y alternativas que podrían ser adoptadas por las organizaciones de normalización del sector vitivinícola.

INTRODUCTION

En un mundo cada vez más interconectado, con el objetivo de proporcionar mayor compatibilidad, intercambialidad e interoperabilidad de productos y procesos, así como para garantizar padrones de cualidad, desempeño y seguridad, y para la protección de la salud y del medio ambiente, la normalización técnica internacional ha asumido un papel de gran relevancia en los más diversos sectores, desde la agricultura hasta la industria de alta tecnología. Al promover el uso de tecnologías de elevada calidad y eficiencia productiva, en nivel mundial, esas normas pasaran a ser consideradas un significativo instrumento para combatir los desafíos de la sociedad global y promover el desarrollo sostenible.

En 1994, reconociendo la importancia de las normas técnicas internacionales, los Miembros de la Organización Mundial del Comercio (OMC) adoptaran el Acuerdo sobre Barreras Técnicas al Comercio (Acuerdo TBT, sigla en inglés) estipulando la adopción de tales normas en el plan domestico. Con todo, su adecuada adopción, sea por países desarrollados o en desarrollo, en muchos casos es perjudicada por la inclusión de tecnologías protegidas por derechos de propiedad intelectual en las respectivas normas.

Conocida ya en los años 1930, la tensión inherente a la relación entre las normas técnicas y los derechos de propiedad intelectual genera un crecente desincentivo a la adopción de normas técnicas internacionales, pues cada día involucran un número mayor de tecnologías protegidas por tales derechos, incluso marcas, patentes e derechos de autor, lo que imposibilita la legal conformación con las normas de los usuarios sin previa licencia de tales tecnologías. Por consiguiente, la tensión produce efectos negativos al comercio, a la transferencia de tecnología y especialmente al desarrollo sostenible.

Frente a ese escenario, el presente artículo tiene como propósito presentar la problemática que emerge de esa tensión y presentar propuestas de medidas que podrían ser adoptadas pelas organizaciones de normalización del sector vitivinícola, especialmente por la Organización Internacional de la Viña e el Vino (OIV), para tratar el problema. Para eso, inicialmente, se abordan los principales conceptos y definiciones a respecto de las normas técnicas internacionales, especialmente las del sector vitivinícola. En la secuencia se analiza la relación entre las normas técnicas y los derechos de propiedad intelectual y la tensión que le es inherente, destacándose la problemática que genera. También se examina el tratamiento dado al problema sobre todo por las organizaciones internacionales de normalización tales como la ITU, ISO e IEC, y países como los europeos, los Estados Unidos y China, que están dedicándose a encontrar soluciones para él. Al final, son presentadas breves consideraciones y las propuestas al sector.

LAS NORMAS TECNICAS INTERNACIONALES

En la actualidad, se encuentran en el mercado un elevado número de productos del sector vitivinícola que cumplen a una o varias normas y reglamentos técnicos o de conformidad con ellos. Tales normas y reglamentos tienen como objetivo asegurar a compatibilidad e interoperabilidad entre productos y procesos, garantizar padrones de cualidad, desempeño y seguridad y/o la protección de la salud y del medio ambiente. En general, los mismos prevén procedimientos, padrones de formas, dimensiones, tipos, usos, terminologías y otros aspectos técnicos, conforme el resultado deseado, en las más diversas áreas tecnológicas, como ingeniería, transporte, agricultura y otros. Además, pueden determinar medidas de implementación y observancia, como exigencias de etiquetado y procedimientos de conformidad (ISO/IEC Guide 2:2004).

En el ámbito nacional, constituye una práctica bastante común tanto de países desarrollados cómo de países en desarrollo la adopción de normas y reglamentos técnicos. La multiplicidad y diversidad de normas y reglamentos nacionales llevaran a la fragmentación del mercado internacional, tornando difícil y oneroso, a los productores y exportadores, desarrollar y/o comercializar productos sujetos a ellos. Además, en muchos casos, establecidos arbitrariamente, pasaran a ser considerados como forma de proteccionismo disfrazado y, así, obstáculos al comercio internacional.

Frente a ese escenario, el criterio estipulado por los miembros de la OMC para que normas y reglamentos técnicos no se transformen en barreras comerciales es que se basen en normas adoptadas por organismos internacionales reconocidos. Se estima de ese modo que las normas técnicas internacionales pueden facilitar el curso del comercio internacional, contribuyendo también para la transferencia de tecnología de los países desarrollados a los países en desarrollo. La armonización es un principio nuclear en al Acuerdo TBT, en el cual se consagra la importancia de las normas internacionales (véase preámbulo y art. 2, Acuerdo TBT).

Por definición, las normas internacionales se constituyen en documentos establecidos por consenso y aprobados por un organismo de normalización internacional reconocido, que fornece, para uso común y repetitivo, reglas, directrices o características para actividades o para sus resultados, con vistas a la obtención del grado óptimo de orden en un dado contexto y que son tornadas accesibles al público (ISO/IEC Guide 2:2004). Cómo un de sus objetivos, buscan asegurar la compatibilidad de tecnologías y sistemas en una base mundial. Para atingir ese objetivo se necesita, por tanto, garantizar que tales normas, sus aplicaciones y uso, estén accesibles a todos, especialmente para que sean adoptadas en un mayor número de mercados.

En el sector vitivinícola, son ejemplos de normas técnicas internacionales las resoluciones aprobadas en el ámbito de OIV, las cuales son convertidas en reglamentos técnicos en sus países miembros. En ese sentido, para ilustrar, se destacan algunas cómo la Resolución OENO 03-2005, que trata del uso de trozos de madera de roble para la elaboración de los vinos; la Resolución OENO n. 353/2009, que trata del método de determinación de la relación de isótopos 18o/16o del agua en vinos y mostos, que determina la presencia del agua en el vino; y la Resolución VITI n. 01/2008, sobre los requisitos mínimos de madurez para las uvas de mesa (OIV, 2010).

En el ámbito nacional, las normas técnicas internacionales pueden ser adoptadas pelos países por medio de su aplicación directa o indirecta, o aún pueden ser usadas como base para el desarrollo de las normas nacionales (ISO/IEC Guide 2:2004). Para que tales normas puedan ser aplicadas en el plan domestico se hacen necesarias condiciones de acceso y uso de las informaciones y tecnologías prescritas en tales documentos. Sin embargo, en muchos casos, las tecnologías se encuentran protegidas por derechos de propiedad intelectual, de modo que el acceso y uso de las normas – que deberían estar disponibles al público – queda restricto y dependiente de autorización de los titulares de los derechos de propiedad intelectual.

LA TENSION INHERENTE A LA RELACION ENTRE NORMALIZACIÓN TECNICA Y PROPIEDAD INTELECTUAL

Los derechos de propiedad intelectual son aquellos asegurados por ley en relación a los frutos de la actividad creativa humana. Corresponden a los derechos exclusivos conferidos a las personas sobre sus creaciones, invenciones, marcas, símbolos, nombres, imágenes y diseños usados en el comercio. Son ejemplos los derechos de propiedad industrial sobre patentes, marcas, indicaciones geográficas y diseños industriales, así como los derechos de autor y conexos. La exclusividad conferida por ley a las patentes, por ejemplo, hace con que terceros no puedan, sin lo consentimiento del titular de los derechos, producir, usar, poner a la venta, vender o importar con tales propósitos, el producto objeto de protección, como también el proceso o producto obtenido directamente por el proceso patentado (véase Acuerdo TRIPS).

Por consiguiente, las normas técnicas que involucran tecnologías protegidas por esos derechos generan una situación complexa y, de cierto modo, conflictiva. Eso porque, mientras las normas procuran establecer padrones comunes, direccionando los esfuerzos para proporcionar el acceso y uso público, libre y colectivo de las tecnologías; la propiedad intelectual busca recompensar los esfuerzos individuales de creación protegiendo con exclusividad los derechos sobre determinada tecnología (*e.g.* invención). Tal situación lida con el hecho de que ambos los instrumentos, aunque relacionados, presentan propósitos distintos (colectivo *versus* individual) (véase OMPI, 2009; LEMLEY, 2002; ZIBETTI, 2007, 2009).

Por lo tanto, hay una tensión inherente a la relación entre normas técnicas e derechos de propiedad intelectual que, en la práctica, proporciona diversos problemas. En la perspectiva del organismo de normalización, la tensión puede emerger en la ocasión en que el desarrollo de una norma involucre una tecnología protegida por patente u otro derecho de propiedad intelectual. En esa situación, el organismo de normalización arriesga infringir los derechos al inclúyelos en norma sin la debida autorización, por el uso no autorizado de la tecnología protegida. Una opción muchas veces elegida por el organismo es la no adopción de normas que envuelvan eses derechos. Sin embargo, actualmente, tal opción se ha tornado cada día más difícil, especialmente debido al elevado avanzo tecnológico y por la ampliación del rango de tecnologías pasibles de protección por derechos de propiedad intelectual – principalmente después del adviento del Acuerdo TRIPS, el cual en materia de patentes, por ejemplo, determina en su art. 27.1 que “cualquier invención, de producto o de proceso, en todos los sectores tecnológicos, será patentable”.

Así, para que un organismo de normalización pueda adoptar una norma que comprenda tecnologías protegidas, *e.g.* por patentes, debe obtener la debida autorización de los respectivos titulares de derechos previamente al estabelecimiento de la norma.

El mismo ocurrirá posteriormente a la adopción de la norma por sus usuarios. Para una empresa fabricar y/o comercializar determinado producto en conformidad con las exigencias de determinada norma, la misma debe tener autorización de uso de las tecnologías patentadas prescritas en la respectiva norma. La ausencia de la autorización del titular de los derechos sobre la patente puede implicar en la infracción del derecho por parte de la empresa, configurada por la simple conformación del producto o proceso con la norma aplicable. En ese caso, para evitar la infracción, la empresa queda dependiente de la licencia del titular de la tecnología patentada (ZIBETTI, 2007; 2009).

El hecho de quedarse al criterio del titular de los derechos la facultad de establecer los términos y condiciones de la licencia puede dificultar y incluso impedir o acceso y uso de la tecnología por la empresa, como ha ocurrido, por ejemplo, en casos como de la *Dell Computer Company*, de la *Qualcomm,* de *DVD*, y otros (LEMLEY, 2002). En el caso de *Dell*, la compañía que ha participado del desarrollo de norma del sector de computación ha faltado en informar que su tecnología era esencial para la misma norma y, al exigir elevados valores en *royalties* de los usuarios, después de la norma haber sido adoptada, ha sido acusada de restringir la competencia industria de computadores. La disputa ha sido procesada en el ámbito de la Comisión Federal de Comercio (FTC, sigla en inglés), en los Estados Unidos. Resultó que, en 1996, la FTC prohibió *Dell* de exigir sus derechos de patente sobre la tecnología requerida la patente (véase FTC, 2010). Además de esa, otras situaciones también pueden ocurrir, como el titular del derecho recusarse a licenciar la tecnología, lo que imposibilita a las empresas competidoras de fabricar u/o comercializar los producto sujetos a normalización. Por consiguiente, las empresas quedan impedidas de ingresar en el mercado relativo al producto.

Eses son algunos ejemplos de situaciones en que la tensión inherente en la relación entre las normas técnicas y los derechos de propiedad intelectual restringen la competencia, perjudican al comercio, la transferencia de tecnología y al desarrollo sostenible. Tales situaciones que afecta principalmente los sectores considerados de alta tecnología, él también puede alcanzar otros, incluso los primarios, como la agricultura, incluso en la vitivinicultura.

Con el incremento del comercio internacional y el aumento de las normas técnicas internacionales, especialmente de normas envolviendo tecnologías protegidas por derechos de propiedad intelectual, el asunto ha ganado mayor destaque en escenario internacional. En 2005, UNIDO publicó el Reporte de Desarrollo Industrial, en el cual se reconoce la existencia de una correlación entre normalización, propiedad intelectual y el comercio internacional. Eso es corroborado en el Reporte sobre el Comercio Mundial de 2005, de la OMC, el cual además señala para el hecho de que la relación entre normas técnicas y propiedad intelectual puede tener efectos negativos al comercio (véase en el Reporte el caso de las normas de telefonía digital de tercera generación (3G) (WTO, WTR 2005)).

Aunque relevante para el comercio internacional, la discusión de la problemática no ha prosperado en la OMC. En ese ámbito, los Acuerdos TBT y TRIPS firmados en 1994, que regulan los aspectos de la normalización técnica y de los derechos de propiedad intelectual relacionados al comercio, respectivamente, no proveen expresamente cualquier medida para tratar de la tensión inherente a correlación de ambos y el comercio internacional (ZIBETTI, 2007, p. 173-202). Frente a ese escenario, tanto los países como las organizaciones internacionales adoptan medidas internas propias para tratar el problema (véase OMPI, SCP/13/2).

MEDIDAS ADOPTADAS PARA TRATAR LAS TÉNSION

Medidas internas de los países

Conscientes de esa problemática, percibida aún en la década de 1930, Estados Unidos de América ha sido uno de los primeros países que adoptaran medidas relacionadas a las patentes involucradas en normas técnicas nacionales. Segundo las Minutas del Encuentro del Consejo de Normas de 1930 y el Reporte del Comité sobre Procedimientos de 1932, se ha recomendado que los diseños y métodos patentados no debieran ser incorporados en las normas técnicas. Sin embargo, cada caso debería ser considerado en sus meritos, pudiendo admitirse en ciertas situaciones la inclusión de tecnologías patentadas en las normas (véase WILLINGMYRE, 2008). Con el pasar de los años los problemas asociados a esa tensión se intensificaran y nuevas medidas pasaran a ser adoptadas principalmente las relacionadas al proceso de normalización interno, así como en materia de propiedad intelectual, compras gubernamentales e defensa de la competencia.

Actualmente, en relación al proceso de normalización, por ejemplo, el Instituto Nacional de Normalización Americano (*American National Standards Institute* - ANSI), que representa aproximadamente 200 organismos de normalización en Estados Unidos, presenta una política nacional de propiedad intelectual. En términos generales, establece medidas para incentivar la divulgación y el comprometimiento de licenciamiento por parte dos titulares de derechos de patentes esenciales relacionadas a las tecnologías a incorporarse en las normas, antes mismo de su adopción, como condición para a inclusión de tecnologías protegidas en las normas. Estipula aún que los organismos de normalización no se responsabilizan por proveer informaciones sobre las patentes y solicitudes de patentes (*e.g.* identificación, validad jurídica y/o extensión de todas patentes) esenciales para cumplir con una norma técnica. Además, se prevé que el licenciamiento de las tecnologías protegidas deben ser justo y/o razonables y no discriminatorios (F/RAND, en inglés *fair, reasonable and non discriminatory*) (véase WILLINGMYRE, 2008).

Medidas en materia de propiedad intelectual, compras gubernamentales y defensa de la competencia (detalles sobre las medidas en las leyes de defensa de la competencia, véase OMPI, SCP/13/2) también han sido estipuladas, especialmente para tratar de las prácticas abusivas relacionadas al licenciamiento de derechos de propiedad intelectual. En ese sentido, en los casos de abusos han sido aplicadas medidas como licencias obligatorias (o compulsorias), suspensiones y restricciones de derechos (como en el caso de *Dell*, en 1996, y de *Qualcomm*, en 2007 (véase LEMLEY, 2002)).

Medidas similares a esas también han sido adoptadas en otros países como los europeos (véanse CEC COM(92) 445 final) y Japón (véase LEMLEY, 2002).

Distintamente, en China – que é uno de los principales mercados de consumo de tecnologías sujetas a la normalización y, de hecho, fato, uno de los mayores adeptos a las normas técnicas internacionales – ha reformulado sus políticas internas de normalización. Segundo los chinos, las cuestiones relativas a los derechos de propiedad intelectual en la preparación y adopción de normas técnicas internacionales pueden ser un obstáculo para la aplicación de tales normas internamente, perjudicando el comercio e el desarrollo (véase comunicación de China a la OMC en OMC G/TBT/W/251). En ese sentido y, aún, considerando el elevado valor pago en *royalties* para el exterior, debido por el uso de tecnologías extranjeras inseridas en las normas técnicas internacionales adoptadas en el país, el país ha establecido una política de normalización que prefiere la adopción de normas basadas en tecnologías nacionales (DELOITTE, 2004).

Tales medidas aplicadas nacional o regionalmente, sin embargo, aunque solucionen algunas cuestiones relativa a la tensión entre las normas técnicas y los derechos de propiedad intelectual en al ámbito interno, no tienen alcance internacional. Aún la variedad de formas usadas para tratar el problema ha resultado en la adopción de medidas diferenciadas e, por veces, incompatibles entre sí y con las normas y principios do comercio internacional. En ese contexto, la incerteza jurídica que permea la adopción y/o aplicación de normas técnicas internacionales proporciona impactos negativos de grande relevo al comercio internacional, a la transferencia de tecnología y al desarrollo. Frente a eso, algunos organismo de normalización internacionales preocupados con el problema adoptaran políticas de propiedad intelectual internas y siguen perfeccionándolas con el propósito de minimizar los problemas inherentes a tal tensión (ZIBETTI, 2009).

Medidas en el ámbito de organizaciones internacionales de normalización

En el ámbito de las organizaciones internacionales de normalización, especialmente aquellas que tratan de sectores que tienden a ser más intensivos en términos de normalización y concomitantemente de protección de derechos de propiedad intelectual, como es el caso de las telecomunicaciones – la *International Telecommunications Unión* (ITU), la *International Standardization Organization* (ISO) y la *International Electrotechnical Commission* (IEC) – preocupadas con la problemática, adoptaran medidas para tratarla.

En los años 1970, el Comité Consultivo Internacional de Telegrafía e Telefonía (CCITT, actual ITU), reconociendo los potenciales efectos negativos de la tensión, ha empezado a discutir la cuestión internamente y en 1976 adoptó informalmente un "código de prácticas" en materia de patentes involucradas en sus normas, basado en las reglas de procedimiento de ISO e IEC de 1970. En 1987, se ha adoptado una "Declaración sobre Política de Patentes de CCITT" y, finalmente, en 1996, se ha creado oficialmente un código de prácticas en ITU (predecesor de CCITT) (Circular n. 183, A. 29/11, de 1975).

En la actualidad, tales organizaciones (ITU, ISO e IEC – que presentan un importante papel en el comercio internacional en general), con el objetivo de remover los desincentivos a la adopción de sus normas, intensificaran los esfuerzos, inclusive pasando a tratar el tema de manera conjunta. En 2007, adoptaran en común un código de boas prácticas materia de patentes. Aún, siguen profundizando las discusiones cada vez más, incluso en otras áreas de la propiedad intelectual, cómo en derechos de autor, protección de programas de computador y marcas (para más informaciones, véase sitio de ITU).

En el código sobre patentes, las organizaciones recomiendan a los titulares de derechos de patente negociar licencias, gratuitas o no, con las partes interesadas sobre una base no discriminatoria y en términos y condiciones razonables (F/RAND, en ingles). Sin embargo, dejan a cargo de las partes interesadas (titular de derechos y usuarios de las normas) acordar los detalles del licenciamiento, una vez que eses acuerdos pueden variar de caso para caso. Esas negociaciones son realizadas fuera del ámbito de las organizaciones. Con todo, en el caso del titular de una patente no disponerse a cumplir tales condiciones F/RAND, las organizaciones no incluyen disposiciones que dependan de tal patente en la norma. En ese código, aún, la organizaciones incentivan la divulgación adecuada de las informaciones relativas a las patentes inseridas en las normas técnicas, pero no se comprometen a proveer informaciones fidedignas o comprensivas sobre la evidencia, validad o alcance de patentes o derechos similares (ITU/ISO/IEC, 2007).

Aunque tales medidas presenten limitaciones, pues no resuelven todos los problemas inherentes a la tensión entre las normas técnicas internacionales y los derechos de propiedad intelectual (véase ZIBETTI, 2009), muchas cuestiones son solucionadas y otras están siendo a cada día mejor entendidas, lo que posibilita pensar en nuevas medidas para tratarlas. La problemática constituye un desafío que necesita ser enfrentado para que la propiedad intelectual deje de ser un factor de desincentivo para la adopción de las normas técnicas internacionales por parte de los países, en los varios sectores de la industria y comercio.

PROPUESTAS PARA LA NORMALIZACÍON EN EL SECTOR VITIVINICOLA

En el sector vitivinícola destacase el relevante papel de la OIV en el que se refiere a la normalización técnica internacional. Sin embargo, en ese ámbito no se identifica medidas internas en materia de propiedad intelectual relacionadas a las normas de la organización. De hecho, el crecimiento del número de tecnologías protegidas en ese sector, sea por patentes u otros tipos de derechos, es notorio. En ese sentido, se estima importante que medidas relacionadas al tema sean discutidas y adoptadas por OIV y demás organizaciones nacionales de normalización del sector.

Una etapa preliminar constituye en la inclusión del asunto en la pauta de discusiones de las organizaciones de normalización del sector. Ese proceso puede ser facilitado si llevar en consideración la experiencia de otras organizaciones e de países que se dedican a tratar del asunto. Para eso, la elaboración de estudios que consideren tales elementos, asociados a las particularidades del sector vitivinícola, puede consolidar una base para subsidiar los debates de manera estructurada y coordenada.

La principal dificultad en ese escenario está en encontrar el adecuando balance de intereses entre os diversos atores involucrados, tales como: las organizaciones de normalización; el sector privado, especialmente los representantes del sector que participan del proceso de desarrollo de normas técnicas internacionales y nacionales; los titulares de derechos de propiedad intelectual; los usuarios de las normas e los consumidores. En ese aspecto, importa destacar el papel relevante de la contribución de los titulares de derechos de propiedad intelectual en las actividades de normalización, tales como la búsqueda de soluciones innovadoras y tecnologías sostenibles para el uso en normas internacionales.

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Country-of-Origin-Effects des georgischen Weines auf die deutschen Weinkonsumenten: Eine Kausalanalyse

Ghvanidze, S.[1)], Fleuchaus, R.[2)], Theuvsen, L.[3)]
[1)]Georg-August-Universität Göttingen, Hochschule Heilbronn, Studiengang Weinbetriebswirtschaft, Max-Planck-Str. 39, 74081 Heilbronn, Deutschland
Email: ghvanidze@hs-heilbronn.de
[2)]Hochschule Heilbronn, Studiengang Weinbetriebswirtschaft, Max-Planck-Str. 39, 74081 Heilbronn, Deutschland
Email: fleuchaus@hs-heilbronn.de
[3)]Georg-August-Universität Göttingen, Department für Agrarökonomie und Rurale Entwicklung, Platz der Göttinger Sieben 5, 37073 Göttingen, Deutschland
Email: theuvsen@uni-goettingen.de

ABSTRAKT

Im vorgelegten Beitrag wird der Einfluss der wirtschaftlichen und politischen Imagedimensionen Georgiens, sowie das Image des Landes als Weinbau- und Urlaubsland auf die Wahrnehmungen des georgischen Weines untersucht. Die Präferenzen der Verbraucher hinsichtlich der Empfindungen zum Herkunftsland des georgischen Weines beziehen sich auf die Faktoren: Emotion, soziale Akzeptanz, Qualität und Preis. Dabei wurde deren Einfluss auf das Kaufverhalten bzw. Kauf- und Probierbereitschaft für den georgischen Wein ermittelt. Die Einfluss- und Erklärungsgrößen wurden unter Verwendung der Partial-Least-Squares Methode (PLS) analysiert. Die Analysen verdeutlichen die kognitive Struktur und damit die bewertungsmoderierende Funktion des Landesimages. Daraus ableitend kann ein Alleinstellungsmerkmal und ein klares Profil Georgiens für den Aufbau der Marke „Georgien" als Herkunftsland geschaffen werden und damit der georgische Wein auf dem deutschen Markt positioniert werden.

ABSTRACT

The purpose of the study is to identify dimensions of country images of Georgia in terms of benefits sought by consumers for Georgian wines. Four consumer motivational factors quality, price, social acceptance and emotional identify the perception of consumers for Georgian wine. The survey was conducted to determine the impact of Country Image of Georgia on the perceptional drivers of consumer preferences for Georgian wine and to examine the influence of consumers' benefits on the purchase behavior for the Georgian wine. The Partial-Least-Squares (PLS) Method was integrated to examine possible relationships that between the consumer preferences and dimensions of country Image of Georgia. The Study found, that the country's image of Georgia strongly impacts on consumers' thoughts related to wine stemming from Georgia and the consumer motivational factors influence the purchase behavior. The results of the present study will allow developing strategic implications for "Georgia" as "brand" for positioning the Georgian wine on the German market.

1 Einführung

Wein ist das bedeutendste Agrarprodukt für Georgien. Die Weinwirtschaft wird als ein Sektor mit potenziellen Marktchancen angesehen. Nach der russischen Handelssperre 2006 ist die Erschließung des deutschen Marktes für den „Hoffnungsträger" der georgischen Wirtschaft von stetig steigender Bedeutung.

Heutzutage sind Qualitätsweine eher die Regel als die Ausnahme. Qualität reicht jedoch nicht mehr als Differenzierungsmerkmal aus. Aufgrund dieser Tatsache sehen sich die

Weinproduzenten einem gnadenlosen Preiswettbewerb ausgeliefert (Orth et al. 2005). Dies steht in starkem Kontrast zu der Erkenntnis, dass der Großteil der Konsumenten, auch wenn diese gar nicht mit der Marke vertraut sind, ihre Kaufentscheidung eher am Ursprung eines Produktes als am Produkt selbst festmachen (Sheth et al. 1991).

Die archäologischen Funde beweisen, dass die Weinkultur als Faktor sozialen und wirtschaftlichen Lebens ihren Ursprung vor 7000 Jahre v.u.Z. in Georgien hatte (Regelmann, 2003). Aufgrund dieser langjährigen Weinbaukultur Georgiens können sich hilfreiche Ansätze für eine verbesserte Positionierung des georgischen Weins auf dem westeuropäischen Markt auftun. Der Aufbau des Ursprungslandes „Georgien" als „Marke" mit all seinen benefits muss im Fokus stehen, um georgischen Wein auf dem deutschen Weinmarkt besser platzieren zu können.

Um die Ansätze für eine Herkunftsmarke für georgischen Wein entwickeln zu können werden zuerst die Konsumentenwahrnehmung in Bezug auf die Effekte des Landesimages Georgiens ermittelt werden. Anhand einer empirischen Studie soll die Wirkung des Landesimages Georgiens auf die Wahrnehmung der deutschen Weinkonsumenten untersucht werden. Auf dieser Datengrundlage können strategische Implikationen für die Positionierung des georgischen Weins auf dem deutschen Markt abgeleitet werden.

2 Theoretischer Hintergrund

2 .1 Zur Bedeutung von *Country-of-Origin-Effects*: Stand der Forschung

Innerhalb der letzten zwei Jahrzehnte hat der Produktursprung als potenzielle Quelle für den Marktwert eines Produktes besondere Aufmerksamkeit von Forschern und Wissenschaftlern erhalten. Diese sind zu dem Schluss gekommen, dass Konsumenten Informationen zum Ursprung eines Produktes nutzen, um seine Qualität besser einschätzen zu können (Peterson, Jolibert, 1995).

Erkenntnisse aus zahlreichen Studien (Knight, Calantone, 2000; Piron, 2000; Lim, O'cass, 2001; Moon, Jain, 2002) konnten so den Einfluss des Herkunftslandes auf die Wahrnehmung der Konsumenten belegen.

Aufgrund der geringen theoretischen Fundierung (Sauer, 1994) in der *Country-of-Origin* (COO) Forschung hat sich bisher keine einheitliche Definition durchgesetzt (Breu, 2002; Al-Sulaiti, Baker, 1998). Für die vorliegende Arbeit soll COO jedoch als normativen[1], affektiven[2] und kognitiven[3] Einfluss definiert werden. Dieser Effekt entsteht durch die Wahrnehmung eines Produkts und ist fest mit einem Land bzw. *Country Image* verbunden (Breu, 2001). Darüber hinaus beeinflusst dieser Effekt Qualitätseinschätzungen, die entweder einzelne Dimensionen eines Produktes oder das ganze Produkt betreffen und damit die Kaufbereitschaft beeinträchtigen (Breu, 2001; Li et al. 2006). Empirische Studien belegen, dass COO mit Faktoren wie Tradition, politischer Status, Wirtschaftslage und/oder die allgemeine Vorstellung über das Land betreffend (Li et al. 2006; Balestrini, Gamble, 2006) als erhebliche Faktoren im Kaufentscheidungsprozess fungieren können.

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[1]Die Normative Komponente spiegelt soziale und gesellschaftliche Normen wider, die auf das Verhalten der Konsumenten wirken können. Es werden Leistungen aus ethnozentrischen und/oder politisch-moralischen Gründen bezogen (Verlegh, Steinkamp, 1999)

[2] Der affektive Einfluss berücksichtigt die Emotionen und Gefühle, die im Umgang mit einzelnen Merkmalen entstehen. Beispielsweise weckt *Country-of-Origin* die Emotionen im Umgang mit der Leistung (Marke, Garantie) des Produkts (Verlegh, Steinkamp, 1999)

[3] Die kognitive Einflusskomponente stellt den sachlich nüchternen Ablauf des Entscheidungsprozesses dar, indem die Leistung anhand von einzelnen Information-*Cues* (z.B. *Country-of-Origin-Stimulus*) beurteilt wird (Breu 2002).

In der Studie von Veale et al. (2006) hat sich das Herkunftsland des Weines als Indikator für die Qualität des Produktes erwiesen. Die Studien von Lockschin und Rhodus (1993), Tustin und Lockshin (2001), Van Ittersum et al. (2003) und Charters und Pettigrew (2006) unterstützen diese These und sind ebenso der Ansicht, dass das Ursprungsland auf dem Weinmarkt als Indikator für die wahrgenommene Qualität im Kaufentscheidungsprozess gilt. Darüber hinaus zeigen neuere Studien, dass der Wert, den der Konsument mit einem bestimmten Ursprung des Produktes verbindet, über die Qualität hinausgehend auch emotionale Assoziationen einschließt (von Alvensleben, 2000; Sheth, 1991; Van Ittersum, 2003; Kleine et al. 1993). Die Faktoren soziale Akzeptanz, emotionales Empfinden, Wert im Hinblick auf die Umwelt und humanistischer Wert haben sich als die stärksten und signifikantesten Größen hinsichtlich der Konsumentenpräferenzen für Weine unterschiedlichen Ursprungs (aus Australien, Chile, Frankreich, Italien, Kalifornien, Oregon, Spanien, Washington und Neuseeland) erwiesen (Orth et al. 2005).

Im vorgelegten Beitrag werden abgesehen von der Qualitäts- und Preiswahrnehmung auch die soziale Akzeptanz und emotionales Empfinden des georgischen Weines und dessen Wirkung auf das Kaufverhalten untersucht.

2.2 Operationalisierung des *Country Images*

Die Erkenntnisse zahlreicher Forschungsstudien über COO belegen, dass die einzelnen Facetten des Landesimages die Assoziationen hervorrufen, die (spontan) auf das Produkt des jeweiligen Landes übertragen werden (Schweiger, 1990). Empirische Studien belegen auch, dass nationale und weitere *Images* von Regionen oder Länder wirkungsvolle Stereotypen sind, die das Kaufverhalten auf allen Zielmärkten beeinflussen.

Zur Definition des *Country Images* werden die Landes- und Produktdimensionen herangezogen. Nach Kühn (1993) ist zwischen dem *„Made-in"-Image* und dem *„Live-in"-Image* differenziert. Das *„Made-in"-Image* umfasst *Country Image* als Herkunftsort wirtschaftlicher Leistungen und das *„Live-in"-Image* bezieht sich auf den soziokulturellen Lebensraum eines Landes. Unter dem *„Made-in"-Image* werden die in der COO-Forschung häufig untersuchten mehrdimensionalen produktbezogenen Vorstellungen, wie z.B. Haltbarkeit und Wirtschaftlichkeit, subsumiert (Weiss-Richard, 2003). Bei *„Live-in" Image* handelt es sich um allgemeine Vorstellungen, die sich auf die Einwohner, Gesellschaft und Kultur sowie (Wirtschafts-)Politik und wirtschaftliche Situation des betrachteten Landes (Kühn 1993) beziehen. Bei der Definition von *Country Image* von Georgien kann auf die Begriffserklärung von *„Live-In" Image* zurückgegriffen werden. Denn Georgien als Weinbauland, in dem der Wein bereits vor 7000 Jahren vor Christus kultiviert wurde, ist in der deutschen Gesellschaft weitgehend unbekannt. Aus diesem Grund kann der Wein nicht als prägnantes Merkmal für die Globalvorstellung über Georgien angesehen werden. Das *Image* Georgiens bei den deutschen Konsumenten basiert einerseits auf der wirtschaftlichen und politischen Situation des Landes, und anderseits auf der Kultur, Gesellschaft, Geschichte und den Traditionen Georgiens. Für die vorliegende Arbeit ist relevant, inwiefern das *„Live-In" Image* auf das *„Made-In" Image* Georgiens als Herkunftsland des georgischen Weines übertragen wird.

Des Weiteren ist empirisch zu überprüfen, ob das Produktimage vom Image des jeweiligen Landes beeinflusst wird Allgemein werden die Produkte aus Entwicklungsländern mit wenig positivem Image assoziiert (Cordell, Victor, 1992; Belk, Rusell, 1993). Je höher der wirtschaftliche Entwicklungsstand eines Landes ist, umso positiver werden seine Produkte bewertet (Heslop et al. 1993). Anderseits hat das Landesimage basierend auf der politischen Situation eines Landes Auswirkung auf die Produktbewertung. Je „westlicher" bzw.

demokratischer die politischen Verhältnisse eines Landes sind, desto besser werden die Produkte bewertet (Häubl, 1995). In der vorgelegten Studie wird der Einfluss des wirtschaftlichen und politischen Images Georgiens, sowie des Images Georgiens als Weinbau- und Urlaubsland auf die Wahrnehmungen, wie soziale Akzeptanz, emotionales Empfinden, Preis- und Qualitätswahrnehmung des georgischen Weines, untersucht. Da es bisher noch keine Studien zu XX und YY in Bezug auf Georgien gibt, trägt die vorliegende Untersuchung dazu bei, eine Forschungslücke zu schließen. Aus diesem Grund soll an dieser Stelle auf die Innovativität dieses Ansatzes und auf den explorativen Charakter der folgenden Studie verwiesen werden.

3 Konzeptionierung der empirischen Studie

3.1 Studiendesign

An der hier vorgestellten Studie haben 825 Probanden teilgenommen. 395 der eingegangenen online Fragebögen waren vollständig verwertbar. Aufgrund des niedrigen Bekanntheitsgrades des georgischen Weines lag der Schwerpunkt der Online-Umfrage auf den Weinkennern. Deswegen wurde der Link zur Umfrage auf fachbezogenen Webseiten wie www.weinakademie.de, www.Wein-Inside.de, www.wein-plus.de, www.weinundmarkt.de veröffentlicht. Die Umfrage wurde im Zeitraum vom 13.01.2010 bis 15.03.2010 durchgeführt. Zur Erfassung der landes- und produktbezogenen Dimensionen wurden überwiegend 7 stufige Likert-Skalen verwendet. Die Datenanalyse erfolgte mit Hilfe des komponentenbasierten Strukturgleichungsverfahrens (PLS) (Götz, Liehr-Göbers 2004). Die Auswertungen wurden mit dem Programm SmartPLS Version 2.0M3 vorgenommen.

3.2 Modellentwicklung und Hypothesenbildung

Im vorliegenden Beitrag wurden 4 Dimensionen der von deutschen Weinkonsumenten empfundenen Werte des georgischen Weines anhand der PERVAL (*perceived value scale*) Skalen (entwickelt von Sweeney, Soutar 2001) gemessen, und deren Einfluss auf das Kaufverhalten bzw. Kauf- und Probierbereitschaft für den georgischen Wein ermittelt. Aus diesen Skalen gingen folgende Dimensionen hervor: Emotion, soziale Akzeptanz, Qualität und Preis.

Konsumenten mit wenig Produktwissen bzw. geringer Produkterfahrung leiten die Qualität des Produkts anhand des produktbezogenen Landesimages ab. Unter dieser Annahme sollen Interdependenzen zwischen dem Produkt und dem produktbezogenen Landesimage bestehen. Produktbezogenes Image Georgiens wäre das Land als Weinbauland zu bezeichnen, das die Qualitäts- und Preiswahrnehmung, sowie emotionales Empfinden und soziale Akzeptanze beeinflusst. In diesem Zusammenhang wurden folgende Hypothesen aufgestellt:

H1a: Je besser das Image Georgiens als Weinbauland, desto positiver die Qualitätswahrnehmung des georgischen Weines.

H1b: Je besser das Image Georgiens als Weinbauland, desto positiver die Preiswahrnehmung des georgischen Weines.

H1c: Je besser das Image Georgiens als Weinbauland, desto positiver emotionales Empfinden des georgischen Weines.

H1d: Je besser das Image Georgiens als Weinbauland, desto positiver die soziale Akzeptanz des georgischen Weines.

Unter der Berücksichtigung, dass die Herkunftswahrnehmung von differierenden inneren Zuständen des Individuums abhängig ist, sollen auf die etwaigen Zusammenhänge des Landesimages und der Produktwahrnehmung hingewiesen werden. Anderseits wurde der Einfluss des wirtschaftlichen Entwicklungsstands und der politischen Situation eines Landes auf die wahrgenommene Produktbewertung bestätigt (Heslop et al. 1993; Wall et al. 1991; Crawford, Lumpkin, 1993; Häubl, 1995). Daraus leiten sich folgende Annahmen ab:

H2a: Je positiver das wirtschaftliche Image Georgiens, desto positiver die Qualitätswahrnehmung der georgischen Weines.

H2b: Je positiver das wirtschaftliche Image Georgiens, desto positiver die Preiswahrnehmung des georgischen Weines.

H3a: Je positiver das politische Image Georgiens, desto positiver die Qualitätswahrnehmung des georgischen Weines.

H3b: Je positiver das politische Image Georgiens, desto positiver die Preiswahrnehmung des georgischen Weines.

Studien über das Landesimage haben nachgewiesen, dass der Prestigewert bzw. soziale Akzeptanz eines Produktes auch von seinem Herkunftsland beeinflusst werden kann (Han, Tepstra 1988; Dichtl et al. 1983). Das herausragende Landesimage transferiert auf das Image des Produktes kann den Konsumenten die soziale Abhebung von ihrer Umwelt ermöglichen und die Darstellung eines gehobenen Selbstimages erleichtern (Möller, 1997:). Landesimages können durch Konsum bzw. Erwerb bestimmter gruppenkonformer Produkte die Integration in eine bestimmte soziale Gruppe vereinfachen und gleichzeitig die Abgrenzung gegenüber anderen Gruppen unterstützen (Malaka, 1991; Mayer, Mayer 1987). Anderseits erzeugt das Landesimage, welches das Selbstkonzept des Individuums anspricht, positives emotionales Empfinden (de Chernatony, 1993; Ambler, 1997). Folglich lassen sich folgende Hypothesen formulieren:

H2c: Je positiver das wirtschaftliche Image Georgiens, desto positiver die soziale Akzeptanz des georgischen Weines.

H2d: Je positiver das wirtschaftliche Image Georgiens, desto positiver emotionales Empfinden des georgischen Weines.

H3c: Je positiver das politische Image Georgiens, desto positiver die soziale Akzeptanz des georgischen Weines.

H3d:Je positiver das politische Image Georgiens, desto positiver die emotionale Empfindung des georgischen Weines.

Der Wahrnehmungen hinsichtlich eines Herkunftslandes kann unter anderem durch verschiedene länderbezogene Dimensionen, wie Nationalcharakter, Mentalität, Lebensstil, kulturelle und traditionelle Gegebenheiten des Herkunftslandes beeinflusst werden (Schweiger, 1992; Lawrence et al., 1992). Die Determinanten werden unter dem Image Georgiens als Urlaubsland zusammengefasst. Daraus können folgende Annahmen getroffen werden:

H4a: Je positiver das Image Georgiens als Urlaubsland, desto positiver die Qualitätswahrnehmung des georgischen Weines.

H4b: Je positiver das Image Georgiens als Urlaubsland, desto positiver die Preiswahrnehmung des georgischen Weines.

H4c: Je positiver das Image Georgiens als Urlaubsland, desto positiver emotionales Empfinden des georgischen Weines.

H4d: Je positiver das Image Georgiens als Urlaubsland, desto positiver die soziale Akzeptanz des georgischen Weines.

Unter der Berücksichtigung der kognitiven Prozesse im Kaufentscheidungsprozess kann das Kaufverhalten eines Individuums direkt durch gefühlsmäßige Empfindungen, sei es Qualitätswahrnehmungen, landes- bzw. produktbezogene Emotionen und soziale Akzeptanz beeinflusst werden (Kroeber-Riel, 2003; Peter, Olson, 1990; Johansson, Nebenzahl, 1986; Möller, 1997). Die Emotionen eines Individuums können sich somit beispielsweise auf dessen

Einstellungen, Präferenzen, sowie Kauf- und Preisbereitschaften auswirken (Kroeber-Riel 2003; Johansson, Nebenzahl 1987; Papadopolous et al. 1990). So kann die gefühlsmäßige Distanz eines Individuums zu einem bestimmten Herkunftsland (Behrens 1982) das Beurteilungs- und Kaufverhalten gegenüber den Produkten dieses Landes positiv oder negativ beeinflussen (Möller, 1997). Folgende Hypothesen ergeben sich daraus für die weiterführende Forschung:

H5a: Je positiver die Qualitätswahrnehmung des georgischen Weines, desto hoher die Zahlungsbereitschaft für den georgischen Wein.

H5b: Je positiver die Qualitätswahrnehmung des georgischen Weines, desto hoher die Probierbereitschaft für den georgischen Wein.

H6a: Je positiver die Preiswahrnehmung des georgischen Weines, desto hoher die Zahlungsbereitschaft für den georgischen Wein.

H6b: Je positiver die Preiswahrnehmung des georgischen Weines, desto hoher die Probierbereitschaft für den georgischen Wein

H7a: Je positiver die soziale Akzeptanz des georgischen Weines, desto höher die Zahlungsbereitschaft für den georgischen Wein.

H7b: Je positiver die soziale Akzeptanz des georgischen Weines, desto höher die Probierbereitschaft für den georgischen Wein.

H8a: Je positiver emotionales Empfinden des georgischen Weines, desto höher die Zahlungsbereitschaft für den georgischen Wein.

H8b: Je positiver emotionales Empfinden des georgischen Weines, desto höher die Probierbereitschaft für den georgischen Wein.

4 Ergebnisse der empirischen Studie

4.1 Stichprobenbeschreibung

Das Durchschnittsalter der Probanden liegt bei 32,5 Jahren. Insgesamt ist die Altersgruppe der 23-42 Jährigen mit 45,6% etwas überproportional vertreten; Interviewpartner unter 22 Jahren sind mit 3,2% im Sample erfasst. Die weiteren Befragten teilen sich auf die Altersklassen zwischen 23-42 Jährigen (45,6%), 43-62 Jährigen (42,8%) und 63-81 Jährigen (8,4%) auf. Der Bildungsstand ist bei einem Anteil von 72,9% von Personen mit akademischem Hintergrund auf hohem Niveau angesiedelt. 17,5% verfügen über das Abitur und 7,3% über die Mittlere Reife. 1,8% haben die Haupt- bzw. Volksschule besucht. 27% der Stichprobe entfallen auf Frauen. 89,3% der Probanden sind deutscher Staatsangehörigkeit, 2,3% stammen aus Österreich, der Rest verteilt sich auf Rumänien, Spanien, Griechenland, Frankreich, Luxemburg, Belgien und die Schweiz. 8,4% der Befragten waren bereits in Georgien und 39% haben bereits georgischen Wein probiert.

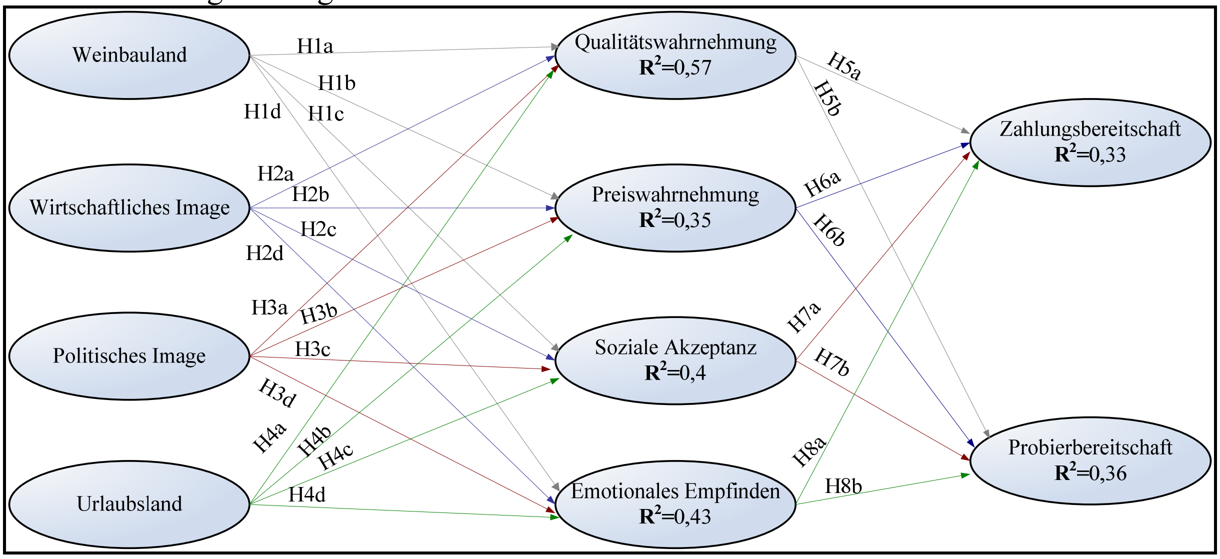
Die Erhebung ist eine Sondierungsstudie, die den Einfluss des Images Georgiens auf die Produktbewertung in der Wahrnehmung der Konsumenten hinsichtlich der Qualität und des Preises sowie des emotionales Empfindens und der sozialen Akzeptanz aufzeigt.

4.2 Überprüfung des Messmodells

Interdependenzen zwischen dem Landesimage Georgiens, dem Konsumverhalten und der Wahrnehmungen des georgischen Weines sowie der Zahlungsbereitschaft und Probierbereitschaft für den georgischen Wein, werden in einem Strukturmodell abgebildet (siehe Abbildung 1). Dabei handelt es sich um theoretische Konstrukte oder auch latente Variablen (LV), die nicht direkt messbar sind. Sie werden durch die Indikatoren bzw. manifesten (beobachtbaren) Variablen gebildet (De Wulf et al., 2001). In der hier vorgelegten Studie wurde eine explorative Faktoranalyse durchgeführt, um die landesimagebezogenen

Dimensionen im Hinblick auf die zugrundeliegende Faktorenstruktur zu untersuchen und um damit eine bessere Übersichtlichkeit und Interpretierbarkeit des Landesimages Georgiens zu gewährleisten. Nach dem Kaiser-Kriterium (Backhaus et al., 2005) konnten vier Faktoren extrahiert werden (vgl. Tabelle 4 [siehe Anhang]).

Abb. 1 Strukturgleichungsmodell



Quelle: Auswertung der eigenen Erhebung

Die zentrale Frage der Untersuchung lautet, welchen Einfluss das Image Georgiens auf die Wahrnehmung des georgischen Weines und hierdurch auf die Zahlungsbereitschaft und Probierbereitschaft für den georgischen Wein hat. Die exogenen LV sind hier wirtschaftliches und politisches Image, sowie Georgien als Weinbau- und Urlaubsland. Emotionales Empfinden, soziale Akzeptanz sowie Qualitäts- und Preiswahrnehmung des georgischen Weines sind die abhängigen (endogenen) LV. Die Zahlung- und Probierbereitschaft sind die endogenen LV. Die Bestimmung der LV erfolgte durch Indikatoren bzw. manifeste Variablen in einem Messmodell und die graphische Darstellung in einem Pfaddiagramm (Ringele, 2004). Statistische Verfahren zur Überprüfung komplexer Kausalitäten werden unter den Begriffen Strukturgleichungsmodelle oder Kausalmodelle erfasst (Wold, 1982). Die Bewertung der Modellgüte erfolgt i.d.R. sequenziell; zuerst wird die Reliabilität der einzelnen Indikatoren und ihrer Konstrukte, sowie der Diskriminanzvalidität bewertet; anschließend wird die Güte des Strukturmodells bestimmt (Hulland, 1999).

Hinsichtlich der Reliabilität (Hair et al. 1998; Fornell, Larcker, 1981) werden Indikatoren im Messmodell (vgl. Tab. 3 [siehe Anhang]) insgesamt als signifikant erachtet, denn ihre Ladung auf den jeweiligen Konstrukten liegen über dem Wert von 0,7.

Im Messmodell wurde ebenfalls das Anspruchsniveau für alle Konstrukte hinsichtlich der *Composite* Reliabilität (Fornell, Lacker, 1981), Konsistenzreliabilität (CRA) (Nunnally, 1978; Bagozzi, Yi, 1988) und der durchschnittlich erfassten Varianz (AVE) (Fornell, Larcker, 1981; Chin, 1998) weitgehend erreicht.

Tab.1: Gütewerte des Messmodells

Konstrukte	**AVE**	**KR**	**CRA**	**R^2**
Weinbauland	1	1	1	
Wirtschaftliches Image	0,89	0,94	0,87	

Politisches Image	0,87	0,93	0,86	
Urlaubsland	0,74	0,92	0,88	
Emotionaler Wert	0,79	0,88	0,74	0,43
Sozialer Wert	0,74	0,93	0,91	0,4
Preisbezogener Wert	0,82	0,9	0,78	0,35
Qualitativer Wert	0,74	0,94	0,93	0,57
Probierbereitschaft	1	1	1	0,36
Zahlungsbereitschaft	1	1	1	0,33

Quelle: Auswertung der eigenen Erhebung

Für eine Beurteilung auf Strukturmodellebene lassen sich das Bestimmtheitsmaß R^2 ermitteln. Bestimmtheitsmaß (Erklärungsbeitrag) gibt die Höhe bzw. den Anteil der erklärten Varianz des latenten Konstruktes wieder. Die Ergebnisse der vorgelegten Studie zeigen, dass der wahrgenommene qualitative Wert zu 52% und der wahrgenommene emotionale Wert zu 43% durch die im Forschungsmodell angenommenen Konstrukte, nämlich durch die Dimensionen des Landesimages Georgiens erklärt werden können. Der Erklärungsbeitrag der dargestellten Wahrnehmungsgrößen kann als moderat erachtet werden (Chin, 1998). Durchschnittlich sind auch die Bestimmtheitsmaße der Probier- und Zahlungsbereitschaft für den georgischen Wein einzustufen. Vor dem Hintergrund des explorativen Charakters dieser Studie stellen diese Werte zufriedenstellende Resultate für das Gesamtmodell dar.

Hypothetische Beziehungen zwischen den latenten Variablen werden durch einen Pfad beschrieben (vgl. Abb. 1) (Götz, Liehr-Gobbers, 2004). Die Reliabilität der Pfadkoeffizienten bezüglich der Schätzrelevanz muss anhand der *Bootstrapping* Methode und einem t-Test festgestellt werden. Pfadkoeffizienten größer 0,1 werden als akzeptabel erachtet (Sellin, 1995). Die Signifikanz der Pfadkoeffizienten wurde in der vorgelegten Analyse durch das *Bootstrapping* Verfahren mit 395 resamples ermittelt. Die Tabelle 2 fasst die Ergebnisse des Strukturmodells zusammen.

Tab.2: Pfadkoeffizienten und t-Werte des Messmodells

H*	**Einfluss von:**	**auf:**	**Pfadkoeffizienten**	**t-Werte**
H1a	Weinbauland	Qualitätswahrnehmung	0,27	2,87**
H1b	Weinbauland	Preiswahrnehmung	0,08	0,63
H1c	Weinbauland	Emotionales Empfinden	0,15	1,45
H1d	Weinbauland	Soziale Akzeptanz	0,12	1,09
H2a	Wirtschaftliches Image	Qualitätswahrnehmung	0,07	0,80
H2b	Wirtschaftliches Image	Preiswahrnehmung	-0,02	0,17
H2c	Wirtschaftliches Image	Emotionales Empfinden	0,17	1,47
H2d	Wirtschaftliches Image	Soziale Akzeptanz	0,34	2,80**
H3a	Politisches Image	Qualitätswahrnehmung	0,17	2,01**
H3b	Politisches Image	Preiswahrnehmung	0,22	1,80
H3c	Politisches Image	Emotionales Empfinden	0,01	0,11
H3d	Politisches Image	Soziale Akzeptanz	0,08	0,79
H4a	Urlaubsland	Qualitätswahrnehmung	0,38	3,37**
H4b	Urlaubsland	Preiswahrnehmung	0,40	2,58**
H4c	Urlaubsland	Emotionales Empfinden	0,42	3,28**

H4d	Urlaubsland	Soziale Akzeptanz	0,21	1,70
H5a	Qualitätswahrnehmung	Zahlungsbereitschaft	0,30	1,55
H5b	Qualitätswahrnehmung	Probierbereitschaft	0,28	1,39
H6a	Preiswahrnehmung	Zahlungsbereitschaft	0,09	0,58
H6b	Preiswahrnehmung	Probierbereitschaft	0,09	0,67
H7a	Soziale Akzeptanz	Zahlungsbereitschaft	0,17	1,20
H7b	Soziale Akzeptanz	Probierbereitschaft	0,12	0,89
H8a	Emotionales Empfinden	Zahlungsbereitschaft	0,07	0,38
H8b	Emotionales Empfinden	Probierbereitschaft	0,17	1,08
*Hypothese, **=signifikant auf 5% - Niveau (t-Wert* 2)				

Quelle: Auswertung der eigenen Erhebung

Das Image Georgiens als Weinbauland mit langjähriger Weinbaukultur weist signifikanten Einfluss auf die Qualitätswahrnehmung des georgischen Weines auf. Die Pfadkoeffizienten bestätigen auch die Wirkung des produktbezogenen Landesimages Georgiens auf die emotionalen und sozialen Empfindungen der Konsumenten. Folglich können die Hypothesen H1a, H1c und H1d bestätigt werden. Ferner zeigt das Strukturmodell, dass der Zusammenhang zwischen der Preiswahrnehmung des georgischen Weines und dem Image Georgiens als traditionelles Weinbauland nicht gegeben ist, wodurch Hypothese H1b nicht bestätigt werden konnte. Der Entwicklungsstand Georgiens nimmt einen erheblichen Einfluss auf die soziale Akzeptanz der Konsumenten und kann ihre emotionalen Empfindungen beeinflussen. Aus diesem Grund können Hypothesen H2c und H2d angenommen werden. Demgegenüber ist die Korrelation zwischen dem wirtschaftlichen Image Georgiens und der Produktbewertung in der preis- und qualitätsbezogenen Hinsicht nicht gegeben. Damit können die entsprechend aufgestellten Hypothesen H2a und H2b widerlegt werden. Im Gegensatz dazu weist die politische bzw. demokratische Situation Georgiens einen signifikanten Einfluss auf die Qualitäts- und Preiswahrnehmungen auf. Allerdings erzeugt die genannte Imagedimension Georgiens weder die positiven Emotionen noch den Prestigewert bei den Konsumenten. Hypothesen H3c und H3d können somit nicht bestätigt werden. Demgegenüber übt Georgien als Kulturnation und attraktives Urlaubsland mit einer interessanten Landschaft und Kultur einen hochsignifikanten Einfluss auf die Wahrnehmungen des georgischen Weines aus. Der stärkste Einfluss des Urlaubslands kommt auf dem emotionalen Empfinden (0,42), gefolgt von der preisbezogenen Empfindung (0,40), der Qualitätswahrnehmung (0,38) und der sozialen Akzeptanz (0,21). Damit können die Hypothesen H4a, H4b, H4c und H4d bestätigt werden. Daneben besitzt die Qualitätswahrnehmung des georgischen Weines einen hochsignifikanten Einfluss auf alle im Strukturmodell dargestellten abhängigen Variablen, die die Kaufabsicht der Konsumenten abbilden. Dadurch werden die a priori postulierten Hypothesen H5a und H5b über die Zusammenhänge der genannten Variable bestätigt. Ferner kann die Annahme über die Beziehung der Preiswahrnehmungen zu dem Konsumverhalten, die sich in der Zahlungs- und Probierbereitschaft im vorgelegten Modell widerspiegelt nicht bestätigt werden. Ebenso müssen die Hypothesen H6a und H6b abgelehnt werden. Emotionales Empfinden der Konsumenten, sowie die soziale Akzeptanz hat eine erhebliche Auswirkung auf das Kauf- und Beurteilungsverhalten des georgischen Weines. Dementsprechend werden die Hypothesen H7a und H7b angenommen. Die emotionale Assoziation in Verbindung mit dem georgischen Wein weist eine moderate Auswirkung auf die Probierbereitschaft auf. Damit wird H8b bestätigt; allerdings wird der Zusammenhang des emotionalen Empfindens zu der Zahlungsbereitschaft der Konsumenten nicht bestätigt. H8a kann somit abgelehnt werden.

4.3 Diskussion

Insgesamt weisen die Ergebnisse auf die kognitive Struktur und damit auf die bewertungsmoderierende Funktion des Landesimages hin. Aus der hier vorgestellten Studie werden folgende Ergebnisse zusammengefasst:

- Das Image Georgiens als Urlaubsland hat einen signifikanten Einfluss sowohl auf die Preis- und Qualitätswahrnehmung als auch auf die emotionalen Empfindungen und soziale Akzeptanz des georgischen Weines.
- Der Entwicklungsstand bzw. die wirtschaftliche Stärke Georgiens beeinflusst die soziale Akzeptanz des georgischen Weines. Im Gegensatz dazu zeigte das wirtschaftliche Image Georgiens keine Auswirkung auf die Preiswahrnehmung auf. Sein Einfluss auf die Qualitätswahrnehmung erwies sich als moderat, was die bisherigen Ergebnisse derStudie von Heslop et al. (1993) widerlegt.
- Der politische Status Georgiens hat keine moderierende Wirkung auf die sozialen und emotionalen Empfindungen des georgischen Weines nachgewiesen. Im Gegensatz dazu hat die genannte Imagedimension eine Interdependenz zu den Qualitäts- und Preiswahrnehmungen, was die Ergebnisse der Studie von Häubl (1995) bestätigt.
- Das produktbezogene Landesimage Georgiens übt den stärksten Einfluss sowohl auf die Preis- und Qualitätswahrnehmung als auch auf die emotionalen Empfindungen und soziale Akzeptanz des georgischen Weines aus.
- Aus der Studie wurde kein Zusammenhang zwischen der Preiswahrnehmung und Zahlungs- und Probierbereitschaft für den georgischen Wein nachgewiesen. Emotionale Empfindungen zeigten ebenfalls keinen Einfluss auf die Zahlungsbereitschaft. Ferner haben sich Qualitätswahrnehmung und die soziale Akzeptanz des georgischen Weines als signifikante Einflussgrößen für das Kaufverhalten für den georgischen Wein erwiesen.

Zusammenfassend lässt sich somit festhalten, dass ein Alleinstellungsmerkmal und ein klares Profil für eine „Herkunftsmarke“ für Georgien durch den Hinweis auf die langjährige Weinbaukultur Georgiens, traditionelle Weinausbaumethoden, sowie die einzigartigen autochtonen Rebsorten geschaffen werden kann. Dabei sollen auf die Eigenschaften des Landes als eine Kulturnation, ein Land mit interessanten Landschaften und einer einzigartigen Natur hingewiesen werden. Die genannten Faktoren haben sich als die stärksten Moderatorvariablen für die Wahrnehmungen des Weines georgischen Ursprungs erwiesen.

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Anhang

Tab.3: Deskriptive Indikatorwerte

Codes	Variables, Statement	r	Ø	σ
	Image Georgiens als Weinbauland*			
GE_04_07	Es ist Weinbauland mit langjähriger Weinbaukultur	1	1	-
	Wirtschaftliches Image Georgiens*			
GE_04_03	Es hat einen hohen Entwicklungsstand	0,95	0,95	0,02
GE_04_04	Es ist wirtschaftlich stark	0,94	0,94	0,03
	Politisches Image Georgiens*			
GE_04_05	Es ist politische stabil	0,93	0,92	0,04
GE_04_06	Seine Staatsform ist freiheitlich und demokratisch	0,94	0,94	0,02
	Image Georgiens als Urlaubsland*			
GE_04_01	Es handelt sich um eine Kulturnation	0,84	0,83	0,06
GE_04_02	Es ist ein attraktives Urlaubsland	0,81	0,81	0,05
GE_04_09	Es hat eine interessante Landschaft und Natur	0,89	0,88	0,04
GE_04_10	Es ist gastfreundlich	0,89	0,88	0,04
	Emotionales Empfinden des georgischen Weines*			
GE_06_04	Georgische Weine bringen mir großen Genuss	0,93	0,93	0,01
GE_06_17	…wecken schöne Erinnerungen in mir	0,85	0,84	0,05
	Soziale Akzeptanz des georgischen Weines*			
GE_06_03	Georgische Weine steigern mein Ansehen bei Freunden und bekannten	0,87	0,86	0,03
GE_06_05	…haben einen guten Ruf	0,89	0,89	0,03
GE_06_06	…liegen im Trend	0,87	0,86	0,04
GE_06_07	…zeichnen mich als Kenner aus	0,82	0,81	0,04
GE_06_09	…sind auch bei meinen Freunden und Bekannten beliebt	0,86	0,85	0,04
	Preiswahrnehmung des georgischen Weines*			
GE_06_01	Georgische Weine haben ein gutes Preis-Leistungsverhältniss	0,93	0,94	0,02
GE_06_13	…sind preiswert	0,88	0,85	0,10
	Qualitativer Wert			
GE_06_10	Qualitätswahrnehmung des georgischen Weines*	0,90	0,89	0,03
GE_06_12	Georgische Weine haben eine große Geschmacksvielfalt	0,84	0,83	0,05
GE_06_08	…sind bekömmlich	0,88	0,87	0,04
GE_06_15	…sind nach den traditionellen Ausbaumethoden hergestellt	0,90	0,89	0,04
GE_06_16	…sind von hoher Qualität	0,83	0,81	0,07
GE_06_18	…sind von gleichbleibender Qualität	0,83	0,83	0,05
	Zahlungsbereitschaft für den georgischen Wein**			
GE_07	Was würden Sie für eine Flasche georgischen Wein ausgeben, auch wenn Sie Ihrem Gefühl folgen?	1,00	1,00	-
	Probierbereitschaft für den georgischen Wein***			
GE_09_01	Wie hoch wäre Ihre Bereitschaft georgischen Wein zu probieren?	1,00	1,00	-

*Skala von 1 = stimme voll zu, 7 = stimme überhaupt nicht zu; ** Skala von 1 = sehr hoch bis 7 = sehr gering;
*** 1 = unter 4,99 EUR 7 = über 30,00 EUR
r= Faktorladung, □ = Mittelwert, σ = Standardabweichung

Quelle: Auswertung der eigenen Erhebung

Tab. 3: Faktorladungsmatrix zur Beurteilung der Landesimagedimensionen Georgiens

	Faktor			
	1	2	3	4
Es hat eine interessante Landschaft und Natur	,800	,070	,058	,222
Es ist gastfreundlich	,754	,094	,042	,130
Es ist ein attraktives Urlaubsland	,548	,085	,333	,119
Es handelt sich um eine Kulturnation	,538	-,039	,183	,371
Es hat einen bedeutenden landwirtschaftlichen Sektor	,468	,288	-,021	,362
Es ist politisch stabil	,009	,812	,321	,019
Seine Staatsform ist freiheitlich und demokratisch	,167	,753	,151	,067
Es hat einen hohen Entwicklungsstand	,242	,334	,763	,073
Es ist wirtschaftlich stark	,020	,551	,655	,008
Es ist ein Weinbauland mit langjähriger Weinbaukultur	,430	,042	,055	,865
Extraktionsmethode: Hauptachsen-Faktorenanalyse. Rotationsmethode: Varimax mit Kaiser-Normalisierung. a. Die Rotation ist in 7 Iterationen konvergiert.				

Quelle: Auswertung der eigenen Erhebung

The expectations of conscious wine consumers[1]

Maurizio Sorbini, Gianluca Macchi
Bologna University, Italy
esorbin@tin.
CERVIM,
Aosta, Italy,
direzione@cervim.it

Overviews
In questa nota sono riportati i risultati di una indagine preliminare sulle aspettative dei consumatori di vino svolta in due periodi (2001 e 2008) con la stessa metodologia in una media città dell'Italia settentrionale.
In questo modo si ritiene di poter fornire un contributo per la conoscenza delle cosiddette "nuove richieste dei consumatori".
Le richieste dei consumatori possono essere valutate solo indirettamente e solo se si considerano le aspettative che il consumatore matura nei confronti del vino. . Il piacere del consumo e le paure connesse con le conseguenze del consumo determinano gli stati d'animo che modellano le aspettative Tali aspettative si trasformano e cambiano i criteri di scelta del vino e concorrono a formare in definitiva le cosiddette richieste del consumatore.
di informazione tecnica per attenuare le paure e riconquistare la confidenza nel consumo. Nell'indagine analizzata è stato possibile isolare i consumatori consapevoli (che riflettono sulle informazioni disponibili sul vino) e ciò ha permesso di riconoscere gli stati d'animo profondi e prevalenti (paura dell'alcool e inquietudine per la produzione iper tecnologica), le conseguenti aspettative
Sono stati infine delineate le aspettative prevalenti che sono state sintetizzate nella ricerca di maggiore naturalità, nella preferenza per sistemi di produzione non ipertecnologici e nella speranza di individuare vini connessi con territori ben riconoscibili e non troppo lontani.
Tali aspettative possono essere considerate come le nuove richieste dei moderni consumatori consapevoli.

In the paper we give the results of a preliminary survey on the expectations of wine-drinkers in an average Northern Italian town over two periods (2001 and 2008) using the same methodology.
In this way we can contribute to identifying the so-called ˝new consumer requirements".
These "consumer requirements" can be assessed only indirectly and only if we take into account the new expectations that consumers are developing towards wine. The pleasure of drinking wine and the fears linked to its consequences create the frames of mind that model the expectations. These expectations change the criteria for choosing a wine and contribute to forming the so-called new consumer requirements.
In the survey it was possible to differentiate conscious consumers (who reflect upon the information available about wine) and consequently to recognize their deep concerns (first of all fear of alcohol and uneasiness about hyper-technological wine production), with the consequent need for information to placate their fears and to rebuild confidence in wine consumption.

[1]Joint authors, G.Macchi was in charge of paragraphs 2 and 3, M.Sorbini paragraphs 1, 4 and 5

Finally, it was possible to identify their main expectations, which can be summarised in the search for more natural, less technological wine, in the hope of finding wine produced in a distinctive, recognisable area not too far from home .
These expectations can be considered basically the “new requirements” of contemporary conscious wine-drinkers.

1.Introduction

The gradual prevalence of conscious wine-drinking over drinking by force of habit makes for interesting dynamics among modern wine-drinkers. Expectations of a wine can no longer be considered as being met by information on production methods and by the complex supply of wine that has become available in the recent past.
Wine-drinkers have become increasingly demanding, their expectations reaching impressively sophisticated and precise levels. However, apart from details on specific aspects regarding national and local situations and features, the sector does not have an overall vision of the basic trends in consumer expectations or of the consequent new requirements for wines.
This paper is aimed at outlining some important associations between consumer expectations and their attitude towards wine. By analysing these associations, we should ascertain the new criteria for choosing wine, over and above the traditional criteria that are still valid.

2.Methodology

This work reports preliminary studies carried out to test consumers’ willingness to take part in a more detailed, thorough survey on wine-drinking among customers of a hypermarket in an average town in Italy, considered to be representative of the behaviour of Italian consumers.
The final aim was to set up a survey plan, the choice of respondents, the type of questionnaire, analytical variables and validity of the results. Since financing was not available for the whole study, only data from the first survey carried out in 2001 have been analysed.[2]
In order to have an idea of how trends are changing, a similar study was repeated in 2008 as part of a survey on various food products carried out partly on the same population (same hypermarket, same average town in Northern Italy, same period and identical survey methods)[3].
The preliminary data, analysed and proposed as giving an indication of consumer expectations, were discussed by a panel of experts (producers, large-scale retail buyers, researchers in the sector) in 2001 and by panels with different members in 2008
In the preliminary survey, shoppers who had bought wine were interviewed (after the checkout) and invited to answer a simplified questionnaire with the possibility of choosing whether to stop or go on to the questions in subsequent paragraphs.
No information was requested on income, profession, origin, family members, drinking outside of the home, packaging preferences, sales point preferences and others considered superfluous for setting up the final methodology, already identified during other previous surveys.
Respondents were divided roughly by age (<30 years old, from 30 to 60, over 60) by the interviewer, without being asked directly.

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[2] Crive Sez Economia Unibo e Unità Economia Alimentare nell’ambito dell’indagine Unibo-Federvini Le strutture del sistema vino italiano cfr M.Sorbini M.,Agosta M. (2004) Les aspects socio-économiques: méthodologie pour le "système vin Italie" (2004) *in* Proceedings (Cr) of OIV GE Analyse des marches et filieres. Paris, 29.03.2004

[3] G. Macchi (2009) *Indagine sui consumi alimentari* DEIAGRA Unibo Rapp Int. Ric final. Reg Veneto/Piave

Information was collected on four days of the week – Tuesday to Friday – both in the morning and afternoon. In 2008 also Monday afternoon was included.
It was estimated that 23% of the hypermarket customers had purchased wine in the 2001 survey week, while in 2008 this figure could not be assessed.

3. Analysis of the preliminary survey

To summarise the analyses and give a complete insight into the trend, the questionnaire will be presented along with the answers from 2001 and 2008.
To limit explanation to topics pertinent to this paper, only subjects concerning expectations are shown (origin, processing, choice criteria and requirements). It should be pointed out that the consumer survey in 2001 (on which no further analyses can be done) recorded the sex of those purchasing wine, without extending the analysis to the single features examined. The 2001 survey showed that 41% of respondents were female, while in 2008 the figure was 48%.

Interest in talking about wine

All customers who were seen to have bought wine (by observing the checkout conveyor belt) were asked if they had time to take part in a social survey and those who accepted were asked if they wanted to talk about wine.

Willingness of customers at the checkout

Despite the inconvenient time (just finished shopping and facing logistical problems) many consumers in the two periods were willing to start talking about wine.

a. Willingness to talk about wine
   2001 (85% out of a total of 508) 2008 (92% out of a total of 267)

Those who refused were not divided into male or female. In 2001 they could be divided by age – 75% over 60 and the remaining 25% under 30. In 2008 all those who refused belonged to the over 60 group.
This could show that there has been an evolution over the years and that young people are less and less afraid of wine and keener to talk about it.

Traditional consumers and conscious consumers

To simplify the preliminary survey, it was assumed that the traditional consumer is one who drinks regularly every day, while the conscious consumer is a person who drinks occasionally and, in any case, not every day.

- b. People who drink every day or occasionally

2001	36%	64%
2008	32%	68%

Most of the people interviewed seemed not to drink wine every day and therefore can be considered "conscious".

Interest in information about wine

All respondents were asked if they were interested in continuing the survey and discussing information about wine, also found on the label.

- c. Interest in talking about wines and information given on the label
  2001 traditional consumers 25% conscious consumers 95%
  2008 traditional consumers 32%...... conscious consumers 91%

The results in the two periods were virtually the same. Most of the conscious consumers accepted to continue talking about wine. Only a small percentage refused because they had little time. The traditional consumers who accepted accounted for only 25% in 2001 and 32% in 2008

Little apparent interest in finding out more about wine

As regards the reasons for not wishing to talk (considering both traditional and conscious consumers together), most people stated that they could not understand the use of having information on the label.

• d. Not interested because	2001	2008
-I haven't time	12%	14%
-I don't know much about wine	6%	3%
-I'm not interested in wine	5%	2%
-I don't know what information there is on the label	10%	9%
-I think the information on the label isn't very useful	67%	72%

In both surveys the main feeling was that *information on the label is useless*.
Whether a result of ignorance or inability to read, the result is the same: a considerable number of consumers do not think the information on the label is helpful.

Associated feelings of conscious consumers

All those who accepted to continue the interview were asked to describe their usual thoughts on a memory of drinking wine, imagining they are a long time between two events of drinking.

Attitudes towards wine-drinking

• e. Thoughts on the wine concentrated on	2001	2008
-the colour of the wine	2%	1%
-the distinctive taste	1%	2%
-the bottle and label	3%	1%
-the cork	1%	2%
-the appreciation of fellow drinkers	5%	2%
-the pairing with the food it accompanied	2%	1%
-the fear of the alcohol content	35%	37%
- the uncertainty about the production technology used	31%	34%
-the sulphur dioxide content	10%	2%
-the origin – from far away countries and unknown wineries	10%	18%

The sensitivity of men and women in 2008

Only during the 2008 survey was it possible to break down the main feelings between men and women.

• f. Distribution of feelings between men and women in 2008		
Fear of the alcohol content	women 66%	men 34%
Uncertainty about the production technology	women 45%	men 55%
Suspicion of wines from unknown countries and wineries	women 48%	men 52%

Fear of alcohol

Again during the 2008 survey it was possible to investigate the reasons behind the fear of alcohol

• Causes of fear of alcohol	2008
Headache after the meal	8%
Drunkenness is socially unacceptable	5%
Knowledge of illnesses it causes	38%
Direct experience of acquaintances who are alcoholics	2%
Fear of accidents and losing driving licence	47%

Concerns about production technology

In 2008 it was also possible to explore the causes of concerns about wine-making technology

• h. Causes of concerns on production technology	2008
-possibility of watering down wine	1%

-possibility of mixing good and bad wine	3%
-use of techniques to correct defects of the wine	23%
-use of chemical additives	62%
-use of machines to break down and remake wine	6%
-use of sulphites	5%

Mistrust of wines from unknown countries and producers

The search for the cause behind suspicion of wines from distant and unknown countries and wineries provided the following reasons.

• i. Causes for mistrust of distant producers	2008
Use of unknown vines	3%
Use of procedures allowed by different laws	21%
Use of different additives from those in Italy	48%
Impossibility of knowing whether the producer is reliable	13%
Size of the winery that exports all over the world	15%

Purchasing preferences

All the people interviewed were then given many purchasing choices, all concerning different features of wines, and asked to state their preferences. Only the most relevant ones are shown here.

Wine and your ideal vineyard

• l. I would choose wine from	2001	2008
an organic vineyard	21%	16%
a family vineyard	64%	72%
a vineyard belonging to a large company	15%	12%

The wine producer

• m. I would choose wine from	2001	2008
a family wine producing business	24%	16%
a medium-sized artisan producer	65%	69%
a large industrial wine-producing cooperative	11%	15%

Wine-making techniques

• n. I would choose a wine produced	2001	2008
with family techniques	15%	8%
with modern artisan techniques	55%	71%
with modern industrial techniques	30%	21%

Technological levels

• o I would choose a wine produced	2001	2008
with chemical processes with additives and preservatives	5%	4%
with only physical processes without additives	76%	83%
with only natural processes	19%	13%

Labels

• p. I would choose a wine	2001	2008
with normal labels	12%	6%
with only descriptive labels	11%	5%
with detailed scientific labels	77%	89%

The alcohol in wine

• Would you choose a wine with an alcohol content	2001	2008
Normal (>12)	32%	27%
Moderate (<12°)	47%	61%
Normal (>12°) but aged in wood	21%	12%

Wine and the WEB

• Would you choose a wine with a label showing	2008
The producer's full address	11%
A description of the vine-growing landscape	15%
Full details of the winery WEBSITE	74%

4. From expectations to consumer requirements

Analysis of the preliminary surveys described in the previous paragraphs (which were just tests to aid preparation of the survey proper) did not detect any novel situations in the two periods. Information from media available well before 2001 and widespread knowledge among experts in the sector had already dealt with issues related to consumer expectations and requirements. The real surprise was the unexpected realization of how many wine-drinkers, even in that random sample without any statistical value, had already developed their own thoughts about wine. It is useful to underline a few points that clearly emerged on the main differences from interpretations by experts in the field.

Information and the division between traditional and conscious wine-drinkers

Since the introduction of the dividing line between habitual and conscious consumers, technical information has been aimed only at conscious consumers, while habitual consumers at most have been given information on what food the wine goes with and on the vine.

Consequently, wineries were advised to use different labels, with just a minimum of information for table wines.

On the contrary, in the survey it became apparent that the majority of habitual wine-drinkers were interested in information. There is no strict discrimination between the drinking habits and desire to know the product of the two groups. It can be concluded that it would be useful to provide technical information, which has always been reserved to high quality wines, also with table wines for everyday drinking. It can be seen that we have to reconsider the exclusion of technical information on wine on the grounds that it is too complicated for the consumer to understand. In fact, what we do not accept is the sad truth that the wine sector has not yet found a common and shared technical language for communicating. (*cf Sorbini ,Agosta ,Pontalti 2006)*

Fear of alcohol

It is not only alcohol that causes fear among consumers (*cf Eurobarometro 2006*). The surveys confirmed the common impressions about people being afraid of alcohol. When consumers think about wine *detachedly from the time of drinking* (in all other situations except enjoyment and even at the time of buying), the fear of alcohol, both for its health and social implications, emerges clearly among their deepest feelings and attitudes connected with wine.

This is the outcome of the century-old campaign against *abuse of alcohol,* which has unfortunately also devastated *normal wine-drinking.*

The *positive effects of moderate wine-drinking* have been excluded and only the terroristic messages about *immediate and future social damage* (the effects of a battle fought with very different media visibility). These messages are implicit also for conscious wine-drinkers.(*cf Folwell 1996)*

All this should make us reflect on the quality and efficacy of media communications on wine, which tend to evoke just the taste of wine, even when attention should be concentrated on other issues to get across with effective *social communication.*

Concerns on the lack of transparency of wine and assymetric information

The survey highlighted growing concerns about poor knowledge of wine-making processes. These concerns are an overflow from other food sectors and are related to the size of the company, hyper-technological processes and the use of additives and preservatives *(cf Matthew 2000, Schmitt 1999).* For wine, however, always considered as being drunk for pleasure, it is essential to have the guarantee of a production process that does not cause even the slightest harm to consumers (*cf Veseth 2008).* This explains the clear uncertainty about the producers' reliability and the tendency to look for wine treated with *mild, non-invasive technologies* in wine-making companies that work *not on an industrial scale but rather as modern artisans*.(*cf Sorbini 2008*)

Preference for Italian wine

The survey noted a latent suspicion towards wine from other countries, as if the winery were less reliable than the Italian producer. This latent mistrust is probably related to the feeling that there are fewer controls than in the Italian system and greater risks related to consumption (*cf Sorbini Costacurta Macchi 2009*) Italian communication could also use this to find features to make domestic wines stand out from the pervasive and homogenous offer from other countries (*cf Aragrande,M.,Macchi G.2008)*

From fears to criteria for choosing

In short, the outcome of the survey is a summary of new consumer requirements.

In choosing a wine, the *frame of mind* is influenced:

- -by insistent *messages about the harm done by alcohol* which induce anxiety and fear,
- -by the feeling of *lack of transparency* generated by imaginative, curt or only descriptive labels that seem to want to keep *information biased* to the disadvantage of consumers,
- -by the instinctive mistrust of industrial techniques, strong additives and other practices for *adjusting* wine
- -by suspicion of the probity of producers from *distant or unfamiliar* regions.

Such fears and concerns mean that consumers *need to regain confidence in the product*.

-their fear of *alcohol and excess of chemicals* induces them to seek *naturalness*,

-their fear of *extreme technologies* urges them to appreciate *craftsmanship,*

-their fear of *distant producers* makes the *origin* become a decisive factor.

To sum up, conscious consumers *choose* if they find the wines have features that can reassure them.

Knowledge and information

For some time now it has been accepted in social food economics that specific knowledge of a food constitutes an effective antidote against fear. Since technical information on processes helps to produce knowledge, it can be deduced that it also serves to rebuild confidence in a product damaged by lack of transparency or by strongly asymmetrical information.

The survey confirmed that the consumers' basic request for information is not new, but becomes a priority when the conscious consumer seeks reasons to be loyal to wine and to its producer.(*cf Aragrande Macchi 2008)*

The relationship of trust between the producer and the consumer is based on the quality and quantity of information. Therefore the *quality of the information* on each wine made available should show the producer's *propensity towards transparency*, the *quantity* of information should testify to his *probity* towards the consumer, the *description of production processes* should provide assurance as to the company's commitment in harmony with the *territory and wine growing and producing tradition.*

5. Conclusions

The simple analysis of a *random sample* of wine-drinkers, aimed at setting up a much wider and more complex study, led to the discovery that wine consumers' expectations and requirements have evolved rapidly over the last decade.

At the same time it highlighted that, as already known, the sector is lagging behind in understanding and communicating.

The widespread awareness that wine is not a staple food but only a drink for pleasure has generated and intensified fear of the consequences of abusing it. The fact that there is a tangible lack of transparency about wine has increased the inevitable concerns about technological wine.

The expectations of more natural products, with production scales that are ideally artisanal, located in well-known areas are becoming criteria for choosing and thus *consumer requirements*.

Precise, reliable technical information emerges as a strategic tool both for attenuating the effect of fear and for regaining consumer confidence.
Consequently, also the quality, quantity and careful management of information can be considered as *new consumer needs*.

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DO CONSUMERS VALUE SUSTAINABLE WINE CLAIMS? AN INTERNATIONAL COMPARAISON

Hervé Remaud[1], Yann Chabin[2], Simone Mueller[3]
[1] Bordeaux Management School and Ehrenberg-Bass Institute
680 Cours de la Libération, 33405 Talence Cedex, France
herve.remaud@bem.edu
[2] Yann Chabin
University of Montpellier 2-CREGOR
Place Eugène Bataillon, 34095 Montpellier cedex 5, France
ychabin@gmail.com
[3] Ehrenberg-Bass Institute / University of South Australia
GPO Box 2471, Adelaide, Australia
simone.mueller@marketingscience.info

ABSTRACT

In the last few months, there hasn't been a single week without an article appearing in one of the various wine magazines and wine news telling the story of a winery or a wine region being engaged in a sustainable programme. At the same time, very little is known about consumers' preferences and acceptance of such claims. We aim to fill this gap by presenting preliminary findings of a cross-cultural research project conducted for the French wine association IGP Pays d'Oc.

A quantitative survey was conducted in five countries (France, UK, Germany, Canada, US) in December 2009 with a total of 11,312 regular wine consumers. Using a discrete choice experiment with visual shelf simulations, we substantiate the relative importance and monetary value of various sustainable wine claims for all consumers on the different markets. Such an approach provides the opportunity to estimate utilities and willingness to pay for each attribute tested and for each market segment. Preliminary findings at the aggregated level indicate that for two countries sustainable claims are valued as highly as French regions of origin and for one country their importance was even higher. Our findings provide wine producers, marketers and policy makers with valuable insights and present the capabilities of a sophisticated and highly valid research method.

RÉSUMÉ

Au cours des derniers mois, il n'a pas été une semaine sans un article dans un magazine / site de vins relatant l'histoire d'une entreprise ou d'une région vitivinicole s'engageant dans un programme dit durable. Dans le même temps, nous connaissons finalement très peu des préférences et acceptante des consommateurs pour des messages durables pour un vin. L'objectif de ce papier est de combler ce manque en présentant les résultats préliminaires d'un projet de recherche conduit pour l'association IGP Pays d'Oc.

Une enquête quantitative a été conduite en décembre 2009 dans cinq pays (France, Angleterre, Allemagne, Canada, Etats-Unis) avec un total de 11312 consommateurs. L'utilisation de la méthode des choix discrets nous permet d'estimer l'importance relative de divers messages durables pour ces consommateurs. Cette approche nous donne l'opportunité d'estimer l'utilité et le consentement à payer pour chaque attribut testé et ce pour chaque segment des marchés considérés. De premiers résultats au niveau agrégé indiquent que pour deux pays, le message durable est tout autant valorisé que la région d'origine, et même plus pour un de ces cinq pays.

INTRODUCTION

Over the last 10 to 15 years, the global wine competitive landscape has changed with the introduction of new world wines. To a great extent, these wines have been successful because consumers perceive them to be easy to drink, to understand, and to purchase. This is particularly true for unique wine concepts such as the Australian Shiraz and more recently the New Zealand Sauvignon Blanc. But competitive pressure and competitor adaptation constantly erodes existing competitive advantages and innovation is required to create new unique selling propositions. Over the last years, wine sustainability grew in popularity and seems to be a message that has potential to gain growing consumer attraction in the future.

Except for organic (at least in France), the first introduction and establishment of sustainable claims for wine were mainly driven by retailers and distributors. They were motivated by a number of reasons such as improving market power against their suppliers, increasing efficiency of their logistic processes and proliferation of a distinct environmental friendly retailer image. This increased supply and store presence of sustainably products increased consumer awareness and created growing consumer demand for eco-friendly food and wine. At this stage for instance the organic wine industry did not grow fast enough to satisfy international consumer demand that in the meantime has slowed down with the economic crisis. While organic is the most successful eco-claim at this stage, it is by no means the only existent sustainable (food) claim. Today one can find wines claiming an eco-friendly practice(s), protecting birds or offsetting carbon emissions and sustainable marketing of wines only seems to be at its beginning.

Today, there is only limited knowledge available to what extent these sustainable claims are valued by consumers and to what degree these claims are in competition or in synergy with other wine attributes and claims. This knowledge is critical for the wine industry to provide those products that maximise consumers' preferences and choice and accordingly shelf listings by retailers. Very few researchers have focused on consumers' preferences and willingness to pay (WTP) for eco-friendly wines and research is limited to a number of countries so far.

Brugarolas Molla-Bauza et al. (2005), using a contingent valuation, reported an average price premium that Spanish consumers are willing to pay for an organic wine that is 16.92% of the average wine price. Barreiro-Hurlé et *al*. (2008) analysed Spanish wine consumers and used a choice experiment with verbal presentation that included five different attributes: origin of the wine (4 levels), production method (conventional versus organic), type of wine (young versus aged), type of grapes (regular versus Resveratol content enhanced), and price (4 levels). Findings indicated that the additional WTP for an organic wine on average was 1.53€. This price premium represents an extra 15% of the maximum price that respondents declared they usually pay for a bottle of wine (10.11€).

Remaud et *al*. (2008) and Mueller and Remaud (2010) utilised graphic shelf simulation choice experiments to analyse Australian wine consumers preferences and choice. Their experiment included four different attributes: region of origin (4 levels), price (4 levels), environmental claims (carbon neutral, environmentally responsible, and no claim), organic claim. Findings indicate that Australian consumers' extra WTP for an organic wine doubled from 2007 to 2009, from A$1.57 to A$3.03. In the meantime, consumer's price premium for an eco-friendly claim remained almost stable for the 'environmentally responsible' wine claim

(A$4.21 and A$4.09) and reduced for the 'carbon neutral' claim (A$1.02 to not significantly different from zero).

All these findings suggest that organic wine is more and more valued by wine consumers in the analysed countries. But the question remains for other sustainable claims and other markets such as the main wine markets in Europe and North America.

Based on a recent research project conducted for the Association des Vins de Pays d'Oc IGP, we provide some answers to this critical question on the willingness to pay for a wider number of sustainable claims in international key markets (France, UK, Germany, US, Canada). At the forefront of the sustainable development practices, the key players of Association des Vins de Pays IGP wanted to better understand wine consumers' preferences and WTP for sustainable claims in the key markets they operate, in order to decide which claims generate the highest value for the consumers when promoting their products.

RESEARCH METHOD

We used a discrete choice experiment (DCE) with visual shelf simulations of product concepts to measure how consumer choice is influenced by a wide array of relevant wine attributes (see Figure 1). In choice experiments respondents are forced to make tradeoffs when choosing from shelf simulations (Louviere, Hensher and Swait, 2000). For food and wine, DCEs have been widely applied (Barreiro-Hurle et *al*.; Lockshin et *al*. 2006; Mtimet and Albisu, 2006; Mueller, Lockshin and Louviere, 2010 and Mueller, Lockshin, Saltman et al. 2010; Teratanavat and Hooker, 2006) and proved to result in predictions that have high validity for real market transactions (Mueller et *al*. 2010). Table 1 presents all attributes included in the experiment, as well as the number of levels for each attribute. This research is unique as it considers a very wide range of attributes for consumer wine choice, thereby ensuring that the measured impact of attributes is not biased by neglecting potentially relevant characteristics.

Table 1: Attributes and levels

#	Attribute	levels	Example
1	Brand	16	Cuvée Mythique, Jeanjean, Arrogant Frog, etc
2	Grape Variety	8	Syrah, Pinot Noir, Chardonnay, etc
3 + 4	Region + sign of quality	8	Pays d'Oc IGP, Bordeaux AOP, Vin de France, etc
5	Alcohol	8	Covering range 10.0% to 14.0%
6	Medal	4	Gold medal, none
7	Price	8	Covering range 3.49€ to 12.24€
8	Sustainability	8	Protect the planet, Carbon Zero, etc
9	Sensory style	4	Fruity and aromatic, complex and well structured
10	Bottling	8	Estate bottled

The *brand* levels were chosen to cover the biggest brands of Pays d'Oc IGP, a few medium-sized brands as well as small brands. Nevertheless, even the biggest brands from the producers of this region are still small brands in any market covered with this study. Including red and white *grape varieties* into the study also allowed the investigation to which extent consumers prefer red or white wine. For red wine, we selected four grape varieties (Syrah, Merlot, Pinot noir, Merlot-Cabernet) and one red wine without indication of the grape variety to represent a blend. Similarly, for white wines, we included two grape varieties (Chardonnay and Sauvignon blanc) and one without indication of the grape variety. The *region* and *sign of quality* were merged into one attribute to reflect realistic combinations. Due to the reform of the EU wine sector, we selected three signs of quality: AOP, IGP and Vin de France. With regards to the region, we selected Pays d'Oc (which was associated with IGP), Languedoc (associated with AOP), Bordeaux (associated with IGP and AOP), and Vin de France (with no reference to a specific region).

The *alcohol levels* were chosen to represent a large range of alcohol content from 10% to 14%. We expected to measure the extent consumers would value wine with lower or medium alcohol content as opposed to strong wines at 14%. We selected the gold *medal* of the annual 'salon de l'agriculture' exhibition in Paris. Because very few wines get this award, we used one level for the gold medal and three levels with no medal claim (to avoid overrepresentation of the model attribute). The *price* levels were chosen to cover the medium and higher priced segment of Pays d'Oc wines, from 3.49€ to 12.24€ in France and Germany, from \$11.90 to \$18.90 in the USA, from C\$11.99 to C\$24.99 in Canada, and from £4.49 to £9.74 in the UK.

The wine *sustainability* levels were chosen to cover claims already available on the market (organic, carbon zero, 10% less glass weight, Protect the planet) and one claim not yet available, namely 'socially responsible'. To avoid overrepresentation of sustainable claims in the design we included three levels with no claims (avoiding having a sustainable claim on each bottle that is presented). The *sensory wine styles* were chosen to cover two main styles: fruity and aromatique, complex and well-structured. The difficulty with that claim was to use claims that could apply to a red and a white wine. To avoid overrepresentation of wine style claims in the design we included two levels with no claims. At last, the *bottling* levels include two claims: 'mise en bouteille à la propriété' and its local translation for each country (estate bottled in the UK/US, im Weingut abgefüllt in Germany). To avoid overrepresentation of bottling claims in the design we included six levels with no claims.

The research survey was designed to simulate a real-life decision-making environment where we asked respondents to imagine themselves at their usual wine store, wanting to purchase a bottle of wine to drink with friends and/or their partner (see Figure 1).

Figure 1: Sample of discrete choice set (French market)

The survey was created as an internet based questionnaire, which has been shown to be superior to traditional offline (paper-and-pencil) method (Sethuraman, Kerin, & Cron, 2005). Accordingly, web-based surveys were found to be an especially suitable enhancement of pictorial elements and result in greater participant attention.

The following table gives a brief overview of all samples of the study. A total of 11,312 wine consumers, recruited via a panel provider, completed the online experiment in December 2009. In order to qualify for the study, respondents were not allowed to work in marketing, market research and in the wine industry, were required to have drunk red or white wines in the last two months, and to have purchased a bottle of red or white wine in the last three months. By extending the period of wine purchase, we wanted to include more occasional / light wine buyers.

Table 2: Samples description (in percentage)

Frequency of consumption	Germany	UK	Canada English	Canada French	France	US Chicago	US New-York
Number of respondents	*2,015*	*2,021*	*1,036*	*982*	*2,027*	*1,614*	*1,617*
≥ once a week	31.7	46.8	36.7	33.6	39.9	42.4	53.9
Once a week	32.4	28.7	29.1	29.8	32.1	30.0	27.5
Once or twice a month	28.1	18.9	25.0	25.1	21.3	21.2	14.0
< once a month	7.8	5.6	9.3	11.5	8.7	6.4	4.7
Total %	100	100	100	100	100	100	100

RESULTS AND DISCUSSION

Analysing how consumers trade off different wine attribute levels in their choices from visual shelves allowed us to estimate the importance attributes have on wine choice. We should remind that these findings relates to the offering of French wines as designed in the experiment.

Table 3 presents the attribute importance on the aggregated (unsegmented) level for each market we analysed. Findings can be read as follows: assuming that the decision to purchase a bottle of wine equals 100% in total, then in market 1 price accounts for about two fifths (40%) and brand for about one fifth (18.8%) of the wine choice decision.

Table 3: (A selection of) Attribute importance[1]

	Market 1	Market 2	Market 3	Market 4	Market 5	Market 6	Market 7
Brand	18,8%	4,8%	4,9%	7,9%	5,6%	8,2%	4,7%
Grape Variety	14,2%	19,9%	9,4%	32,5%	21,2%	41,0%	19,1%
Price	40,5%	65,1%	82,2%	53,6%	64,8%	36,6%	62,5%
Sustainable	3,2%	3,7%	0,4%	0,5%	3,1%	6,1%	3,8%

Not surprisingly, *price* is the most important attribute for consumers (except in market 6) when selecting a wine to purchase to drink with friends and/or the partner. The importance of price varies quite a lot from one market to another: price accounts for 82% of the selection/buying process in the market 3 as opposed to 36.6% in market 6. Except for market 1, *grape variety* is the second most important attribute for consumers when selecting wines. Grape variety is even the most important attribute in market 6, accounting for 41% of the choice process, as opposed to 9.2% in market 3. *Brand* (and brand specific packaging) is not as important as producers might expect (potentially because of the small relative

[1] Countries cannot be identified here and their order is not identical with Table 2.

international size of the brands we used). At most, brands account for 18.8% of the buying process in market 1 (which is quite high), compared to about 5% in markets 7, 2, and 3.

Sustainable claims, which are the key components investigated in this project, have the highest impact in market 6, where with 6.1% of attribute importance, sustainable claims are even more important than the French region in association with a sign of quality (AOP/IGP/Vin de France). On the other hand, sustainable claims are not perceived as important at all for a few markets (3 and 4) at the aggregated level, accounting only for about 0.5% of the choice process.

After this overview regarding the attribute importance over all markets analysed, we would like to focus on one specific market (market 2) to illustrate the relative utility concept as well as consumers' willingness to pay (in €) for specific claims. To preserve the confidentiality of companies involved in this project, we selected three important attributes for this market: price, grape variety and sustainable claims (Figure 2).

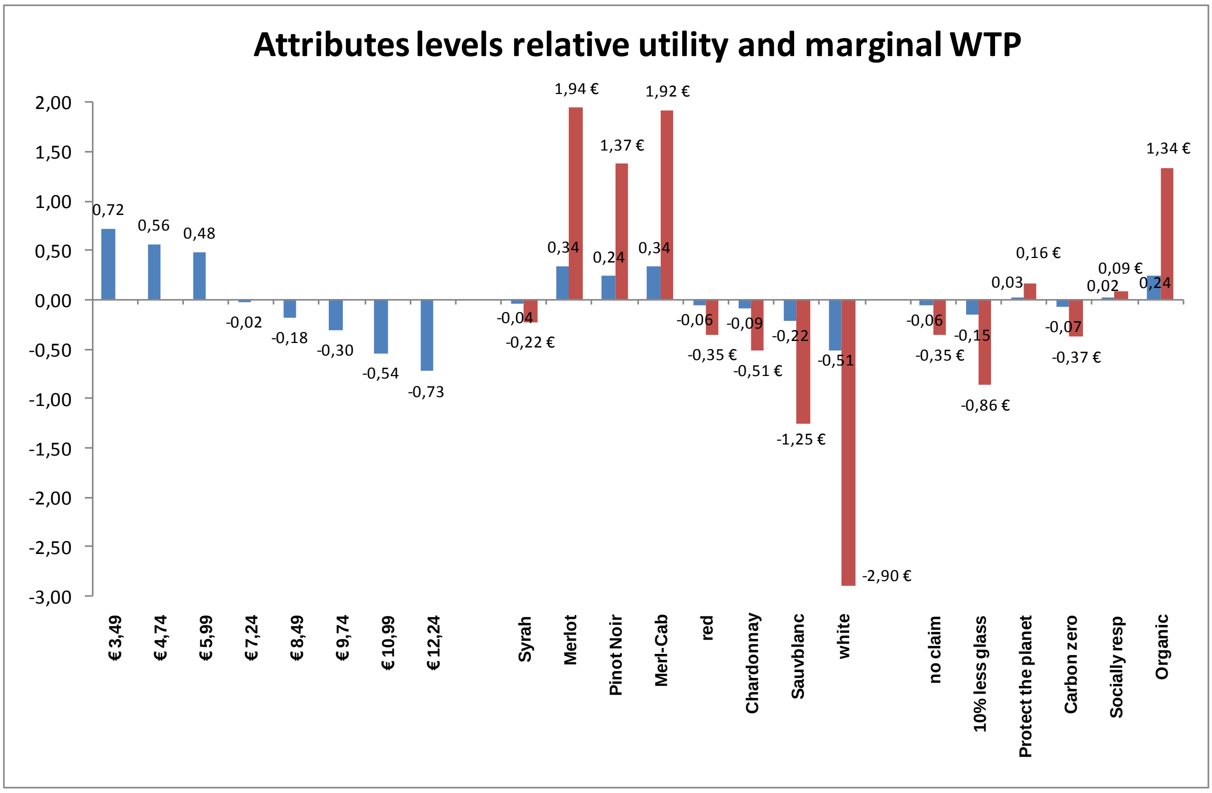

Figure 2: Attribute levels relative utility and marginal WTP

With regards to *price*, one can observe a rational economic reaction towards lower vs. high priced wines in this country. The cheaper the more likely a wine is chosen and consumers value wines that are priced 6€ and below. Wines priced 7.24€ are still acceptable in the sense that its attached relative utility is almost zero (-0.02). Higher prices generate negative utilities that have to be offset by other attribute levels that are very positively valued.

With regards to the *grape varieties*, consumers of market 2 have a greater preference for red wines, that can possibly be related to the timing of the survey (red wines are more strongly demanded over winter than in summer). Specifically for reds, Merlot, Pinot noir and Merlot-Cabernet generate a positive relative utility whereas Syrah and unspecified red wine generate negative utilities. Especially the later finding indicates that wine businesses would gain from mentioning the grape varieties on their label when more than one grape variety is used. The relative preference is lowest for unspecified white wine, generating a negative utility of -0.5. Specific white varieties Chardonnay and Sauvignon Blanc are less preferred than red varieties but Chardonnay is preferred over Sauvignon Blanc on this market

The marginal willingness to pay (WTP) is a direct translation of these utilities combined with the average price elasticity in that market. This marginal WTP should be added to the average price of a bottle of wine in this study ($7.87 with all available wines, corrected by the no-choice constant): about 5.01€. This marginal price premium (or discount) represents an advantage of a wine with this attribute level over wines that don't have it. Translating this advantage into a price value allows a comparison of the advantage over all attributes in one country and within different countries. It should be kept in mind that the choice advantage of a wine is lost once the price is indeed increased by the marginal WTP (the likelihood to choose is then the same as a wine that doesn't have this characteristic but is less expensive). Accordingly a company adding a favourable attribute to its wine can choose to increase its prices (and sell the same units as before) or to leave the price and sell more units.

Among all wines presented, Merlot is the one generating the highest marginal WTP for *grape varieties* (1.94€), followed closely with a blend Merlot-Cabernet (1.92€). Pinot Noir, less preferred by the consumers, generate a lower marginal willingness to pay (1.37€). All other grape varieties with negative utilities generate negative marginal WTP. This means that consumers would purchase a bottle of syrah at 4.79€ (5.01 – 0.22), and a non-specified white wine at 2.11€ (5.01 – 2.90).

With regards to *sustainable claims*, as found in previous studies, organic is one of the three sustainable claims that generates a positive utility (+0.24). Protect the planet and socially responsible are the two other claims generating a small positive utility. On the other hand, carbon zero, 10% less glass, and no claim generate negative utilities; the least preferred being 10% less glass weight. The marginal WTP for a consumer to purchase an organic wine is 1.34€, which is a price premium of about 26.7% for the average price of a bottle of wine of 5.01€. This price premium is very close to the extra cost required to convert a vineyard into an organic one, which account for about 25 to 30% higher costs relative to a conventional wine business. One should keep in mind that our analysis so far only reflects the aggregated level, and segmentation will certainly locate a segment with a higher price premium for organic wine. The marginal WTP for a consumer to purchase a wine that is labelled 'protect the planet' is 0.16€, which is a price premium of about 3.1% for the average price of a bottle of wine of 5.01€. The other sustainable claim that adds value to a wine is socially responsible. This claim does not exist today, and was created (designed) especially for that study. The marginal WTP for a consumer to purchase a wine that is labelled 'socially responsible' is 0.09€, which is a price premium of about 1.8% for the average price for a bottle of wine of 5.01€.

CONCLUSION

Except for two markets (at the aggregated level) where consumers do not perceive sustainable claims as important in the decision process to purchase a bottle of French wine, all other markets do value such claims. In one market, a sustainable claim is even higher valued than the French region of origin associated with a sign of quality and in three other markets, a sustainable claim is as important as origin. The detailed presentation of market 2 gave insights into the relative monetary valuation of sustainable claims by consumers. Not all sustainable claims generate a positive utility. Organic is the one that generates the highest marginal willingness to pay. However, these specific findings only relate to market 2 and differ for other markets. This result indicates that wine businesses would have to consider and analyse markets separately when promoting sustainable claims. At this stage, a number of markets are very promising to promote sustainable wine profitably while others need to mature further to become viable.

ACKNOWLEDGEMENT

This research project was funded by the Association Vins de Pays d'Oc IGP and the European Union. We would like to especially thank Linda Filone, Communication and Promotion Officer at Vins de Pays d'Oc IGP, for her contribution and commitment to the project.

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Integrating Environmental Attributes into Marketing Strategies of Wine Producers

David Kbilashvili, MBA and MA graduate
VIII mk.region; Bulding 11; App 81. Rustavi, Georgia
d.kbilashvili@gmail.com

ABSTRACT 1st LANGUAGE

Due to fast technological advancement, pressures regarding environmental sustainability and social responsibility have become inseparable part of every industry. Wine industry which is based on agricultural products has the direct impact on environment and consequently is involved in the general trend and increased concern of environmental sustainability. Together with high quality, contemporary and future consumers will expect the wine-growing and producing practices to be environmentally sustainable. This trend has the direct influence on the obvious shift of wine producers from corks to alternative twists as the top closures of the bottle. Occurring without actual tree removal, cork harvesting retains wildlife habitat in a uniquely biologically diverse landscape. Consequently important notion exists that fate of many species is tied to that of cork industry. This trend creates interesting relationship among cost reduction, environmental sustainability and customer perception and segmentation. More specifically, while alternative twist tops, other than corks, increase the ease of consuming the product, it decreases the customers' perception of oldness, which represents the important aspect of wine consumers purchasing behaviour. This indicates on the necessity of careful analyses of different segments of the market regarding the preferences of ease of usage or age of wines keeping in mind the environmental sustainability. Consequently the main focus will be made on presenting the findings, which will demonstrate the interesting correlation of above mentioned aspects of viti/viniculture, followed by recommendations on proper integration of environmental attributes in overall marketing strategies of wine producers, as the mean of motivation for maintaining environmental sustainability.

ABSTRACT 2ND LANGUAGE

Aufgrund des raschen technologischen Fortschritts, dem Druck in Bezug auf ökologische Nachhaltigkeit und der sozialen Verantwortung, sind zu einem untrennbaren Teil jeder Branche geworden. Die Weinindustrie, die auf landwirtschaftlichen Erzeugnissen basiert, hat direkte Auswirkungen auf die Umwelt und ist infolgedessen am allgemeinen Trend beteiligt und erhöht das Interesse an der ökologischen Nachhaltigkeit. Gemeinsam mit hoher Qualität, gegenwärtige und zukünftige Verbraucher erwarten, dass der Weinbau und die Weinherstellungspraktiken ökologisch nachhaltig sein werden. Dieser Trend hat direkten Einfluss auf die offensichtliche Umstellung der Weinerzeuger von Korken auf alternative Wendungen wie die Top-Verschlüsse der Flasche. Dadurch dass tatsächlich kein Baum mehr entfernt wird, erhält die Korkernte wildlebenden Tieren eine einzigartige, biologische Landschaft. Folglich kann man die wichtige Schlussfolgerung ziehen, dass das Schicksal vieler Arten mit der Korkindustrie verbunden ist. Dieser Trend schafft interessante Beziehungen zwischen Kostensenkung, ökologischer Nachhaltigkeit sowie Kundenwahrnehmung und Segmentierung. Genauer gesagt während alternative Twist Tops, andere als Korken, die Leichtigkeit des Produktverzehrs erhöhen, verringert es die Wahrnehmung der Kunden für das Alter, die den wichtigen Aspekt des Kaufverhalten des Weines der Verbraucher darstellt. Dies zeigt die Notwendigkeit einer sorgfältigen Analyse der verschiedenen Segmente des Marktes in Bezug auf die Präferenzen der einfachen Nutzung oder das Alter der Weine unter Berücksichtigung der ökologischen Nachhaltigkeit. Daher

wird der Schwerpunkt auf der Präsentation der Ergebnisse, die interessante Korrelation der oben genannten Aspekte der viti / Weinbau, gefolgt von Empfehlungen zur ordnungsgemäßen Einbeziehung von Umweltbelangen im gesamten Marketing-Strategie Prozess der Weinerzeuger, als Motivationsmittel zur Erhaltung der ökologischen Nachhaltigkeit.

Introduction

Gaining and maintaining market positions under increasing level of globalization, puts wine producers in a condition, where they should consider integrating "none-traditional" attributes in the marketing strategies. As the result of increased pace of local or international competition, more and more target markets become saturated. This is backed by the worldwide overproduction of grape and inappropriate usage of available grape varieties. This general characteristic of the industry, contradicts the strengthening trend of preserving environmental sustainability, creating indirect opportunity for wine producers to differentiate themselves from that of competitors. Usage of corporate citizenship as a strategic tool for differentiation requires empirical evidences of actual benefits expressed by higher growth margins and increased number of loyal customers. At the present, there is an obvious dearth of literature concentrated on evaluations of practical positive outcomes for the businesses as the result of being socially responsible corporate citizen. On the other hand, there is a lack of awareness among consumers, about what it really means for a company to operate pro-environmentally. This leads to the situation when wine producers ask themselves how far they need to go to facilitate their products' positioning (Nowak, Washburn, 2002). The research project "Social Responsibility of Business – Challenges and Prospects", implemented by the Centre for Strategic Research and Development of Georgia, indicated the low level of awareness regarding environmental responsibility. Majority of companies assumed that measures regarding protection of the environment should primarily be taken by companies, which produce a harmful impact on the environment in their business operations. These findings create the background for presenting the concrete environmental issue, which could improve the appreciation of corporate social responsibility from both – businessmen and consumer sides – by demonstrating the usefulness of careful analyses of environmental aspects in wine production and deliberate delivery of "green messages" to target markets. The Georgian wine industry will be chosen as the matter of focus, taking into consideration its old traditions of wine-making and current interesting transitional period. The position of Georgian wine industry and the ongoing developments in international wine market strengthen the interest.

Georgian Wine Industry

Level of civilization of the culture can be measured by its viticulture and oenology (Tyler, 2008). Based on the significant number of archaelogical evidences, Georgia is regarded as the birthplace of wine. The word "wine" itself is believed to come from "gvino" - the Georgian word for wine (georgianwinesociety.co.uk, 2008). Representing the most integrated culture of the country, wine production is perceived as much more than just business industry. Possessing 525 different varieties of grapes, out of which only 37 is commercially used, indicates the vast potential of the industry. Historically, being the main source of economic wellbeing of the country, the industry was always involved in political matters. "The first thing the conquerors were doing when invading Georgia, was cutting down vineyards 'grown like a child' (as the folk poem says) by Georgian wine-growers. The enemy believed that they could defeat the country by cutting down the vineyards. But vineyards were replanted and refreshed again and again (Georgian Journal, 2009)".

Russia's ban on Georgian wine can be considered as the modern example of historical experience. The independent French laboratory failed to find any trace of the chemicals based on which the official Russia was announcing the decision (Smith, 2006). Majority of political and even wine experts concluded that Russia's ban on Georgian wine was politically motivated economic blockade. The loss of biggest penetrated market made basic difficulties of Georgian wine industry more obvious. They can be summarised as: Lack of experience in integration of the industry outside the Soviet Union space; Lack of awareness of Georgian viticulture and oenology in other markets than post Soviet Union space; Lack of modern production facilities (Krigbaum, USAID 2006). However, with an active participation of Georgian government in the international promotion of country's wine, the initial vulnerable situation has been overcome and currently Georgian companies are targeting more developed markets with the plans of further modernization of production facilities. The 33rd World Congress of Vine and Wine in Georgia represents clear outcome of ongoing reforms within the industry involving the effective cooperation between private and public sectors.

According to the criteria based on which international wine industry is divided in OW and NW producers and which covers long, uninterrupted history of winemaking, Georgia - known as the cradle of winemaking - can be referred as OW producer. On the other hand, existing reality of current condition prevents the industry from being included in OW producers and puts it as the potential NW producer. Another criterion, describing OW and NW producers as countries within and outside Europe, cannot be referred to Georgia, taking into account its geographical location on the crossroad of Europe and Asia. Above discussed situation can be translated into Initial phase of strategic flexibility, based on the careful observation of intense competition between OW and NW producers.

As the result of graduate research undertaken by the author, aimed to analyse the current competition between OW and NW wine producers, ongoing trends in global wine market and the key factors for success were distinguished. The following trends: - Consolidation of producers, distributors and retailers; The shift in consumer preferences towards high quality wines; Overproduction of grapes; Increasing concern of environmental attributes of wine industry, and increasing focus on emerging markets – were accompanied by such key factors for success as effective network within the industry, between private and public sector; strategic approach to international wine industry; R&D as the basis for new technologies and variety combinations; stringent regulatory and appellation control system; sufficient marketing of available viticulture; attracting the foreign investments; and economies of scale and scope. History and tradition, rich repository of grape varieties (525 different varieties and only 37 studied and used in production), characteristics of health-beneficial microsomas in Georgian grape varieties, pure ecology, high level of involvement of foreign international organizations in the development of Georgian wine industry represent number of competitive advantages. Merging the trends of international wine industry and key factors for success, together with competitive advantages of Georgian wine industry, stand for the crucial direction before concentrating on particular issue, in this case the cork stopper, to ensure the sustainable integration in international wine market.

Cork Industry

Obvious trend in international wine industry covering the shift from corks to alternative twists as the top closures of the bottle can be considered as the opportunity for improving the level of awareness of importance of environmental issues in penetrating the target markets.

Becoming more cautious regarding environmental attributes, represent one of the main characteristics of contemporary wine-consumer. "Consumers want to know what you are doing to make the environment or your community the better place and how it is embedded in the company culture. Consumers are savvy enough to know if you are doing it for company reasons (winebusiness.com, 2009)". Together with high quality, contemporary and future consumers will expect the wine-growing and producing practices to be environmentally sustainable.

Covering around 2.7 million hectares of Portugal, Spain, Algeria, Morocco, Italy, Tunisia and France, cork oak landscapes represent a main source of income for more then 100,000 people and maintain one of the richest biodiversity among forest habitats, including globally endangered species such as Iberian Lynx, the Iberian Imperial Eagle and the Barbary Deer (Rainforest Alliance Report, 2009). Harvested without actual tree removal, cork production belongs to the unique system, balancing the needs of biodiversity conservation and people (earthtrends.wri.org, 2007). The cork is extracted from the thick bark of the oak without damaging the tree. Actually, regular cork stripping prevents a bark from aging and maintains the health of the tree and overall ecosystem of the forests (Non-Wood News, 2009). Water retention, soil conservation, carbon storage, desertification and soil erosion represent other important characteristics of cork industry. Ecological values of cork can be summarised by the following table:

Tab. 1. Ecological Value of Cork

Cork
A natural resource, renewable, biodegradable and recyclable
• **Natural cork is an environmentally-friendly material that completely biodegrades or can be readily recycled without creating any significant secondary waste.** • **After use as a stopper, the cork biodegrades without producing toxic residues or may be recycled into other products.**
High environmental values, low environmental impact
• **1 tonne of natural cork stoppers (357,143 stoppers) comes from 10 ha of *dehesa* or 1.3 ha of dense cork oak forest.** • **To obtain 10t of cork harvesters strip 400 young trees (25 kg/tree/cycle) or 167 mature trees (60 kg/ tree/cycle).** • **Trees don't die after harvest.** • **1 t of virgin cork and 7.3 t of waste cork are produced and used for granulate or cork board for insulating or other uses. None of the cork is wasted. Even the fine particles of cork dust are collected and used as fuel to heat the factory boilers.** • **2t of CO_2 is fixed per 1t of natural cork stoppers** Source: Torres, 2006.

Cork is used for the variety of products, from floor and wall coverings to fishing buoys. In spite of number of segments existing in cork industry, bottle stoppers represents 70% of the total value of the cork market (corkqc.com), emphasising the crucial importance of wine industry in maintaining its economic value, which ensures the sustainability of the unique ecosystem discussed above. WWF (World Wildlife Fund) 2006 report indicates that in spite of the growth in international wine production since 2000, the cork stoppers market has not experienced the same increase, and tendency of decline in production becomes more and more obvious. The report outlines number of factors negatively influencing the cork industry: Strong marketing of alternative closures; General tendency of blaming cork for tainting the wine; Appearance of cheaper wines in global wine market produced for quick consumption; Lack of awareness among consumers regarding importance of a cork industry leading to the confusion in expression of preference and choice.

But the most influential factor, under which above mentioned issues can be summarised, is the intensified global competition, which divided the international wine market into NW and OW, involving fundamental structural changes in production, consumption and trade (Berneti/Casini, 2006). Challenges of current environment of the industry basically include threat of overproduction of grapes; consolidation of forces among producers, distributors and retailers and shift in consumer preferences (Jenster/Cheng, 2008). And those who adapt faster to this reshaping environment are gaining significant competitive advantages. The fast adaptation to the developing environment is perceived by number of international competitors as the signal for integrating alternative top closures to the production practices. Exactly this can be considered as an additional opportunity for Georgian wine industry to differentiate its products from that of competitors.

Material and methodology

The paper concentrated on: Conducting literature review on cork industry developments, searching, collecting and analysing secondary data about environmental attributes involved in wine production. Collecting and analysing information about Georgian wine industry, based on the cooperation with Wine and Vine State department under the Ministry of Agriculture of Georgia. Relevant articles and researches were available based on the access to Cambridge and Anglia Ruskin University libraries, as the master graduate of Anglia Ruskin University, Cambridge. Cooperation with leading Georgian wine-producer companies represents another important part of the paper.

Qualitative research was undertaken, using questionnaire designed for Georgian wine companies. The basic goal of the questionnaire was to obtain information about the impact of the ongoing tendency in cork industry on the leading Georgian wine producers, also to find out the level of awareness among companies regarding the importance of maintaining the cork as the top closure. Consisting of Open-Ended questions, qualitative research concentrated on outlining the future plans of the companies regarding alternative twists. Current presence in international market and potential target markets were important subject of interest as well.

Results and Discussions

According to leading companies of Georgia, cork stoppers needed for wine bottles are imported basically from Portugal and Spain, two major cork exporter countries. This indicates to the direct influence on the ongoing shift occurring in the cork industry. In spite of being 100% importer of cork, the lack of awareness among respondent companies regarding its ecological and economic values was obvious. Therefore majority of the companies were quite tolerant regarding alternative twist tops, referring to the tendency as the result of intensified global competition, which put them in the condition where they should take measures for reducing costs necessary for competing in different segments of saturated international markets. However, leading players of the Georgian wine industry understood the importance of aged wines in the perception of consumers, primary threat of which is top closures other than corks. Representing one of the obvious competitive advantages of Georgian wine, history and tradition stand for the basic barrier for rapid substitution of cork stoppers in local production.

Presence in the international wine market, which is divided in Old World and New World, represented other subject of research. Basic goal of this was to find out how Georgian companies are meeting the tendency of increasing wine consumption in developed and emerging markets. Post-Soviet space, the EU, Asia, the USA represent the basic markets where Georgian companies are exporting their products and trying to get the niche market positions taking into account the low production capacity. According to respondents, Eastern Europe and the USA belong to priority target markets, based on the relatively more obvious increasing rate of wine consumption. General characteristics of contemporary customer, which is becoming more and more environmental-oriented, should be outlined here. With the increasing rate of wine consumption, Georgian companies should take into consideration the increasing environmental concerns, and focusing on the specific environmental attributes involved in cork industry, would create an opportunity for differentiating themselves from competitors.

Being the cradle of wine making is the most important massage that Georgia can deliver to the rest of the world, which will, at least, create the interest of contemporary consumer. If this message is backed by "green" notes based on the cork, the image of the Georgian wine will be strengthened as the product of the industry, which is oriented towards the combination of tradition and innovation in both production and marketing, especially when cork is considered to be the "green" product which itself fits with the current marketing and consumer trends. The basic threat when targeting consumers with pro-environmental marketing strategies, is the underestimation of the worthiness of changing their consumption behaviour based on the benefits to themselves and to society as a hole (Rangan, et al, 1996). This threat is relatively eliminated when referring to the priority segment of the Georgian wine, consisting of uniqueness and age oriented wine consumers, who do not have to change much in their purchasing behaviour. Informing them about coherence between their consumption pattern and environmental attributes would ensure their loyalty and motivate them to promote the same purchasing behaviour to other segments as well, which can be considered as the additional prospects for Georgian companies, target markets of which are determined by available unique grape varieties and limited production capacity.

Conclusion

The purpose of this paper was to emphasise the importance of cherishing environment once again by stakeholders of wine industry. Intensified globalization, resulting in hyper competition, tempts companies to underestimate the consequences of their competitive moves. Obvious shift from cork stoppers to alternative twist closures represent pure example of this. Lack of awareness of importance of cork industry for the unique ecosystem creates inadequate perception of this shift among consumers. Informing contemporary customers about specific environmental issue involved in wine industry fits current marketing and consumer trends, which can be considered as the motivation for companies to formulate pro-environmental marketing strategies.

Ongoing developments in global wine market, which involves obvious trends and key factors for success of OW and NW producers, together with the competitive advantages of Georgian wine industry represented the important subjects of interest. Merging of above mentioned factors was needed to create sound background for concentration on specific environmental attribute and its integration in marketing strategies of wine producers.

Known as the cradle of the winemaking, Georgian wine industry can effectively use “green” messages by demonstrating its pro-environmental approach, especially when the whole volume of the industry’s output is using the cork stoppers. This is backed by consumption behaviour of target consumers, who, despite lack of knowledge, still prefer wines with cork closures, which are associated with quality and age.

Based on the issues discussed in the paper, Georgian wine can gain the competitive place in international market. With proper integration strategy, relevant policy and Strong international promotion, which will make the potential consumers aware of the country with ancient culture of winemaking, providing completely different taste to them, using the unique grape varieties with the pro-environmental production practices, it will be possible to place Georgian wine among the most prestigious wines of the world.

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

GIOVANNI CARGNELLO[1*], LUCIANO PEZZA[1], NICOLA BELFIORE[1], GIUSEPPE GALLO[1], GIANNI TEO[2], STEFANO SCAGGIANTE[2], LISSA VEILLEUX[2], FAUSTO BASSOTTO[3], ROSARIO DI GAETANO[3], SERGIO FORNO[4]

[1*]Vice Presidente GiESCO - Direttore di Ricerca: C.R.A. – Centro di Ricerca per la Viticoltura. Viale XXVIII Aprile, 26 - 31015 Conegliano (Treviso) (I). Ufficio : Tel. +39 04384567.47; Fax +39 043864779; Port. 3496614876; E-mail: giovanni.cargnello@entecra.it;. Personale : tel+39 043862128 ; Port. 3477191342; E-mail: cargnellogiovanni@libero.it
[2]Università di Padova - Campus di Conegliano - Treviso – (I).
[3]Già docente alla Scuola Enologica di Conegliano-Treviso – (I). Libero professionista.
[4]Già collaboratore della SOP di Asti– (I). Libero professionista.

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".

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ENOLOGIA SOSTENIBILE E RESPONSABILITÀ INTEGRATA DELLA FILIERA DELLA VITE E DEL VINO. UN PROGETTO SPERIMENTALE DI MISURA DELLA ACCOUNTABILITY INTEGRATA.

Pierstefano BERTA, Giusi MAINARDI
OICCE - Via Corrado del Monferrato, 9 - 14053 Canelli (ASTI) ITALIA - info@oicce.it
Gruppo di ricerca marketing enologico - Facoltà di Agraria - Università degli studi di Torino
Corso Enotria, 2/c - 12051 Alba (CUNEO) ITALIA

RIASSUNTO

La filiera vitivinicola è un modello chiave per comprendere il ruolo delle piccole imprese ed è fondamentale per la loro sopravvivenza futura. Allo scopo di studiare il processo d'integrazione è stato definito un modello di autovalutazione per analizzare le singole entità aziendali. Questo modello comprende gli aspetti di Governance, Ambientali, Sociali ed Economici, in modo da dare una valutazione globale su indicatori collegati allo sviluppo sostenibile. Permette anche di misurare il processo di avvicinamento ad una situazione ideale, e rappresenta un buon inizio per le aziende che desiderano affrontare lo schema ISO26000.

Utilizzando il modello, si sono intervistate 22 aziende, divise tra vitivinicole, meccaniche e di governance. I risultati sono stati analizzati sia in modo quantitativo sia qualitativo.

Questo studio ha permesso di dimostrare che i principi del Global Compact e la ISO 26000 possono servire come guida per introdurre il concetto di responsabilità sociale nelle attività quotidiane delle PMI della filiera enologica.

The vine and wine network is a key model for understanding the role of small businesses and it is fundamental to their future survival. In order to study the process of integration we developed a self-assessment model to analyze the business entities. Our model incorporates aspects of governance, environmental, social and economic activity, to give an overall assessment on indicators related to sustainable development. It allows measuring the process of coming close to an ideal situation, and represents a good start for companies wishing to approach the CSR reality.

Using the model, we have interviewed 22 companies, between wine, mechanical and governance companies. The results were analyzed in a quantitative and qualitative way.

This study has shown that the principles of the Global Compact and ISO 26000 can be used as a guide to introduce the concept of CSR in the daily activities of small and medium enterprises in the wine industry.

INTRODUZIONE

Una analisi molto rara nel dibattito internazionale è il potenziale legame tra la singola impresa che si propone di raggiungere l'obbiettivo dello sviluppo sostenibile e la rete più ampia della quale fa parte, sia questa costituita da una filiera, da un consorzio di imprese, da cooperative, oppure da reti professionali o sociali.

La filiera vitivinicola è un modello chiave per comprendere il ruolo delle piccole imprese e la filiera è fondamentale per la sopravvivenza futura delle singole organizzazioni.

Il Progetto SEVEN nasce da un'idea del gruppo di lavoro OICCE per lo sviluppo sostenibile.

Si tratta di un progetto dedicato alla sostenibilità sociale, ambientale ed energetica, dalla produzione dell'uva alla distribuzione del vino, condotto attraverso un'analisi della gestione della filiera vitivinicola e della tracciabilità dei prodotti, sperimentando e realizzando nuove tecnologie per la qualità e la sicurezza delle uve e dei vini e nuove tecnologie vitivinicole dedicate alla salute e al benessere del consumatore.

Il progetto si rivolge all'analisi dell'intera filiera vitivinicola, iniziando dal vigneto fino all'imbottigliamento ed al confezionamento dei prodotti, e si ispira al concetto dello sviluppo sostenibile e al Patto Globale delle Nazioni Unite, utilizzati come modelli preventivi per promuovere, realizzare e gestire degli interventi che si intendono realizzare.

Il Progetto SEVEN ha portato alla nascita di alcune attività già avviate per l'anno 2010.

Una di queste attività era la messa a punto di un sistema di autovalutazione che permettesse di verificare l'avanzamento delle singole organizzazioni sulla strada dello sviluppo sostenibile. Questo modello comprende gli aspetti di Governance, Ambientali, Sociali ed Economici, in modo da dare una valutazione globale su indicatori collegati allo sviluppo sostenibile.

Permette anche di misurare il processo di avvicinamento ad una situazione ideale, e può rappresentare un inizio per le aziende che desiderano affrontare lo schema ISO26000.

Si tratta volutamente di un modello generale, in grado di prendere in considerazione le diverse realtà del complesso mondo dell'enologia: laboratori, produttori di macchine, fornitori di servizi, oltre che naturalmente aziende agricole, cantine sociali o imbottigliatori.

MATERIALI E METODI

Si sono predisposte delle schede da somministrare a persone che fanno parte del top management. Agli intervistati si è chiesto di dare un giudizio di conformità tra il comportamento aziendale ed alcuni modelli predisposti, di complessità crescente.

Utilizzando il modello, si sono intervistate 22 aziende, divise tra vitivinicole, meccaniche e di governance. I risultati sono stati analizzati sia in modo quantitativo sia qualitativo.

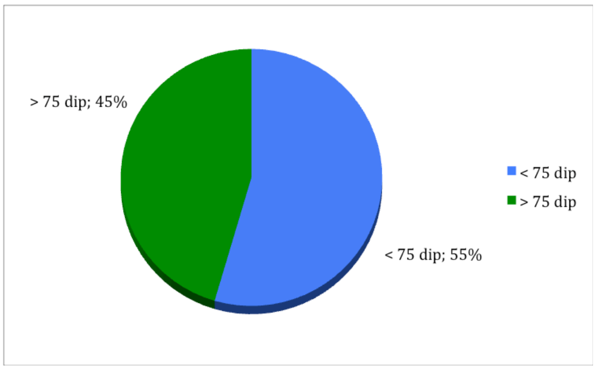
I risultati ottenuti permettono di presentare il livello di coinvolgimento aziendale rispetto a otto aree che corrispondono a punti importanti di un percorso verso lo sviluppo sostenibile:

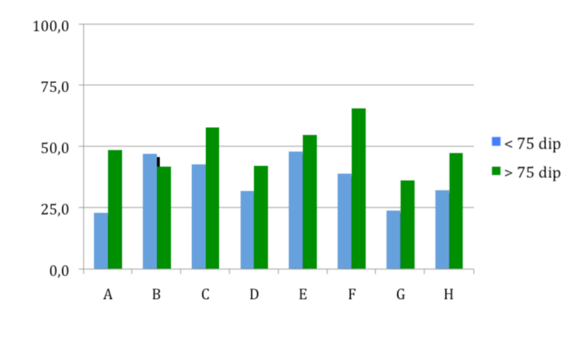
A: La governance del sistema di sviluppo sostenibile.
B: La politica della produzione e il suo impatto sull'ambiente.
C: Gli indicatori di prestazione che portano alla creazione dei risultati.
D: I rapporti con gli stakeholders esterni e il territorio.
E: I rapporti con gli stakeholders interni: il personale.
F: La gestione della leadership e l'organizzazione del lavoro.
G: La ricerca della performance attraverso la metodologia 5S.
H: La ricerca della performance attraverso le metodologie del problem solving.

Ogni area è declinata su domande (da 4 a 6 per area, per un totale di 41).

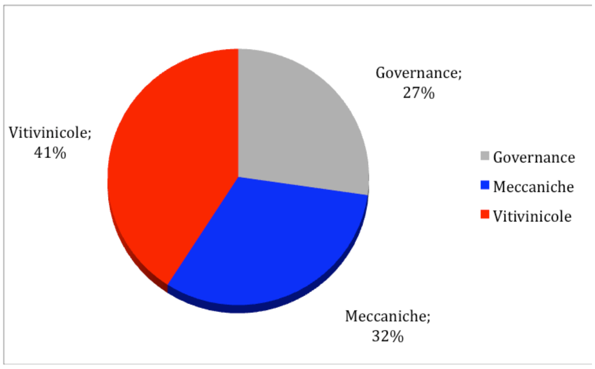
Le singole risposte per ogni domanda sono classificate su uno schema preordinato su 5 livelli. Le risposte ricevono quindi un punteggio, riportato nella scala da 0 a 100 per la creazione delle medie complessive. Questi valori medi sono rappresentati nei grafici successivi. L'insieme dei punteggi dati da tutte le organizzazioni per una certa domanda fornisce la media globale per quella specifica domanda, che assieme alle altre domande costituisce la media globale dell'area. Nell'analisi qualitativa si sono operati due tipi di separazione dei dati, raggruppando in un modo diverso l'insieme delle organizzazioni.

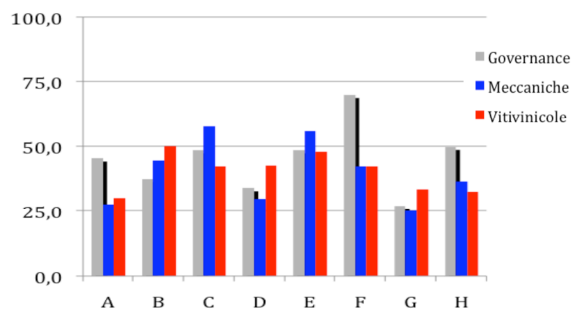
La prima forma di raggruppamento è stata fatta separando le aziende superiori a 75 dipendenti (verde) da quelle inferiori a 75 dipendenti (azzurro). In questo modo è possibile verificare l'influenza della grandezza dell'organizzazione sui processi.





La seconda ripartizione è stata fatta separando le organizzazioni in tre gruppi: organizzazioni di governance (istituzioni, gestione e controllo, etc) (grigio); aziende meccaniche (blu); aziende alimentari (produttori di vino, distillati, liquori, etc) (rosso).





La somministrazione del questionario è semplice. È stata comunque sempre compiuta da certificatori esperti, che oltre a registrare le risposte in modo quantitativo, hanno anche seguito qualitativamente le operazioni, in modo da registrare anche gli elementi non specificamente descritti. I punteggi di una organizzazione o di un gruppo di organizzazioni per una specifica domanda, o un'area, si possono interpretare in questo modo:
punteggio inferiore a 25: l'organizzazione è agli inizi dell'analisi;
punteggio da 25 a 50: si sta sviluppando l'aspetto, che non ha ancora raggiunto una solidità;
punteggio da 50 a 75: si sono messi in atto processi o procedure gestionali solide;
punteggio superiore a 75: esempio di eccellenza in quell'area.

RISULTATI

AREA A: La governance del sistema di sviluppo sostenibile.

La prima serie di domande riguarda il punto fondamentale della gestione aziendale nel suo complesso, cioè l'insieme di regole definita "governance" aziendale, considerata specificamente sotto l'aspetto dello sviluppo sostenibile.

Le domande sulla governance riguardano i seguenti 6 aspetti:

A1 Politica riguardo lo sviluppo sostenibile
A2 Coerenza della politica aziendale con gli standard internazionali
A3 Visione chiara da parte dei dirigenti che si occupano di farne condividere l'impresa

A4 Analisi delle priorità sugli argomenti e soggetti dello sviluppo sostenibile
A5 Processo di presa in conto di buone prassi
A6 Approccio concreto nell'applicazione della politica dello sviluppo sostenibile

L'analisi dell'implementazione dei processi di governance fornisce risultati coerenti con quanto presentato da ricerche precedenti. Le aziende più piccole sono meno propense rispetto a quelle grandi a mettere a punto strategie oggettive e strutturate di responsabilità ambientale e sociale, operando piuttosto tramite processi informali, che dipendono molto da una dinamica personale degli individui coinvolti.

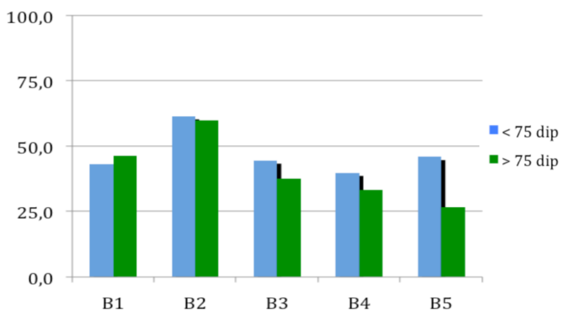
Le organizzazioni che operano nell'ambito del controllo appaiono naturalmente più attive nel definire la politica di governance (Risposte A1, A2, A3). Le aziende meccaniche hanno un elevato livello di presa in conto delle buone prassi di lavoro (Risposta A5).

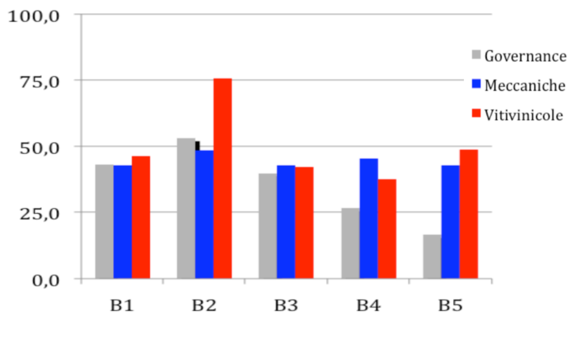
AREA B: La politica della produzione e il suo impatto sull'ambiente.

La seconda serie di domande coinvolge un aspetto di politica "pratica" cioè la gestione della produzione. Lo scopo delle domande è misurare la capacità delle organizzazioni a monitore e governare il processo di produzione, in particolar modo seguendo un'ottica di verifica dell'impatto ambientale.

Le domande sulla politica di produzione riguardavano i seguenti 5 aspetti:

B1 Progettazione dei processi e delle attività
B2 Analisi dei punti critici
B3 Gestione del processo di produzione
B4 Utilizzo dell'energia
B5 Ciclo di vita dei prodotti





La dimensione aziendale non gioca un particolare ruolo in questa area.

I risultati mettono in evidenza un aspetto specifico dei produttori di vino: il fatto che le cantine siano coinvolte nella pratica del metodo HACCP si nota per l'alto punteggio sulle metodologie di determinazione di punti critici.

AREA C: Gli indicatori di prestazione che portano alla creazione dei risultati.

Gli indicatori di prestazione (in inglese Key Performance Indicators o KPI) sono indici che monitorano l'andamento di un processo.

Le domande sugli indicatori di performance riguardavano 5 punti generali:

C1 Uso di audit come strumento di valutazione
C2 Monitoraggio dei Key Performance Indicators
C3 Misura autonoma della performance da parte dei dipendenti
C4 Utilizzo della metotodologia "Visual management"
C5 Gestione delle idee per il miglioramento del sistema

Si nota chiaramente che le aziende di minori dimensioni non riescono a superare la fase di un puro monitoraggio degli indicatori (Risposte C1, C2), e non utilizzano i dati per avviare un vero processo di miglioramento continuo.

Le organizzazioni di governance hanno un approccio più evoluto nella gestione delle idee di miglioramento (Risposta C5). Possono quindi essere il motore per il miglioramento continuo della filiera. Le aziende meccaniche usano specificamente audit e KPI in modo molto diffuso.

AREA D: I rapporti con gli stakeholders esterni e il territorio.

Con il termine stakeholder si individuano i soggetti "portatori di interessi" nei confronti dell'azienda, siano essi interni o esterni.

La serie di domande della IV area riguarda i rapporti tra l’azienda e tutte le interfacce esterne con le quali ha dei rapporti strutturali ed affronta 5 punti:

D1 Individuazione degli stakeholders e delle loro esigenze e aspettative
D2 Processo di identificazione delle sfide sociali
D3 Unione dei criteri economici con i principi dello sviluppo sostenibile
D4 Identificazione con il territorio
D5 Processo di verifica delle sue sfide ambientali

Le piccole e medie imprese operano in modo primario attraverso una rete di relazioni interpersonali e quindi, almeno in teoria, dovrebbe essere chiaro per loro il valore dell’investimento nel sociale. In realtà si vede una maggiore attenzione per gli stakeholders esterni fatta dalle aziende più strutturate (Risposta D2). Una piccola azienda considera più importante correlarsi alla società che la circonda in termini di relazioni B2B e di singoli progetti per la comunità, piuttosto che costruire un complesso rapporto strutturato.

L’identificazione con il territorio è nettamente più forte per organizzazioni di governance e aziende vitivinicole, rispetto alle aziende meccaniche (Risposta D4).

AREA E: I rapporti con gli stakeholders interni: il personale.

La quinta serie di domande riguarda i rapporti con il personale, visto sia da un punto di vista sociale sia da un punto di vista di risorsa produttiva. Riguardano 5 punti:

E1 Gestione delle competenze individuali
E2 Comunicazione interna
E3 Consultazione del personale
E4 Salute e sicurezza
E5 Formazione

L’importanza essenziale per le PMI delle risorse umane è ben noto. Il fatto di non avere un processo strutturato, spinge necessariamente ad avere un forte legame e una grande dipendenza dalle competenze personali dei dipendenti. Le aziende della filiera utilizzano diversi strumenti per consultare il personale, riqualificarlo ed aggiornarlo. Però si nota come ci sia spazio di miglioramento riguardo salute e sicurezza (Risposta E4).

Una particolare attenzione è data dalle aziende meccaniche nelle aree della gestione delle competenze e formazione (Risposte E1, E5).

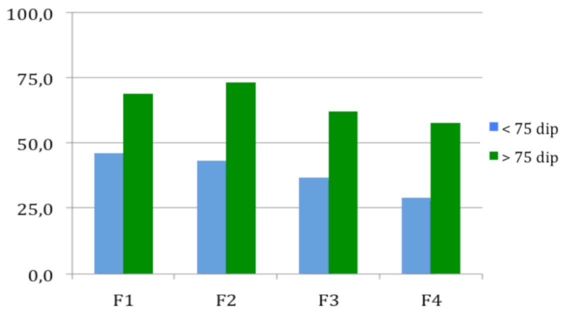
AREA F: La gestione della leadership e l’organizzazione del lavoro.

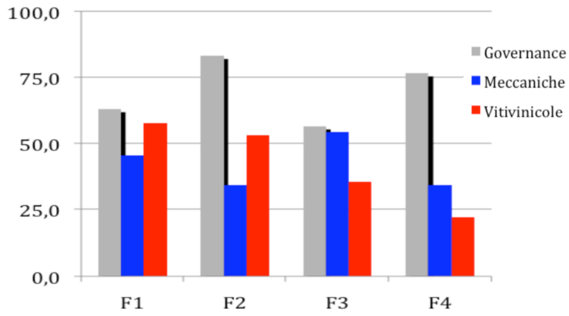
Quest’area analizza la gestione della leadership, cioè come l’organizzazione tiene sotto controllo i suoi processi in senso generale.

Le risposte evidenziano il quadro di rapporti interni in azienda, che si riflettono sull’efficacia operazionale. Le domande sull’organizzazione del lavoro riguardano 4 temi:

F1 Organizzazione delle squadre di lavoro

F2 Ampiezza dell'organizzazione del lavoro in squadre organizzate
F3 Riunioni di squadra e comunicazione
F4 Flusso di comunicazione





In questo caso si nota una stretta correlazione tra l'evoluzione operativa e le dimensioni aziendali, su tutti i punti.
Le strutture di governance appaiono particolarmente attive nella creazione di gruppi di lavoro e nell'attivare un flusso di comunicazione (Risposte F2, F4)

AREA G: La ricerca della performance attraverso la metodologia 5S.
La settima serie di domande mette in luce le metodologie operative rivolte al miglioramento delle performance economiche, attraverso l'utilizzo della metodologia 5S.
Le domande sul metodo 5S riguardano 6 punti:
G1 Gestione e coinvolgimento del personale nel processo 5S
G2 Ampiezza della partecipazione al processo 5S
G3 Descrizione delle aree di lavoro
G4 Employee empowerment
G5 Uso di schede di comunicazione visiva
G6 Progresso nella diffusione del programma 5S
Le dimensioni aziendali hanno una chiara influenza sulla capacità di implementazione della metodologia 5S. Non vi sono invece particolari differenze tra le tipologie di aziende

AREA H: La ricerca della performance attraverso le metodologie del problem solving.
L'ottava e ultima serie di domande riguarda le competenze specifiche nel problem solving.
Le domande sulla metodologia del Problem Solving riguardano 5 punti:
H1 Approccio PDCA
H2 Uso degli strumenti del Problem solving
H3 Utilizzo della metodologia del Problem solving
H4 La routine del Problem solving
H5 Frequenza nella creazione di gruppi di Problem solving
Le dimensioni aziendali sono discriminanti rispetto la capacità di implementazione della metodologia del problem solving. Le aziende meccaniche e di governance mostrano un approccio più evoluto rispetto alle aziende vitivinicole.

DISCUSSIONE E CONCLUSIONI
Il significato profondo del lavoro svolto con il Progetto SEVEN, significato che è coerente con quanto pubblicato in precedenza dai ricercatori che hanno studiato il ruolo delle PMI, è che le aziende più piccole: sono meno propense rispetto a quelle grandi a mettere a punto

strategie di responsabilità ambientale e sociale; operano tramite processi molto più informali; dipendono molto da una dinamica personale del top management coinvolto.

I risultati che sono stati ottenuti hanno messo in evidenza come il risparmio di costi e l'aumento di efficienza siano solo una parte, relativamente marginale, tra le motivazioni che una PMI considera interessanti per andare verso un processo di sviluppo sostenibile. Una piccola azienda considera più importante correlarsi alla società che la circonda in termini di relazioni B2B e di progetti per la comunità.

Uno dei problemi che si sono verificati nel corso dell'analisi è il fatto che le aziende della filiera non si identificano sempre con il vocabolario utilizzato normalmente a livello internazionale. Termini come "CSR", "sviluppo sostenibile", "Global Compact" non sono usati internamente dalle aziende della filiera enologica, e in generale sono poco conosciuti. Al posto si fa piuttosto riferimento ad altri termini, che però non corrispondono al linguaggio internazionale dello sviluppo sostenibile.

Le PMI che sono collegate in rete riescono ad avere una capacità enormemente maggiore di cogliere e rispondere alle sfide della responsabilità sociale rispetto alle aziende isolate da un contesto. Il fatto di formalizzare e strutturare la rete ha permesso immediatamente di far nascere sinergie altrimenti molto difficili. Una rete di imprese come quella costituita dal Progetto SEVEN si è dimostrata un buon incubatore di progetti collegati alla Responsabiltà Sociale delle piccole e medie imprese.

È ben documentato il fatto che le PMI soffrono di una costante mancanza di tempo per affrontare questioni che stanno al di là delle loro preoccupazioni operative quotidiane. Per esempio Oshani Perera (2008) ha messo chiaramente in evidenza il problema della mancanza di risorse discrezionali nelle PMI. La disponibilità di risorse discrezionali consente alle aziende di pianificare in anticipo, di innovare i prodotti anche senza attendersi un immediato ritorno economico e sperimentare miglioramenti sulle produzioni in corso.

Le PMI in genere si focalizzano sulle scelte immediate e sono difficilmente in grado di dedicare tempo alle opportunità che si pongono al di là dell'orizzonte immediato degli eventi. Diverse tra le organizzazioni coinvolte nel Progetto SEVEN hanno presentato la mancanza di tempo come uno dei principali ostacoli per il miglioramento delle prestazioni nell'ambito della responsabilità sociale.

Strettamente collegata alla mancanza di tempo, la mancanza di sufficienti competenze interne è un altro grande ostacolo per le PMI. In altre parole, non è tanto importante il tempo realmente impiegato nell'attuazione di una determinata pratica sociale, ma piuttosto il tempo speso per capire che cosa si deve fare.

Le aziende della filiera condividono in modo generale il concetto che le buone pratiche ambientali potrebbero ridurre l'utilizzo di risorse, diminuire i rifiuti e quindi permettere alle aziende di realizzare risparmi notevoli.

Tuttavia, sono poche le piccole e medie imprese che analizzano in modo oggettivo i dati in loro possesso e che possono quindi fornire dettagli sul loro modo di operare. Ciò indica che le PMI, anche quando sono impegnate attivamente nell'ambito della responsabilità sociale, non hanno il tempo o la possibilità di capire esattamente come le attività di responsabilità sociale possano contribuire da un punto di vista economico alla loro attività. Questi dati confermano l'ipotesi che le PMI per la maggior parte si impegnino in un formale rispetto dei principi, piuttosto che incorporare le prestazioni sociali e ambientali nelle attività quotidiane.

La nostra analisi è stata in grado di confermare come le PMI più grandi abbiano una maggiore probabilità di ottenere miglioramenti commerciali e finanziari, attraverso una politica strutturata di responsabilità sociale, piuttosto che le piccole imprese o le microimprese. Le aziende più grandi si sono anche dimostrate meglio informate e più proattive durante le interviste.

È interessante notare il ruolo del rapporto B2B nello sviluppo interno dei concetti collegati alla responsabilità sociale. Le aziende inserite attivamente nella filiera, a causa del coinvolgimento congiunto con i loro clienti ed i loro fornitori, diventano partecipi anche delle necessità ambientali e sociali complessive.

Questo studio ha permesso di dimostrare che i principi del Global Compact e la ISO26000 possono servire come guida per introdurre il concetto di responsabilità sociale nelle attività quotidiane delle PMI della filiera enologica.

Ha anche permesso di evidenziare come al momento non tutte le aziende siano pronte ad unirsi al dibattito internazionale sullo sviluppo sostenibile. Ci si può aspettare una maggiore attenzione dalle aziende rivolte all'esportazione, da quelle collegate a reti internazionali, e da quelle fortemente collegate ad una filiera di produzione, e soprattutto dalle aziende guidate da persone che sono consapevoli dell'urgenza di mettere in atto delle procedure per lo sviluppo sostenibile.

Questo studio indica anche che le pratiche di responsabilità sociale delle aziende possono passare da una pura conformità formale ad un approccio proattivo, se si mettono in evidenza i vantaggi commerciali e se questi sono realmente compresi da un management dotato di acume commerciale, che capisce la necessità di mantenere una posizione sul mercato internazionale e di differenziarsi dai concorrenti.

Per rimanere competitive, le filiere in generale, e in particolare quella enologica, sono sempre più destinate a modificare il loro punto di vista, abbandonando una pura visione di costi e conformità di prodotto verso una più ampia visione del rispetto delle necessità della intera filiera. Le norme ambientali e sociali molto probabilmente porteranno al consolidamento delle catene di approvvigionamento e contribuiranno a ridurre il numero di fornitori in grado di partecipare alle filiere. Fornitori, che non saranno in grado di rispettare le specifiche ambientali e sociali, e contemporaneamente non saranno in grado di avere costi corretti di produzione, saranno lasciati fuori. Le aziende che invece potranno rispondere a queste richieste si uniranno e collaboreranno tra loro, contribuendo così a creare filiere solide che avranno una continuità nel tempo.

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TRADICION Y CALIDAD DEL VINO EN EL MARCO DEL DESARROLLO SUSTENTABLE. PARADIGMAS EN EL URUGUAY.

E.de Frutos[1], S. Schaffrath [2] C. Altezor[3], A. Berreta[4] M. Terzano[5]

[1] Ingeniera Agrónoma Enóloga Instituto Nacional de Vitivinicultura
Montevideo, Uruguay

[2] Licenciado en Economía, Universidad ORT
Montevideo, Uruguay

[3] Arquitecto Investigador del Patrimonio Histórico Natural
Montevideo, Uruguay

[4] Historiador, CEIL, Universidad de la República
Montevideo, Uruguay

[5] Directora de Arquitectura de la Com. Nac. del Patrimonio Cultural, MEC
Montevideo, Uruguay

ABSTRACT

Compared to other sectors, the industry of wine production in Uruguay shows a lower profitability which has caused in recent times a slower development and induced, in response, a moderate process of concentration. Nevertheless, the industry of wine production makes relatively more important social contributions mainly through its compensation concept. Additionally, the consumer habits inherited by the important migration processes still secure high levels of individual consumption. For its part, the Uruguayan Government has passed the Law of Historical y Cultural Heritage (since 1971) and is currently analyzing a new bill which considers comprehensively the concept of current sustainability. As an illustrative example, all three wineries declared “Historical National Monuments” have been included in the analysis.

RESUMEN

La vitivinicultura de Uruguay presenta, en términos comparativos, una rentabilidad menor a la observada en otros sectores, hecho que propendió en los últimos tiempos a un desarrollo más lento e incentivó como respuesta un moderado proceso de concentración. No obstante, la vitivinicultura hace aportes sociales comparativamente más profundos, principalmente a través del concepto remuneraciones. Asimismo, los hábitos de consumo heredados de los importantes procesos inmigratorios todavía aseguran que el consumo unitario se mantenga en niveles elevados. Por su parte, el Estado uruguayo ha promovido la Ley de Patrimonio Histórico y Cultural (en vigencia desde 1971), así como actualmente se encuentra en estudio un nuevo proyecto de ley, cuyo espíritu contempla cabalmente el concepto de sostenibilidad actual. A modo de ejemplo se incorporan las tres bodegas declaradas “Monumento Histórico de la Nación”.

Una vista a la economía del país

Uruguay es un pequeño país (180.000 Km^2 y 3,5 millones de personas) ubicado al sur de América del Sur y comparte fronteras con dos “gigantes”, Argentina y Brasil. Es un país con amplia influencia europea (a través de los significativos procesos inmigratorios observados a lo largo de su historia) y por tanto mantiene ciertos rasgos de consumo asimilables a los europeos.

Uruguay y sus principales poblaciones

Fuente: CIA, World Factbook

Entre los años 2002 y 2009 Uruguay logró crecer a una tasa promedio anual de 5,2%, así como fue capaz de capturar importantes procesos de flujos de inversión internacional que permitieron reducir significativamente el desempleo y aumentar el empleo (ambos ubicándose actualmente en niveles históricamente positivos, 7,2% y 58,5% respectivamente). Asimismo, Uruguay es, a pesar de ser un país en vías de desarrollo, una nación de ingreso medio (USD 13.163 por año por habitante) y se ubica en la tercera posición de los países con mayor ingreso per cápita del Cono Sur, únicamente detrás de Argentina y Chile.

Desde el punto de vista productivo, Uruguay es un país eminentemente agroexportador y altamente especializado en el desarrollo de su cadena agroindustrial. A su vez, el rubro turismo también es de gran importancia. Es necesario que cualquier diseño de estrategia de desarrollo sostenible tenga en cuenta las posibles sinergias entre ambos sectores. Un aspecto relevante es que el país destina prácticamente un 84% de su territorio a uso agrícola. En línea con lo anterior, cerca de un cuarto del valor agregado es aportado por las actividades primarias y el complejo industrial (que como se dijo previamente está altamente ligado al primero). En particular, el valor agregado por los productos frutícolas (entre los que se encuentra la vid) y la industria de la bebida generaron 3 de cada 100 unidades de ingreso en el 2009.

Surge de la última Encuesta de Actividad Económica (con datos al 2007) que la industria alimenticia produce directamente cerca de un quinto del valor bruto de producción nacional (sin contar el sector primario) y ocupa cerca de 42.000 personas, lo que representa más del 10% de la mano de obra ocupada en el 2007.

Valor Bruto de Producción, Valor Agregado Bruto, Remuneraciones (miles USD) y Puestos Ocupados (datos al 2007)[1]

Sector CIIU R3	VBP	VAB	Remuneraciones	Puestos de Trabajo
TOTAL - A,B,C	20.552.885	8.406.930	3.243.482	384.438
D	10.050.553	3.017.021	852.903	100.576
D 15	4.186.819	1.120.780	307.922	41.745
D 155	408.784	195.660	37.594	2.269
D 1552	35.470	10.150	3.614	705

Fuente: INE, Encuesta de Actividad Económica 2007

Participación del Subconjunto
(datos al 2007)

Sector CIIU R3	VBP	VAB	emuneracione	Puestos de Trabajo
D (% Total - A,B,C)	48,9%	35,9%	26,3%	26,2%
D 15 (% Total - A,B,C)	20,4%	13,3%	9,5%	10,9%
D 15 (%D)	41,7%	37,1%	36,1%	41,5%
D 155 (% D 15)	9,8%	17,5%	12,2%	5,4%
D 1552 (% D 155)	8,7%	5,2%	9,6%	31,1%

Fuente: INE, Encuesta de Actividad Económica 2007

El cuadro siguiente muestra como se distribuyó el ingreso obtenido por cada agrupación de interés. En promedio, los sectores secundarios y terciarios gastan i) cerca de un 59% de sus ingresos en adquirir insumos, ii) 2% en impuestos (netos de subsidios directos e indirectos), iii) 5% en adquirir nuevo capital fijo, iv) 16% en remuneraciones a la mano de obra y v) 18% en remuneraciones al capital (excedente de explotación). Sin embargo, la industria elaboradora de alimentos gasta 23 puntos porcentuales más que el agregado en insumos y 8 puntos porcentuales menos en remuneraciones.

El cuadro siguiente muestra que tanto la industria de alimentos y bebida (D 15) y la rama "Elaboración de Vinos (D 1552)" destinan siete de cada diez unidades de ingreso al pago de insumos, por lo que sólo tres corresponden a valor agregado. Sin embargo, esta situación no se da en el caso del resto de la industria elaboradora de bebidas, dónde prácticamente la mitad de los ingresos representan ganancias para alguno de los agentes involucrados. Otro elemento que se destaca es la pequeña porción de ingresos que logra capturar el capital (aproximada a través del excedente de explotación) en la industria elaboradora de vinos (12,9% vs. 18,5%, en bodegas y a nivel agregado respectivamente). A pesar de ello, el monto que capturan las remuneraciones es significativamente más alto que el que captan en la industria agregada (10,2% vs. 8,5%). Este hecho, junto con el anterior, sugiere que el sector incide positivamente en la distribución de la riqueza. Esto es, en tanto los dueños del factor capital acceden a una remuneración menor a la promedio y el factor trabajo es remunerado por encima del promedio, la brecha existente entre los dos se ve reducida.

[1] *"Total – A,B,C"* refiere a todas las ramas de la actividad económica, excluyendo el sector agropecuario, la pesca y la explotación de minas y canteras. *"D"* incluye todas las ramas dentro de la industria manufacturera. La rama *"15"* se refiere a las elaboradoras de alimentos y bebidas en especial, mientras que la *"155"* incluye únicamente la industria elaboradora de bebidas. Finalmente, la rama "1552" es la rama específica de la industria vitivinícola. Sin embargo, debe tenerse en cuenta que las uvas que son procesadas en el lugar se incluyen en el capítulo *"A"* y no en la rama *"D 1552"*.

Distribución del Valor Bruto de Producción generado
(datos al 2007)

Sector CIIU R3	Insumos	Imp Netos	Capital Fijo	Remuneraciones	Exc. Expl.
TOTAL - ABC	59,1%	2,1%	4,6%	15,8%	18,5%
D	70,0%	3,6%	2,5%	8,5%	15,4%
15	73,2%	0,2%	2,1%	7,4%	17,2%
155 - 1552	52,1%	13,2%	4,7%	9,2%	20,7%
1552	71,4%	0,6%	4,9%	10,2%	12,9%

Fuente: INE, Encuesta de Actividad Económica 2007

La vitivinicultura en el marco del análisis económico

La siguiente tabla ubica el sector vitivinícola uruguayo en el mundo. En particular se destaca que los indicadores “en niveles” muestran que Uruguay no es, ni remotamente, un actor preponderante del mercado internacional. Sin embargo, una vez se calculan indicadores “relativos” se observa que el país se encuentra en una posición relativamente privilegiada e incluso muestra estándares de consumo similares a los de los principales actores de este mercado.

Hectáreas plantadas, Producción de Uvas y Producción y Consumo de Vino
(datos al 2008)

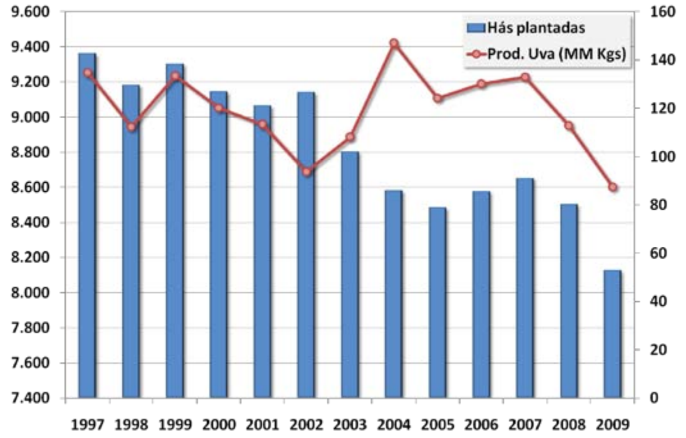
2008	Has plantadas (miles)	Producción Uva (MM Qx)	Producción Vino (mill. HL)	Consumo Vino (mill. HL)	Habitantes (miles)	Territorio (miles Km2)	Productividad (Kgs por Ha)	Has cada 1000 Hab	Producción (Litros por Hab)	Consumo (Litros por Hab)
Alemania	- -	14	10	21	82.330	357	- -	- -	12,1	25,1
Argentina	227	28	15	11	40.914	2.737	5.674	5,5	35,9	26,2
Australia	173	20	12	5	21.263	7.682	5.318	8,1	58,3	23,0
Chile	198	25	9	- -	16.602	744	5.808	11,9	52,4	- -
China	470	72	12	14	1.338.613	9.570	7.047	0,4	0,9	1,0
EEUU	398	67	19	29	307.212	9.162	7.744	1,3	6,2	9,3
España	1165	57	35	13	40.525	499	2.251	28,7	85,4	31,6
Francia	852	57	41	32	64.058	640	3.077	13,3	64,2	49,6
Italia	840	81	49	26	58.126	294	4.436	14,5	83,6	44,7
Portugal	246	- -	- -	5	10.708	91	- -	23,0	- -	42,0
Rumania	207	- -	7	5	22.215	230	- -	9,3	30,6	24,3
Uruguay	9	2	1	1	3.494	175	13.280	2,4	26,9	22,9

Fuente: OIV (2008), INAVI

Como fuera mencionado previamente, existen ciertas presiones sobre el sector vitivinícola que incentivan el desarrollo de la profesionalización del negocio, así como el aprovechamiento de escalas. El resultado de estas presiones se observa claramente en dos indicadores. El primero es el aumento de la productividad. La figura presentada a continuación muestra una significativa reducción de la superficie plantada, pero no un quiebre considerable en la producción de uva. Ello sugiere entonces que el desarrollo y aplicación de técnicas más intensivas redundó en aumentos de productividad que logaron contrarrestar en parte la reducción en las hectáreas plantadas.

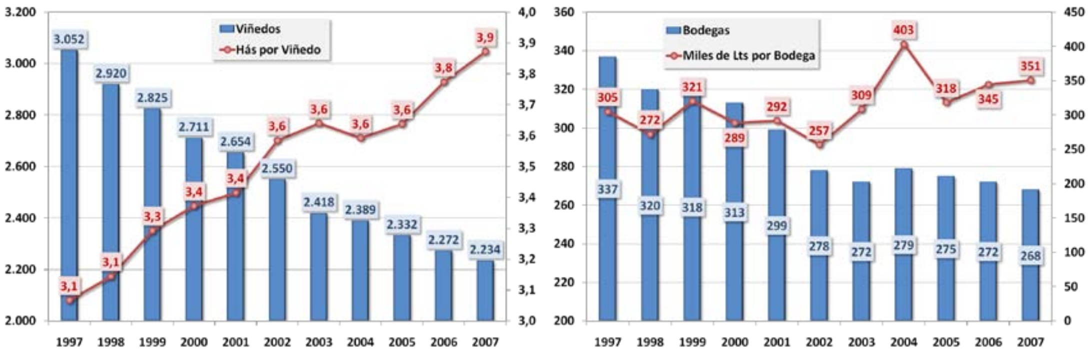
Por otro lado, la profesionalización del negocio vitivinícola y la necesidad de aprovechar escalas está redundando en un moderado proceso de concentración del área plantada. En línea con lo anterior, es posible comentar que entre 1997 y 2007 el tamaño medio de una viña pasó de 28 Hás a 32 Hás, lo que en términos relativos implica un crecimiento del orden del 16%.

Hectáreas plantadas y Producción de Uva (MM Kgs)
(datos al 2007)



Fuente: INAVI

(a) Número de Viñedos y Plantación Media, (b) Número de Bodegas y Producción Media



Fuente: INAVI, IICA

Desarrollo sustentable

En este contexto se entiende como desarrollo sustentable al espacio dónde se obtienen resultados positivos en los tres pilares del desarrollo: i) económico, ii) social y iii) ambiental. Sólo en los casos dónde se desarrollan satisfactoriamente los tres aspectos de una sociedad se está ante un desarrollo sostenible y perdurable en el tiempo.

Legislación sobre Patrimonio

En Uruguay, la Ley N° 14.040 de 27 de octubre de 1971 crea la Comisión del Patrimonio Histórico y Cultural de la Nación. Por esta Ley (Artículo 5°) pueden ser declarados monumentos históricos, los bienes muebles o inmuebles vinculados a acontecimientos relevantes, a la evolución histórica nacional o a lo que sea representativo de una época de la cultura del país.

Estos preceptos y algunos nuevos (como el Patrimonio Cultural Inmaterial) están siendo reorganizados en un Proyecto de Ley que está en la etapa de aprobación del Parlamento uruguayo. Sin perjuicio de la normativa vigente el Proyecto de Ley del Patrimonio Cultural de la República Oriental del Uruguay plasma una realidad social, económica y ambiental basada en el actual concepto de sostenibilidad. Asimismo, contempla la política nacional de desarrollo del turismo.

En este sentido, se define al Patrimonio Cultural de la República Oriental del Uruguay como el conjunto integrado por todos los bienes materiales e inmateriales a los que la sociedad le atribuye valores significativos de interés histórico, artístico y cultural en sentido amplio, incluyendo los bienes de interés arqueológico (terrestres y marítimos); paleontológicos; científicos y antropológicos.

Integran el Patrimonio Cultural entre otros:

1. **Los conjuntos:** entendiéndose por tales, grupos de construcciones aisladas o reunidas, cuya arquitectura en unidad e integración en el paisaje les dé un valor excepcional desde el punto de vista de la historia, el arte o la ciencia.

2. **El paisaje cultural:** entendidos como la superficie territorial continental o marina, en la cual las interacciones del ser humano y la naturaleza, a lo largo de los años, han producido una zona de carácter definido, de singular belleza escénica o con valor de testimonio natural, y que podrá contener valores ecológicos o culturales. Los componentes significativos de esta categoría son:

   - El sustrato natural (orografía, suelo, vegetación, agua)
   - Las acciones humanas: modificación y/o alteración de los elementos naturales y construcciones para una finalidad concreta.
   - Las actividades desarrolladas (componente funcional en relación con la economía, formas de vida, creencias, cultura en sentido amplio)

3. **Paisaje Activo:** aquel que conserva un papel social activo en la sociedad contemporánea asociado con el modo de vida tradicional y cuyo proceso de evolución sigue activo.

4. **El "patrimonio cultural inmaterial"** que se puede manifestar, a modo de ejemplo, en los siguientes ámbitos:

   - tradiciones y expresiones orales, incluido el idioma como vehículo del patrimonio inmaterial;
   - usos sociales, rituales y actos festivos;
   - conocimientos y usos relacionados con la naturaleza y el universo;
   - técnicas artesanales tradicionales.

Tradición y Calidad del Vino

Uruguay exhibe ante el mundo del vino los resultados de una producción que apuesta a la calidad sobre la cantidad en la competencia internacional actual. A través de la presentación de tres ejemplos demostraremos como la permanencia de una tradición de cultivo establecida en el época colonial española, enriquecida por los aportes inmigratorios de fines del siglo XIX, desarrollada por el conocimiento científico técnico nacional durante el siglo XX (particularmente vinculado a los aportes académicos y del Estado), han construido ese marco de calidad. Tradición y cambio entendido como un desarrollo productivo sustentado en la actividad económica familiar y empresaria, en una acción mancomunada de individualidades y colectivos de trabajadores, el respeto a la naturaleza, el medio ambiente y la cultura del trabajo denodado, transmitido de padres a hijos así como respetuosa de la mano de obra ocupada.

Las calidades de los primitivos inmigrantes europeos, su cultura de trabajo, su sacrificio y tesón cimentaron el desarrollo industrial empresario de las bodegas en el Uruguay en el siglo XX, sumada a la presencia de una actividad familiar que hasta hoy se mantiene. Pero es en los años finales del pasado siglo que la vitivinicultura realiza una transformación profunda de sus estructuras productivas, industriales y comerciales en un proceso denominado “la revolución del vino uruguayo” con el objetivo de obtener vinos de calidad y de reconocimiento internacional[2]. Empresarios, enólogos de prestigio, investigadores académicos, técnicos diversos y personal del cultivo y la industria imprimen un nuevo dinamismo al sector productivo, además del surgimiento en el ámbito universitario de estudios histórico críticos sobre la cultura vitivinícola en el Uruguay.

Tradicionalmente, en el Uruguay, el cultivo de la vid – ocupando menos del 1% del área agrícola nacional - se realiza con gran respeto a la naturaleza y el medio ambiente. Desde los inicios del siglo XX los cronistas de época reconocen que los viñedos ocupan las zonas más pintorescas del país, por la configuración de los terrenos y la belleza del paisaje natural y cultivado, adornados con amplios jardines y villas. El cultivo de la vid se asociaba al de la huerta y frutales a demás del olivo. Actualmente el desarrollo empresario tiende al monocultivo de la vid en la mayoría de las bodegas, aunque la presencia arbórea en monte natural y plantaciones bordeando una caminería a la vez funcional y de paseo y otros detalles del equipamiento del territorio, incorporan valores inconmensurables de imagen, tradición y patrimonio cultural a los establecimientos.

Paradigmas que dan testimonio

De particular atención ha sido para el Estado uruguayo el reconocimiento de “Monumento Histórico Nacional” para los viñedos plantados en el siglo XIX y de producción sostenida hasta hoy día, como son los casos de 20 há. de 1887 en la Estancia La Cruz y de 1 há. en Los Cerros de San Juan. Deben ser además mencionados los aportes de una arquitectura para la producción, de época, todavía funcional y conservada como emblema empresarial.

En esa tríada de consideraciones que armonizan tradición y cambio: economía con sostenibilidad social, cultura de trabajo y respeto al medio ambiente es que actualmente se sustenta el concepto de calidad en la industria vitícola uruguaya. Entre otros ejemplos que testimonian este aserto, es de toda justicia destacar como complejos vitícolas representativos a aquellos que han merecido reconocimiento por su carácter patrimonial para el Uruguay. A estos efectos el Estado uruguayo declara a través de la Ley Nº 14.040 Monumentos Históricos Nacionales a tres bodegas y viñedos que ameriten aquel reconocimiento.

Los Cerros de San Juan, en el Departamento de Colonia, ***Juanicó*** en el Departamento de Canelones y ***La Cruz*** en el Departamento de Florida.

Los Cerros de San Juan fue declarado Monumento Histórico en el 2004, Nació como consecuencia de la inmigración alemana en la segunda mitad del siglo XIX. A partir de 1854 se fue conformando un establecimiento agropecuario constituido por estancia, cabaña y más tarde bodega familiar, que será el fundamento del desarrollo de la Bodega Los Cerros de San Juan en el correr del siglo XX y hasta nuestros días. La empresa

[2] De Frutos, E., Beretta, A.: Un siglo de tradición. Primera Historia de Uvas y Vinos del Uruguay. Aguilar . 1999.

conserva núcleos de vivienda – reconocidos como “pueblos” – para los trabajadores y una escuela para los niños.

Juanicó fue declarada Monumento Histórico en el año 2009. Nació como establecimiento agropecuario en 1835 y hoy día constituye una empresa vitivinícola de avanzada con fuerte incorporación de tecnología e innovación Dio lugar desde sus orígenes a una población de trabajadores –en este caso- externa al territorio del establecimiento llamada Pueblo Juanicó vecina a la vía férrea.

Estancia La Cruz. fue declarada Monumento Histórico en el 2010, nació en 1887 y produce una particular simbiosis entre un poblado, el ferrocarril y una unidad agroindustrial vinculada a la producción vitícola. En efecto, La Cruz es una población asentada a la vera de la vía de ferrocarril (un nuevo medio de comunicación para la época al servicio de la población y de la economía) y un emprendimiento productivo cooperativo “Sociedad Vitícola Uruguaya”.

Consideraciones finales

- En términos comparativos, el sector evidenció una rentabilidad moderadamente baja, hecho que al volverse estructural acabó enlenteciendo su desarrollo.
- El presente proceso de concentración de viñedos y bodegas permite aumentar la eficiencia y aprovechar escalas, mejorando los resultados, fortaleciendo el pilar económico del desarrollo sustentable.
- Aprovechamiento de sinergias con el turismo permite rentabilizar y valorizar la industria a la vez que se desarrolla una actividad más intensiva en mano de obra, fortaleciendo los pilares económico y social.
- Uso de la marca “Patrimonio Histórico de la Nación” puede facilitar el proceso. Exigencias de mantenimiento y desarrollo de la Ley permiten afianzar el pilar económico y el medioambiental.
- El marco de aplicación de la norma a “patrimonio cultural inmaterial” permitirá preservar y promover las manifestaciones tradicionales como el Día de la Vendimia, celebrado desde 1888, y es a su vez, un importante sustento cultural de la producción vitícola uruguaya.
- Otro tanto, con la protección de “paisaje activo”. Ello sería aplicable a los viñedos, favoreciendo la adecuada conservación del activo papel social del “viticultor” en las sociedades contemporáneas.
- El reconocimiento de las expresiones orales propias como identidades culturales permitirá fortalecer la posición cultural del vino en la sociedad uruguaya, así como propender a una divulgación más profunda y efectiva del arte vitivinícola. Entre estas expresiones se pueden encontrar por ejemplo: “vino lija” para el vino muy tánico (en uso hoy por las más jóvenes), aunque se utiliza desde 1920, “vino criollo” (al vino uruguayo), utilizada desde 1923, vino Harriague como denominación previa del tannat (desde 1877), entre otras.
- La cifra de consumo unitario de Uruguay de 28 litros por habitante año es un indicador inequívoco de la incorporación del vino y su arraigo en la dieta habitual de los uruguayos.

- Los conceptos vertidos –como el de DO- recorren el camino de preservar la diversidad, identidad, tradición y cultura del vino, bajo un sistema sustentable en todos los aspectos de la vida productiva en armonía con la gente y la naturaleza.

- Bodegas y viñedos como centros productivos en el territorio, constituyen bienes patrimoniales que representan valores históricos, científicos y culturales que respaldan un compromiso de conservarlos y transmitirlos. El trabajo de rescate, valoración y preservación se hace siempre imprescindible y compromete a toda política cultural de un país.

- En el siglo XXI el Estado comienza a reconocer esas empresas, incluyéndolos en la lista de Monumentos Históricos Nacionales, por sus valores arquitectónicos, por la calidad del producto logrado y como patrimonio intangible (el know how).

Anexo Fotográfico

Ilustración 1. Cava de la Bodega Juanicó
Declaración 2009

Ilustración 2. Cava de la Bodega Juanicó
Declaración 2009

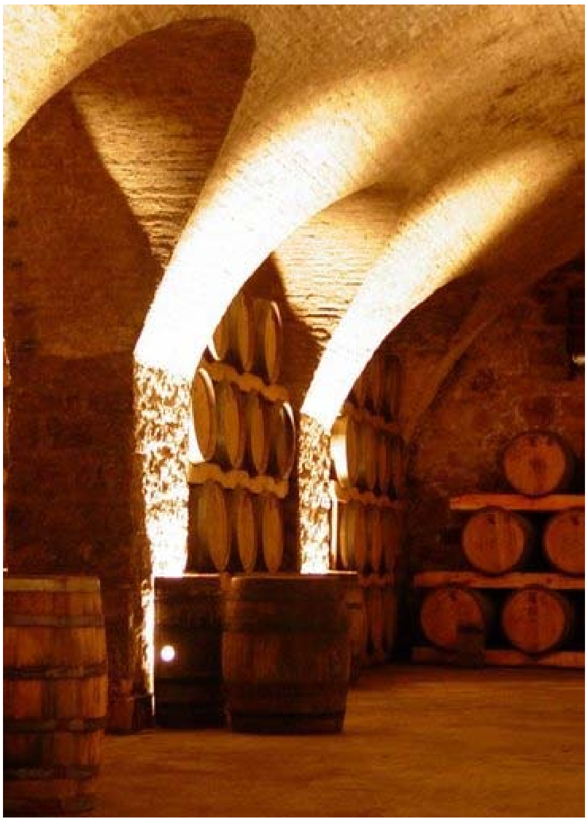


Ilustración 3. Cepa Tannat 1890
Cerros de San Juan - Declaración 2004

Ilustración 4. Cava de la Bodega
Cerros de San Juan - Declaración 2004

Ilustración 5. Cava de la Bodega
Cerros de San Juan - Declaración 2004

Ilustración 6. Viña Tannat La Cruz 1887
Declaración 2010

Ilustración 7 a 11. Tannat de Estancia La Cruz
Declaración 2010





DAS VERBOT EINES GUTEN ÖNOLOGISCHEN VERFAHRENS

Wolfgang Haupt
Bundesministerium für Ernährung, Landwirtschaft und Verbraucherschutz,
Rochusstrasse 1, 53123 Bonn, Deutschland, wolfgang.haupt@bmelv.bund.de

ABSTRACT

The evolution of the addition of glycerol as an oenological practice is described on the basis of studies of literature. The technical advantages of this practice and its appreciation by scientists of oenology, chemists and experts of the appraisal of wine are explained. With regard to the whole oenology of the end of the 19^{th} century, the author tries to explain the refusal of this practice, for that he identifies essential reasons in the fear to loose the authenticity of the wine and the confidence of the consumer in the original quality, without misconceiving the problems of the concurrence of the producers in quality.

KURZFASSUNG

Die Entwicklung des Zusatzes von Glycerin als önologisches Verfahren wird auf der Grundlage von Literaturstudien dargestellt. Die technischen Vorteile des Verfahrens und dessen Beurteilung durch Wissenschaftler der Önologie, Chemiker und Weinsachverständige werden erläutert. Im Hinblick auf die gesamte Önologie am Ende des 19. Jahrhunderts versucht der Autor, die Ablehnung des Verfahrens zu erklären, dessen wesentliche Gründe er in der Furcht vor dem Verlust der Authentizität des Weines und des Vertrauens des Verbrauchers in die originäre Qualität erkennt, ohne die Probleme des Qualitätswettbewerbs der Erzeuger zu verkennen.

EINLEITUNG

In den Gremien der Internationalen Organisation für Rebe und Wein (OIV) wird zurzeit ein „Wissenschaftliches Verfahren zur Prüfung eines Vorentwurfs für ein neues önologisches Verfahren und/oder seiner Änderung“[1] entwickelt. Dabei werden auch technisch-wissenschaftliche Elemente berücksichtigt, die für die Beurteilung der Zulassung von Bedeutung sind, zum Beispiel „Ziel des önologischen Verfahrens“ und „Informationen bezüglich Sicherheit und Gesundheit“.
In den Rechtsvorschriften[2, 3, 4] über önologische Verfahren werden verschiedene Kriterien für die Beurteilung von önologischen Verfahren genannt. Ich möchte beispielhaft einige Kriterien nennen, die einen großen Spielraum für Auslegungen eröffnen:

- die Beschränkung auf technisch sinnvolle und notwendige Verfahren,
- Beschränkung der Verfahren oder Zusätze auf das Mindestmaß,
- die Erhaltung der natürlichen wesentlichen Merkmale des Weins,
- die Wahrung der Echtheit des Erzeugnisses,
- die Vermeidung der Irreführung der Verbraucher,
- die Sicherung der Akzeptanz des Weins bei den Verbrauchern.

Diese Kriterien sind für die aktuelle Situation der Weinwirtschaft formuliert, die durch eine arbeitsteilige Wirtschaftsweise sowie ein gesichertes und qualitativ zufrieden stellendes Angebot gekennzeichnet ist.

Mit der vorliegenden Arbeit sollen diese Kriterien im Hinblick auf ein konkretes önologisches Verfahren betrachtet werden. Als önologisches Verfahren wurde der Zusatz von Glycerin zu Wein ausgewählt. Dafür gibt es zwei Gründe. Erstens gibt es in der Literatur zahlreiche technische Beschreibungen und Hinweise über dieses Verfahren. Zweitens ist die technische und rechtliche Beurteilung dieses Verfahrens schon seit mehr als einem Jahrhundert abgeschlossen und heute nicht umstritten.

MATERIAL UND METHODEN

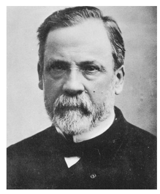
Für die Arbeit wurden vornehmlich Bücher und Zeitschriften aus der Periode des wachsenden Einflusses der Wissenschaft und Technik auf die Weinwirtschaft verwendet, die in der Zeit von 1770 bis 1920, von Scheele bis Günther veröffentlicht worden sind. Wegen begrenzter Sprachkapazität konnten nur die Arbeiten in deutscher, englischer, französischer und italienischer Sprache berücksichtigt werden. Aufgrund der Fülle des Materials musste die Auswertung auf die wichtigsten Veröffentlichungen beschränkt werden.

Scheele
1742-1786

ERGEBNISSE UND DISKUSSION

Die Entdeckung des Glycerins

Glycerin wurde von Carl Wilhelm Scheele entdeckt und beschrieben[5]. Im Rahmen seiner Studien über die Gärung isolierte Louis Pasteur das Glycerin als Nebenprodukt der alkoholischen Gärung mit einem Gehalt von etwa 3 Prozent des vergorenen Zuckers[6]. Auf der Grundlage der Erkenntnisse Pasteurs wies Giuseppe Ubaldini Glycerin in Weinen aus der Toskana nach[7]. Jacques Brun[8] und Carl Friedrich Mohr[9] haben schon früh versucht, die Entstehung des Glycerins während der Gärung in einer chemischen Formel darzustellen.



Pasteur
1742-1786

Nach ersten Arbeiten von Hermann Müller-Thurgau[10] wurde die Bildung des Glycerins in den Trauben durch Botrytis cinerea von Jean Laborde untersucht und beschrieben[11]. Diese zweite Möglichkeit war von großer Bedeutung für die Beurteilung der Weine, die aus edelfaulen Trauben erzeugt worden sind, da diese Weine sehr hohe Glyceringehalte aufweisen können.

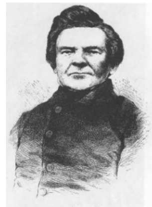
Laborde
1865-1920

Die Eigenschaften des Glycerins

Carl Wilhelm Scheele hatte schon die zwei wichtigsten Eigenschaften von Glycerin beschrieben: Glycerin hat einen süßen Geschmack und gerät nicht in Gärung[12].
Die Toxizität des Glycerins wurde im Jahr 1876 von Dujardin-Beaumetz und Audigé untersucht. Toxische Wirkungen wurden nur bei Aufnahme extrem hoher Mengen festgestellt, sodass Glycerin als gesundheitlich unbedenklich galt[13].
Schon früh wurden Anforderungen an die Beschaffenheit des Glycerins gestellt, das für önologische Zwecke verwendet wurde. Nach Foelix[14] sollte Glycerin von reinem Geschmack sein und keinen Fettgeruch besitzen. Er wies auch darauf hin, dass schon bleihaltiges oder mit Zuckersirup verfälschtes Glycerin gefunden worden ist. Daher empfahl Dal Piaz dem Önologen eine Prüfung des Glycerins auf seinen Gehalt an Blei, um die Anwendung bleihaltigen Glycerins zu vermeiden[15].

Was die chemische Zusammensetzung und Beurteilung des Weines angeht, so ist Glycerin Bestandteil des Extrakts[16].

Der Zusatz von Glycerin zu Wein



Gall
1791-1865

Kurz nach der Entdeckung des Glycerins als natürlicher Bestandteil des Weines hat Ludwig Gall den Zusatz von technischem Glycerin zur Verbesserung der Weinqualität empfohlen[17]. J. Beyse wies 1866 auf einen Pflanzenstoff hin, mit dem man Wein markig und ölig machen könne; 1869 teilte er mit, dass er Glycerin als Geheimmittel empfohlen habe[18]. Der „Zusatz von Glycerin“[19], „addition of glycerol“[20], wird in der Fachliteratur als önologisches Verfahren beschrieben und erläutert. Für dieses önologisches Verfahren entstanden Eigennamen, die aus dem Namen der verwendeten Substanz oder aus dem Namen ihres Entdeckers abgeleitet waren, zum Beispiel „glycérinage“[21], „glicerinaggio“[22], „Scheelesieren“[23], Scheelisieren[24, 25] und „schéelisation“[26].
Ludwig Gall[27] empfahl Glycerin für die Verbesserung des Geschmacks der Weine. Mit einer Menge von 2 Prozent oder mehr Glycerin sollte die Süße des Weines erhöht werden. Der besondere Vorteil lag darin, dass keine Gefahr des Abbaus von Glycerin durch eine Gärung des Weines bestand. Dieser technische Vorteil war von großer Bedeutung, da es damals kein sicheres und wirtschaftliches Verfahren für die Konservierung süßer Weine gab. Beyse entwarf eine Tabelle, in der dem Säuregehalt des Weines eine bestimmte Menge an Glycerin-Zusatz zugeordnet wurde[28]. Das Ziel der Geschmacksverbesserung des Weines wurde von vielen Autoren unterstützt.
Karl Maier schilderte einen weiter gehenden Anwendungsbereich des Glycerins, nämlich die Erzeugung von Ausbruchweinen, zum Beispiel auch Malaga, Xeres, Capwein, Tokayer. Dabei bezeichnete er Glycerin als eines der Urmaterialien, die für die Herstellung unbedingt erforderlich seien, und als „unentbehrlicher Körper in der Weinpraxis“. Mit dem Auftritt von Glycerin habe die „Fabrikation der Ausbruchweine“ einen „völligen Umschwung erlitten“[29].
Die Herstellung von Kunstwein war ein weiteres Feld für die Anwendung von Glycerin. Als Beispiel für diese Praxis können die Empfehlungen von Friedrich Jakob Dochnahl genannt werden[30]. Für die Herstellung von Kunstwein, der dem Normalwein ähnlich ist, empfahl Dochnahl mehrere Rezepturen auf der Basis von Wein, Rosinen, Weingeist und Zitronensäure. Dabei wird auch auf Wein als Grundsubstanz oder auf die Gärung verzichtet. Für jede Rezeptur war technisches Glycerin vorgesehen. Die Glycerin-Menge hing ab von dem Grad der Verdünnung und von der Notwendigkeit, den Extrakt künstlich nachzubilden.
Im Zusammenspiel mit den anderen önologischen Möglichkeiten, insbesondere der Chaptalisation, der Gallisation und der Petiotisation, ermöglichte der Glycerinzusatz eine technische Beherrschung aller wichtigen Weininhaltstoffe. Dies bedeutete, dass die Zusammensetzung des Weines hinsichtlich der Gehalte an Zucker beziehungsweise Alkohol, Säure und Wasser technisch steuerbar wurde.

Die Beurteilung des Zusatzes von Glycerin zu Wein

Vor dem Hintergrund der durch die Reblaus (Phylloxera vastatrix Pl.) verursachten Ertragsverluste und dem Wunsch, die Bevölkerung mit billigem Wein zu versorgen, wurde die technische Verbesserung der Weine von vielen Autoren begrüßt und durch Publikationen unterstützt[31, 32]. Bemerkenswert ist eine ausdrückliche Empfehlung, die nach dem gesetzlichen Verbot erfolgte[33]. Der Zusatz von Glycerin zu Wein ist in technischem Sinne ein gutes

önologisches Verfahren, da unter der Voraussetzung ordnungsgemäßer Anwendung reinen Glycerins die Qualität des Weines verbessert wird und keine Nachteile entstehen.
Es gab aber auch Ablehnungen des Verfahrens durch Önologen, zum Beispiel Neßler[34], und mehrheitlich von Sachverständigen für die Beurteilung der Weine[35, 36, 37, 38, 39].
Das Kaiserliche Gesundheitsamt, Berlin, hatte den Zusatz von Glycerin als unzulässig eingestuft[40]. Jedoch revidierte es seine Auffassung und billigte einen Zusatz, mit dem der Gehalt des Wein auf 14 g/l erhöht wurde. Damit wurde eine Erhöhung der Weine auf das Höchstniveau normaler Weine als zulässig anerkannt[41].

Das Verbot des Zusatzes von Glycerin zu Wein

Der Zusatz von Glycerin zu Wein wurde in Deutschland im Jahr 1892 verboten[42]. Für das Verbot wurden zwei Gründe angeführt[43]:
Erstens galt der Glycerinzusatz als gesundheitsschädlich, weil die Anwender nicht in der Lage seien, reines Glycerin von gesundheitsschädlichem Glycerin zu unterscheiden. Der Zusatz von gesundheitsschädlichem Glycerin könne nicht ausgeschlossen werden. Darauf gründete man ein Verbot des Zusatzes zu Wein, aber auch zu allen weinhaltigen oder weinähnlichen Erzeugnissen. Zweitens wurde der Glycerinzusatz als Verbrauchertäuschung eingestuft, weil die technische Veränderung eine höhere Qualität des Produkts vorspiegele. Ferner könne der Glycerinzusatz die Verdünnung des Weines insofern verdecken, als der Extraktgehalt angehoben wird. Dieser Aspekt war wichtig, da die Gallisation durch die Festlegung eines Mindestgehalts an Extrakt beschränkt wurde[44]. Diese Beschränkung hätte umgangen werden können, wenn die Erhöhung des Extrakts durch Glycerinzusatz zulässig geblieben wäre.
Das Verbot wurde in vielen Ländern erlassen. In den Verbotsvorschriften wurde der Glycerinzusatz teils konkret benannt, teils wurden Stoffgruppen benannt, zu denen Glycerin gehörte. Die Tabelle gibt Auskunft über die dem Autor zugänglichen Rechtsvorschriften und ist insofern nicht abschließend[45, 46, 47].
Einzelheiten der Hintergründe und Motive für die Verbote in den einzelnen Staaten können wegen fehlender Dokumente hier leider nicht erörtert werden. Allerdings liegt die Vermutung nahe, dass alle Staaten ihre Verbote aus ähnlichen Gründen erlassen haben, auch wenn der Schwerpunkt der Beweggründe zum Teil stärker beim Verbraucherschutz, zum Teil bei der Sicherung des fairen Wettbewerbs gelegen haben mag.

spezifisches Verbot des Glycerinzusatzes		allgemeines Verbot des Glycerinzusatzes	
im Staat	im Jahr	im Staat	im Jahr
Spanien	1888	Argentinien	1904
Deutschland	1892	Italien	1905
Ungarn	1897	Frankreich	1907
Belgien	1899	Japan	1909
Portugal	1899	Luxemburg	1909
Dänemark	1903		
Südafrika	1906		
Österreich	1907		
Rumänien	1908		
Schweiz	1909		

SCHLUSSFOLGERUNGEN

Die Einführung des Glycerinzusatzes als önologisches Verfahren schaffte die Möglichkeit, die Zusammensetzung des Weines technisch zu steuern. Die Folgen dieser Möglichkeiten wurden aber aus Gründen des Verbraucherschutzes abgelehnt, sodass dieses önologische Verfahren in den meisten Staaten mit weinwirtschaftlicher Bedeutung verboten wurde.

DANKSAGUNG

Ich danke meiner Ehefrau für ihre ständige Unterstützung, Herrn Prof. Alain Bertrand sowie - für ihre bibliothekarische Beratung und Hilfe - Frau Renate Schoene, Frau Ursula Kleinheyer-Thomas und Frau Beate Frohn.

ANMERKUNGEN

[1] Vorläufiger Resolutionsentwurf der OIV CST/07/356 „Vorläufiges wissenschaftliches Verfahren zur Prüfung eines Resolutionsentwurfs für ein neues önologisches Verfahren, seiner Änderung und/oder seiner Aufhebung".

[2] Artikel 4 Absatz 1 des Abkommens zwischen der Europäischen Gemeinschaft und den Vereinigten Staaten von Amerika über den Handel mit Wein vom 10 März 2006 (Amtsblatt der Europäischen Union Nummer L 87 vom 24. März 2006, S. 2). http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2006:087:0002:0074:DE:PDF

[3] Artikel 7 des Abkommens zwischen der Europäischen Gemeinschaft und Australien über den Handel mit Wein vom 1. Dezember 2008 (Amtsblatt der Europäischen Union Nummer L 28 vom 30. Januar 2009, S. 3), http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2009:028:0003:0087:DE:PDF
Dieses Abkommen ist von der Europäischen Gemeinschaft, jedoch nicht von Australien gebilligt.

[4] Artikel 120f und Anhang XVb Abschnitt A der Verordnung (EG) Nr. 1234/2007 des Rates vom 22. Oktober 2007 über eine gemeinsame Organisation der Agrarmärkte und mit Sondervorschriften für bestimmte landwirtschaftliche Erzeugnisse (Verordnung über die einheitliche GMO) (Amtsblatt der Europäischen Union Nummer L 299 vom 16. November 2007, S. 1) in der Fassung der Änderung durch die Verordnung (EG) Nr. 491/2009 des Rates vom 25. Mai 2009 zur Änderung der Verordnung (EG) Nr. 1234/2007 über eine gemeinsame Organisation der Agrarmärkte und mit Sondervorschriften für bestimmte landwirtschaftliche Erzeugnisse (Verordnung über die einheitliche GMO) (Amtsblatt der Europäischen Union Nummer L 154 vom 17. Juni 2009, S. 1). http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=CONSLEG:2007R1234:20090801:DE:PDF

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POTENTIAL IMPACTS OF ORGANIC WINE REGULATION AS A TECHNICAL BARRIER TO TRADE

Milton C (1), Battaglene T (2)

(1) Winemakers' Federation of Australia
Level 1, NFF House, 14-16 Brisbane Avenue, Barton, Canberra, Australia
(2) Winemakers' Federation of Australia
Level 1, NFF House, 14-16 Brisbane Avenue, Barton, Canberra, Australia

Abstract

When a label says "organic", in countries with organic regulation, it means the wine has met certain standards that are set by a government agency or standard setting body. Different nations have their own certification criteria, so what's organic in one country may not be so in another.

For example, one of the largest misconceptions about organic wine in Australia is that it is preservative free. Certification requirements vary across the world from one country to another and from one certifying organisation to another. Under organic and biodynamic certification in Australia, the amount of preservative allowed in the final product is about 50% of what can be used under conventional food standards for wine. However, in the US, to label your wine as "Organic Wine" no preservative can be added at all.

Certification bodies have a vested interest in maintaining separate organic standards as they all scramble for market share and attempt to see off their competitors. Retailers may also seek to provide their backing for a particular organic standard for a perceived market advantage over retailers who choose to use a different certification standard that has a weaker public (brand) profile. The wine sector and consumers have an equal interest in seeking harmonisation of regulations so that when a customer buys a bottle of wine off the shelf that it is labelled as 'organic' that consumer knows exactly what that means. This provides customer certainty and reduces producer costs as well as potentially adding value.

In this paper, we overview the potential for growth in the organic wine sector, discuss some of the recent developments in this field and suggest potential OIV involvement in the development of an international organic wine standard.

INTRODUCTION: WHAT IS ORGANIC WINE?

The simple answer to this question is that there is no single internationally accepted definition. In this section we outline a number of the commonly proposed definitions.

Organic refers to the application of agricultural and processing practices that emphasise the use of renewable resources; conservation of energy, soil and water; recognition of livestock welfare needs; and environmental maintenance and enhancement, while producing optimum quantities of products without the use of artificial fertiliser or synthetic chemicals and non-essential food additives and/or processing aids. (FSANZ, 2009)

When a label says “organic”, in countries with organic regulation, it means the wine has met certain standards that are set by a government agency or standard setting body. Wines may use organically grown grapes but the process of making the wine can be completely opposite of organic. It is often best to look at organic winemaking in two parts: winemaking and viticultural practices. According to the standard certified organic, organic vineyards are managed without the use of fungicides, insecticides, herbicides or synthetic fertilizers. Organic winemaking practices have definitions that differ from country to country, but generally speaking, it usually involves wine made from organic grapes, containing less than 100-120mg/L of total sulphur dioxide, which appears naturally during fermentation and is also added for stability (Ansems 2009). Other natural products such as egg whites and milk may be added for clarification. Historically true organic wines have been stigmatized due to their lack of sulphites, which alters flavour and reduces shelf life, but in many cases a modern view of organic incorporates low levels of sulphur dioxide as being acceptable.

Organic wines can also incorporate sustainable wines and biodynamic wines as well as organic wine itself. However, an organic wine is not necessarily sustainable.

Biodynamic refers to an agricultural system with a very holistic approach to organic farming which introduces specific additional requirements to an organic system, therefore pesticides; herbicides and synthetic fertilizers are also prohibited. They are based on the application of preparations introduced by Rudolf Steiner and subsequent developments for management derived from practical application experience and research based on these preparations (FSANZ 2009).

Biodynamic wine, therefore, is wine produced with these activities in mind. Biodynamic farming also notes the interdependence of all nature and the lunar effect on all active soils. Biodynamic winemakers allow the vineyard to operate as a self sustaining entity within the surrounding ecosystem, placing emphasis on all living organisms contributing to the farm’s success. Biodynamic wineries rely on manure, crop rotations and biological pest control to revitalise the soil with nutrients and protect the vineyard from predators (Organic Wine Trade Company 2010)

Sustainable wine involves wine that is made in such a manner that it will allow the vineyards and environment to continue to produce and undiminished product for all future generations. Threats to sustainability in vineyards come in the form of soil depletion, erosion and water pollution amongst others (Ansems 2009).

Another branch of organic wine is 'natural wine', which is considered as a wine made, in small quantities, by an independent producer, on low-yielding vineyards, from handpicked, organically grown grapes, without added sugars or foreign yeasts, without adjustments for acidity and without micro-oxygenation or reverse-osmosis (Morethanorganic.com 2010). However, there is no internationally acceptable definition of 'natural' and this is currently under consideration by the Codex Alimentarius Commission.

DEMAND FOR ORGANIC WINE AND POTENTIAL FOR GROWTH

There has been an increasing demand for organic and inherently 'green' produce in recent times, which started with solid growth in the nineties and continues up to today (Geier, 2006). This is generally attributed to a variety of reasons.

Firstly the increased awareness of climate change and global warming has seen a push for manufacturers and producers to explore more sustainable ways of making their products and show their green credentials. Organic farms may sometimes have lower overall input costs as opposed to conventional farms, due to a lack of need for fertilizers and chemicals, but conversely, often have higher labour costs and are not as easy to farm as conventional farms (Wheeler, Crisp 2009).

Second, growing consumer concern over some agricultural production methods and a demand for products that they perceive as cleaner and greener has lead to an increased demand for organic products. In a more environmentally conscious environment as seen in recent times, people want to look after not only the planet, but also themselves.

Worldwide demand for organic products is reported to be increasing rapidly (especially in Germany, England, Switzerland, New Zealand, Japan, and the United States). The European Union has shown an increasing demand for organic wines. More and more new organically based wineries are being established and old traditional wineries are adding organic wine to their product line (Azabagaoglu et al. 2007).

This means organic wines are no longer the province of small family wineries with no market share. Many stores, particularly in the United Kingdom are becoming extremely eco and health conscious in the wake of public demand and now stock large ranges of organic varieties of wine. Examples of stores which have a strong focus on providing organic food are Tesco's, Sainsbury's and Waitrose.

The Tesco Organic food range consists of over 1200 competitively priced organic foods ranging from fruit and vegetables to milk and dairy (Tesco 2007). Its organic wine range currently sits at 8 bottles. Waitrose has over 1600 organic items across its organic range and over 22 bottles of organic wine listed on its webpage (Waitrose 2010). Sainsbury's has 450 lines in their SO organics range (Sainsbury's 2010). Marks and Spencer is experiencing significant growth rates of 48% for its organic food range and doubling to 500 products (Marks and Spencer 2010).

In Australia, Coles now has over 170 organic brands in store (Coles 2010), with Woolworths also in on the action with Woolworths Organic, which is constantly looking to expand (Woolworths 2010). Aldi supermarkets have a commitment to its 'Just Organic' range of bringing food 'grown just as nature intended' (Aldi 2010).

In North America, all the major grocery retailers' websites have a positive fresh image regarding the food they sell and are proudly displaying prominent advertising for their organic lines. Wal-Mart currently has a very eco-friendly image as depicted on their webpage, obviously keen to display their green credentials with over 200 organic products listed (Walmart 2010). Supervalu launched its own line of natural foods, Wild Harvest, in late 2008, in an attempt to boost sales. With prices more than 15% lower than brand-name organic products, their 'organify your world' advertising seems to be paying off (Peer 2008). Safeway has created '*O* Organics', a new line with 300 organically certified products (Safeway 2009). Finally Kroger has a brand called 'Private Selection Organics' in their line of stores. All the above organic lines carry the USDA certified organic seal (Kroger 2010).

In recent times organic produce has experienced explosive growth, with more than 11% of all fresh fruit and vegetable sales in the United States in 2009 being organic totalling more than US$9.5 billion. The organic share of all food sales in the United States has also grown to 3.7% of all sales, up 5.1% over 2009 (Nelson 2009). 54% of all organic food sales occurred in conventional grocery stores, whilst 38% occurred at natural food retailers.

IFOAM released a report in early 2009 analysing the world of organic agriculture and prominent trends and statistics. Australia accounts for the largest organic certified area (12m ha) followed by Argentina (2.8m ha) and Brazil (1.8m ha), combining with other organic countries to reach an ever increasing total of 32.2 million hectares worldwide, 1.5 million more hectares than previously reported. The strongest growth has been in Africa and Latin America. The global market for organic products reached US$46 billion in 2007 with the vast majority of goods consumed in Europe and North America. IFOAM states, "The growth in developing countries shows that organic agriculture can contribute to meaningful socioeconomic and ecologically sustainable development, especially in poorer countries (Bowen 2009).

Another driving force behind the current increase in organic produce is booming demand and policy support (Hills 2009). Not only has the market for organic food has grown very fast, which has an enormous impact on production, but governments are becoming increasingly interested and support schemes are being established for the organic market. Many developing countries also see organic markets as a way of gaining access to the European and American markets, the largest consumers of organic produce.

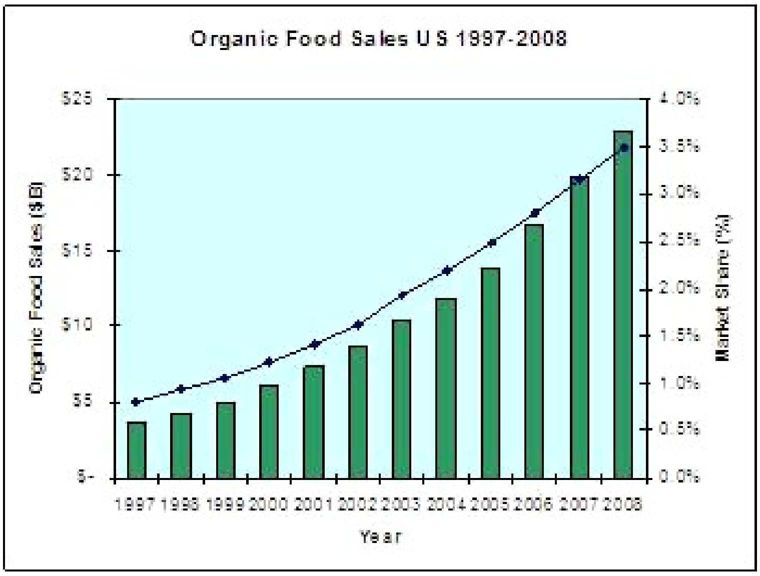

Fig 1: This shows the significant growth rate achieved by organic food in the United States from the late 1990's to 2008. With a total market share of just over 0.5% and US$5 billion to approximately 3.6% of the total market share and $22 billion dollars eleven years later, this demonstrates how impressive organic growth sales have been, and continue to be (Martin 2009)

RECENT DEVELOPMENTS IN ORGANIC WINE

There have been many instances over the past few years which have ensured that Organic wine is being noticed.

BioFach also known as the World Organic Trade Fair is the world's largest exhibition of organic wines and is growing in popularity each year. It recently had 318 exhibitors of organic wines, the majority of which were either from Italy, Germany, Spain and France (BioFach 2009).

Market instability, as seen in the past several years, especially the Global Financial Crisis of 2009, has put a strain on trading of certain product worldwide. Organic wine showed zero to slight growth rates, and seems to have avoided the downturn in sales that many other industries have experienced (BioFach 2009). In fact in the second half of 2009, organic wine sales were expected to rise 10% in Europe (BioFach 2009). Australia, which is currently experiencing oversupply issues, has seen increasing sales and demand for organic and biodynamic wine during this difficult time of industry upheaval (Biological Farmers of Australia 2010). The US has seen wine sales increase in recent years, especially with organic wine replacing conventional table wine sales at a rate nearly four times higher (Baisley 2009). BioFach recently declared that estimated worldwide organic market sales in 2008 were at US$ 50.9 billion, with Europe accounting for $26b and the USA $23b and Asia $950 million (BioFach 2010).

Overall demand for organic produce, however, is reported to be declining, due to effects of the recession as people look for cheaper options. This may mean that there is still potential for growth in the organic wine sector.

BARRIERS TO ORGANIC TRADE

The Organic Trade Association (US) lists the main trade barriers to organic produce as being (Organic Trade Association 2010):

- Foreign government regulations and national organic standards or procedures regarding the import of organic products (particularly with EU and Japan)
- Cultural references
- Language problems
- Market transparency of foreign markets
- Pricing as information problem
- Problems of the mutual acceptance of certification procedures and documentation between certifiers from different countries
- A perceived lack of support by government and industry organizations
- Subsidies and foreign government support
- Competitiveness of certain US products
- High currency exchange rate
- Customs tariff structure, especially for exports of processed products in EU
- Amount of work for export operations

In a 2008 study analysing organic wine market needs, four significant obstacles were apparent that were inhibiting development of the market. These included:

- Low consumer knowledge about organic wines and organic wine production.
- Problem of Business to Consumer marketing.
- Strong competition between conventional wines and organic wines. When quality is equal, organic wines are less competitive because of their higher price.
- High prices of organic wines (Jonis et al. 2008).

As awareness, availability and demand of organic produce grows, as too does serious infringements involving fraudulent behaviour. This has a negative effect upon consumer confidence in organic produce, and ultimately the market (Codex Alimentarius 2010). There is a need for exchange of information between control bodies, control authorities and competent authorities between all countries involved in the import and export of organic wine to protect against this behaviour, which has detrimental effects for many parties involved.

In regard to organic trade with Asia, there is a low level of, and little barriers to, organic trade with Asia (Saminathan 2004). The FAO suggests that we need more mutual recognition, collaboration for recertification and mutual acceptance of other countries' standards based on differing local agricultural situations and agreement on equivalency among the regulatory and certification standards need to happen if we are ever to achieve a harmonization of standards and a truly universal approach to organic trade with no barriers (Saminathan 2004).

While we have seen an increased prominence of organic wine in supermarkets and large retail environments of late, it has often been harder to locate organic wines outside of specialised organic shops or direct sales through the winery themselves (Jonis et al. 2008).

PROBLEMS WITH ORGANIC CERTIFICATION

There are several issues in relation to organic certification and accreditation worldwide which are listed below:

- **Lack of a single authority**

There is no singular body which accredits and monitors organic produce on a world scale. It is up to each individual nation or group of nations to decide on the standards for which organic produce is deemed acceptable. In several of the major wine producing nations there are multiple agencies which approve certification and each are scrambling for their market share and thus vehemently oppose a standardized international accreditation scheme as they would cease to exist.

- **Those who choose not to be certified organic**

There are also issues with products which are organic in nature that do not want to sign up for certification for reasons such as the cost of obtaining certification for something they have been routinely doing for years. Also, some certification organisations charge a tariff for the use of the logo on each bottle sold, which for smaller-scale wineries is not a viable option (Duggan 2008). Others see it is unnecessary, keep in a subtle view that all wines should be minimizing their impacts on the environment and the incidence of pesticides, without feeling the need to brag about it.

- **Length of time involved**

The process of obtaining certification of organic status can be quite laborious and time consuming for those involved. Firstly land which is being converted to organic farming must rest before a number of years before an inspector from a certification authority can test the soil, which then must be approved before organic certification can be issued (Tesco 2007).

- **Issues with standards and labelling**

There is constant debate about how the standards should be displayed on labelling of organic products and many of the regulations are very explicit. Some producers are potentially getting around organic certification by using similar largely undefined terms such as 'green', 'natural', 'eco-friendly' and 'locally grown'. As such, due to some clever wording by advertisers and producers of non-organic products, some market share for organic produce could be capitalised by non-organic producers.

- **Commercialisation of organic produce**

Some critics argue that organic produce has become a gimmick due to big businesses and as a result, adherents to the early organic movements of small scale sustainable environmentalism consider the benchmark of what it means to be organic has been significantly lowered (Childs 2006).

ORGANIC AND BIODYNAMIC LEGAL STATUS

The legal status of organic and biodynamic produce in various countries differs.

In the United States, 'organic production' is a system that is managed in accordance with the *Organic Foods and Production Act* (OFPA) 1990 and regulations in Title 7, Part 205 of the Code of Federal Regulations provide for site-specific conditions by integrating cultural, biological and mechanical practices which bring about recycling of resources, promote ecological balance, and conserve biodiversity. This serves as the legal definition of 'organic' in the United States and is developed and implemented by the National Organic Program.

The issue of numerous standards in Australian organic certification was made clear by the Hon. Justice Gray when he acknowledged in an Australian Federal Court decision in August 2007 that there is an absence of any recognized legal definition of the term 'organic' (Leu 2009). The Australian Standard 6000-2009 was produced to fulfil this need for a definition of 'organic' in Australia.

Although Canada has had an organic standard since 1999, it was not codified into law until the Organic Products Regulations in 2006, which took a further three years to be implemented. As of June 11, 2009, Canada's Organic Products Regulations have attained legal status (CFIA 2010).

HOW OIV CAN BE INVOLVED

In the OIV Strategic Plan leading up to 2012, its clear vision is to be the world scientific and technical reference organisation on vine and wine (OIV 2008). OIV can support the organic and biodynamic wine industries and fulfil its vision by coordinating the development of a credible international standard which can be used reliably amongst member countries. By taking on this leadership role, they have a chance to assist in the construction of a standard which can be an enforceable standard across many countries worldwide. Thus, even though it must be far reaching in scope, taking into account many different factors relating to all member countries, OIV still has to acknowledge that each country has a right to develop its own principles in organic production and as such, the standard can not be mandatory. With news that the European Union is moving ahead with formulating wine processing regulations as part of their organic standards as the next step in overhauling the EU organic standards, this increases the timeliness of the opportunity for OIV to capitalise on the development of a worldwide standard.

The development of an internationally recognized organic standard will:

- Provide consumers and producers with the certainty they need to continue investment in organic produce and avoid fraudulent claims from exports and imports.
- Minimize the need for creation of multiple labels to qualify for the requirements of standards in various nations, enabling easier trade of organic produce across international markets.
- Allow OIV to demonstrate leadership in bringing together a market which has developed in many directions.

CONCLUSION

Although organic wine is growing in size, it still covers a relatively small market share of worldwide food sales, but it is a prominent trend which needs to be looked at carefully, and will only expand over time as community awareness in regard to the environment and health continue to occur. Organic and biodynamic winemaking is shaping up to become very important in the development of the wine industries in this coming era.

Organic and biodynamic winemakers are keen to be universally recognized as quality winemakers; producing wines which are comparable to, if not better than conventional wines, just made in a more carefully constructed and focused way, taking into account harm minimization to the environment and using naturally derived products and processes. The sheer technicality of adhering to these systems will not appeal to everybody, but there is certainly a market which shows a significant degree of prosperity over the coming years. There are certainly many conventional winemakers who are viewing organic wine's current success and evaluating their own winemaking processes and techniques to see if there is any way to improve their own techniques in a quest to make their best possible wines, which can only benefit the wine industries throughout the world.

The potential for growth in organic and biodynamic products is significant and it is a very important arena for OIV to become firmly involved in regulating. The implications of so many different options regarding standards and certifications is that it can become confusing and very time consuming for producers in choosing which avenue to pursue. By coordinating the development of an umbrella like standard that encompasses many countries' various standards and harmonising this into an acceptable and credible document, which can be enforceable at a minimum level, OIV will be making the international entire organic wine industry much less complicated. As such, OIV will continue to show true leadership in scientific and technical expertise as per its vision and mission statement. Furthermore, it is up to the countries involved in organic wine production to assist in the development of this standard and ensure that the best practices are conveyed to achieve the highest possible industry standards.

ANNEX 1: CERTIFICATION AND LABELLING REQUIREMENTS BY COUNTRY

It is commercially attractive in recent times to use expressions such as ‘green’, ‘eco-friendly’ and ‘sustainable’ claims on almost all types of products including wine. People want to feel as though they are doing the right thing by the environment. Organic certification is the primary mode of protection for producers and consumers as it acts against anyone falsifying claims due to its rigorous and systematic testing.

INTERNATIONAL

Internationally there are several schemes which are greatly important in shaping the certification and labelling of organic products. These include:

- BEUC – The European Consumers’ Organisation has a strong focus on sustainability and food labelling ensuring that information about food and drink products is not misleading and that sustainability of products is improved by reducing their impacts on the environment.
- International Federation of Organic Agricultural Movements (IFOAM) – IFOAM’s mission statement is declared as being ‘leading, uniting and assisting the organic movement in its full diversity’. Their goal is ‘the worldwide adoption of ecologically, socially and economically sound systems that are based on the principles of organic agriculture’. These principles are listed as being the principles of health, ecology, fairness and care:
    - Principle of Health. Organic agriculture should sustain and enhance the health of soil, plant, animal, human and planet as one and indivisible.
    - Principle of Ecology. Organic agriculture should be based on living ecological systems and cycles, work with them, emulate them and help sustain them.
    - Principle of Fairness. Organic agriculture should build on relationships that ensure fairness with regard to the common environment and life opportunities.
    - Principle of Care. Organic agriculture should be managed in a precautionary and responsible manner to protect the health and well-being of current and future generations and the environment.
- International Organic Accreditation Service (IOAS) – An independent, non profit organisation working on behalf of all involved in organic agriculture to ensure trust and fair trade in products labelled as organic. They verify the competence of certification bodies active in the field of organic and sustainable agriculture and work worldwide.
- Organic Trade Association (OTA) – The mission of North America based OTA is to promote and protect organic trade to benefit the environment, farmers, the public and the economy and envisions organic products becoming a significant part of everyday life, enhancing people’s lives and the environment.
- The Soil Association – This UK based organisation monitors the standards for organic produce in this region and often meets and exceeds the minimum standards set out by the UK government. They also provide certification to producers who meet their organic standards.
- Demeter – Demeter coordinates certification of bio-dynamic produce internationally.

EUROPEAN UNION

European Union (EU) –In June 2007, Council Regulation (EC) No. 834/2007 on organic production and labelling of organic products was published and it came into force on January 1, 2009. This repealed the former Regulation (EEC) No 2092/91. The accompanying implementing rules Commission Regulation (EC 889/2008) and (1235/2009) were adopted shortly after setting the detailed rules for operators. These documents together form the new rules on organic farming for the European Union. In drafting EC 834/2007, it was agreed between Member States that Community rules for organic winemaking should be developed. New rules for wine will be elaborated as a next step for these regulations. Current EU law provides for no definition of organic wine and as such does not allow it to appear on any wine label. Wine can be labelled as 'made from organically grown grapes' if the grower has certified organic status but there is no guarantee as to how the wine has been made. The old rules will continue until such time as new detailed rules are developed.

Labelling Requirements in the new EU regulations:

- Indication of any organic ingredients in the ingredients list becomes mandatory.
- Due to trouble drafting the new and mandatory EU logo which includes labelling requirements, this has been postponed to July 1, 2010.
- EU Community Logo may be used for imported goods from a third country. It may not be used for 'in-conversion' produce, products that contain less than 95% organic ingredients or products derived from hunting and fishing with organic ingredients. As such, the 70% labelling rule has since been deleted.
- Labelling of origin will become mandatory on July 1 2010. 'EU agriculture' must contain 98% of local ingredients.

UNITED STATES

The United States Department of Agriculture (USDA) has developed the National Organic Program (NOP) which delivers organic certification to produce in the United States. NOP coordinates the certification and accreditation of all American organic produce. You must apply to get organic certification by one of either 56 domestic certification agents or 44 foreign certification agents**.** Every domestic or imported organic product sold is required to adhere to the USDA's National Organic Program.

Certification

Any product in the United States that is labelled as "100% organic", "organic", or "made with organic ingredients", must be certified. Certification provides assurance that the product complies with the standards as directed by the NOP. These standards are comprehensive and cover every aspect of organic farming, processing, transportation, labelling and packaging. The standards specifically preclude the use of synthetic pesticides, herbicides and fungicides, antibiotics, growth hormones, sewage sludge, artificial fertilizers, GMOs and irradiation.

Labelling

The USDA's strict labelling rules are designed to appear only on organic food and will assist in understanding the exact organic content. Only products that have at least 95 percent of their ingredients derived from organic production methods are permitted to use the USDA Organic seal. The USDA Organic seal is basically an assurance to the customer that the food purchased is organic and protects both the consumer and producers from fraudulent claims. The United States also has no 'in conversion / transition category, unlike other nations, which makes their market quite inaccessible to newcomers to the organic trade. Food that is organic with only one ingredient may use the label. Labelling of foods with more than one ingredient will fall into one of four categories which include:

- **100% organic products** – must only contain organically produced ingredients. Eligible to display USDA organic seal but must display the certifying agency's name and seal.
- **At least 95% organic products** - must consist of at least 95 percent organically produced ingredients**.** Any remaining ingredients must be approved on the National List. These items are eligible to display the USDA Organic seal and must display the certifying agency's name and seal.
- **At least 70% organic products –** must consist of at least 70% organically produced ingredients. They may use the words "made with organic ingredients" and list the three main organic ingredients on the principal display panel. However. They are not eligible to display the USDA Organic seal.
- **Less than 70% organic products –** These are not permitted to use the term organic anywhere on the principal display panel. They are, however, entitled to identify, in the ingredients list, the specific ingredients used that have been produced organically. Still, they are not eligible to display the USDA Organic seal.

CANADA

Certification

Mandatory certification to a revised National Organic Standard was proposed in 2006 and implemented in 2008. The Canadian Food Inspection Agency is the Federal Government authority responsible for regulating the organic sector. The revised National Organic Standard, known as the Canadian Organic Production Systems Standard, comprises two sets of standards which describe the principles and management standards required of organic production systems as well as the list of substances permitted to be used in organic production. The standards are very comprehensive and cover every aspect of organic production, from planning and production right through to labelling and packaging.

The standards will protect consumers against false claims and will govern the use of the new Canada Organic logo. The logo will be voluntary to use, however, all products bearing the logo are required to comply with the Organic Product Regulations. Use of the logo will indicate that the product to which it is affixed has been certified as meeting the Canadian standard. Canada has produced general principles of organic farming which promote healthy and responsible agricultural practice.

Currently, compliance with the National Standard of Canada for Organic Agriculture is voluntary. Producers, however, choose to certify their produce as a way of validating and ensuring they retain their organic status. Certified products must comply with the

NSCOA and be independently certified as such by one of the certification bodies accredited by the Standards Council of Canada. In an agreement with USDA, CFIA will consider USDA certification equivalent to its own and vice versa.

Labelling

The following labelling conditions are enforced in Canada:

- Any product that refers to the term “organic” or similar on its principal display panel must have 95% or more of its ingredients derived from certified sources of organic production that are in accordance with the Standard.
- Any product that contains between 70% and 95% of its ingredients from certified sources of organic production is permitted to refer on its principal display panel to the actual percentage quantity of ingredients contained that have been derived from certified sources of organic production.
- Any product that contains less than 70% of its ingredients from certified sources of organic production is only permitted to list, on its ingredients panel, the particular organic ingredients it contains.

AUSTRALIA

The National Standard for Organic and Bio-dynamic Products AS 6000-2009 (the Standard) provides a framework for Australia’s organic industry. The Standard, which is produced by Food Standards Australia New Zealand (FSANZ) and is administered by the Australian Quarantine and Inspection Service (AQIS), clearly sets out minimum requirements for organic production, processing, transportation and labelling.

Certification

There are currently seven certification bodies accredited by AQIS to implement the Standard in regard to Australian produce. Many have differences based on whether the applicant is a primary producer (manufacturing or distribution) or in retail trade. The certification bodies include:

- National Association for Sustainable Agriculture (NASAA) – They have developed their own standard and certification steps for both within and outside Australia, accredited with IFOAM, JAS and USDA.
- Australian Certified Organic, the certification arm of Biological Farmers of Australia (BFA) – This is currently the largest in Australia certifying 55% of the organic industry.
- Organic Growers of Australia, their standard is developed and maintained by BFA and they provide certification for farmers and processors.
- The Organic Food Chain - gives access to USA and Japanese markets and use of their logo
- Safe Food Queensland
- Tasmanian Organic-Dynamic Producers (TOP) – uses the National Standard.
- Bio-dynamic Research Institute (BDRI)

Each of these certification bodies is responsible for ensuring that both the general principles and specific requirements of the Standard are being adhered to by the farmers, processors, transporters and retailers certified under their inspection system.
Certifiers randomly and periodically audit producers with approved organic statuses to ensure they are complying with the Standard.

Demeter is an organisation which provides certification and labelling regulation on biodynamic products. It encompasses four partner organisations, the Bio-Dynamic Agricultural Association of Australia (BDAAA), the Bio-Dynamic Research Institute (BDRI), the Bio-Dynamic Marketing Co Ltd. (BDMC) and the Bio-Dynamic Gardeners Association Inc. (BDGAI). To achieve Demeter Biodynamic Certification, a farm must demonstrate significant development of soil structure and humus building.

Labelling

Under the Australian Standard, certified organic products are required to display both the details of the certified operator, and the certifying organisation's details or logo/trademark. The following are the different types of organic labelling currently in Australia:

- 100% organic products must have all of their ingredients derived from organic production methods.
- Organic products must have at least 95% of their ingredients derived from organic production methods. Any remaining product ingredients must consist only of approved substances, meaning that genetically modified technology is banned and anything which disrupts the natural metabolism of livestock and plants is not approved. Any ingredient not derived from organic production must be clearly indicated as such in the ingredients
- Made with organic ingredients means that the product must have at least 70% of its ingredients derived from organic production methods. Any remaining product ingredients must consist only of approved substances. Any ingredient not derived from organic production must be clearly indicated as such in the ingredients
- Products containing less than 70% organic ingredients cannot use the term organic on the principal display panel. However, reference can be made in the ingredients list of the product to any ingredients used that have been derived from organic production methods
- In-conversion refers to a production system that complies with the Standard for at least one year but has not yet achieved full organic certification. It takes up to three years before organic certification can be fully obtained by producers.

NEW ZEALAND

The Official Organic Assurance Program (OOAP) provides a framework for New Zealand's organic industry. The OOAP, which is administered by the New Zealand Food Safety Authority (NZFSA), was originally introduced as a means of ensuring New Zealand's organic exporters would have continued access to markets in the United States and Europe. Legislative changes in these markets resulted in strict criteria being set for imports of organic products. The NZFSA OOAP complies with the very strict and legally enforceable European Community Regulations EC 2901 governing organic production. It has also been accepted by the USDA for recognition of organic certifying bodies.

Certification

While New Zealand does not currently have a uniform domestic standard for organic production, each of the three certification bodies involved with the OOAP provide domestic certification services. The three certification bodies include:

- AsureQuality – owned by the New Zealand Government.

- Bio Dynamic Farming and Gardening Association – works with Demeter to certify biodynamic produce. Inspectors will visit operators annually to gather information about methods. Assessors containing experienced biodynamic farmers / processors will then convene to decide whether to grant certification.
- BioGro NZ (New Zealand Biological Producers and Consumers Council Inc) – works internationally with IFOAM And JAS

Their aim, in doing so, is to protect New Zealand consumers against deception and fraud in the market place by ensuring that all stages of domestic organic production, preparation, storage, transport and marketing adhere to predefined standards and are subject to both routine and random inspection. Each of the three certification bodies has established minimum standards which must be adhered to by domestic producers.

ARGENTINA

Certificadora Argentina (OIA) is the certification agency responsible for organic produce in both domestic and international markets for Argentina. The certification program covers primary production, processing and marketing. OIA is registered at SENASA (National Agri-food Health & Quality Service - Argentina) and is recognised by the EU and accredited by IFOAM (International Federation of Organic Agriculture Movements), which provide for the certification. OIA has agreements with Biosuisse, Soil Association UK and the Conseil D'Acreditation du Québec (CAQ) to allow its associated certified producers, processors and distributors to access other organic markets like the State of Québec (Canada), or to use recognized seals like Biosuisse (Switzerland) or Soil Association (United Kingdom) in displaying organic logos on their produce. OIA has the power to certify organic projects in Argentina, Brazil and Nicaragua and is a major player in the certification of organic produce in much of South America.

Argencert is also a certification body that allows all certified operators in Argentina sell products both domestically and throughout Europe. They comply with SENASA domestically and also maintain compliance with IFOAM’s (International Federation of Organic Movements) specific requirements which allows the use of the IFOAM seal on Argentine products. It has close ties with NOP and much of its inspection programs are undertaken by Bio Suisse (Switzerland) or JONA (Japanese Organic and Natural Food Association)

CHILE

In Chile there are three main active certification bodies; one national (Certificadora Chile Organico, CCO) and two international (the Swiss company IMO and the German company BCS Oko-Garantie). Chile’s organic certification and labelling requirements are monitored by the Chilean SAG (Agriculture and Livestock Service) to certify organic production in Chile under Law 20.089, Regulation DS N°36 and Technical Norms DS N°17. This legislation was created as the National Certification System for Organic Agricultural Products and has been in force since December 24, 2007.
NSF International also provides organic certification in Chile through Quality Assurance International (QAI). The organic producers in Chile produce wine mainly for exportation into the international market.

SOUTH AFRICA

There are currently six organic certification organisations active in South Africa:

- Africa’s Farms Certified Organic for the South American Market (AFRISCO) - connected with French certifier ECOCERT
- Organic Agriculture Association of South Africa, now known as Organics SA (OSA)
- The Bio-Dynamic and Organic Certification Authority (BDOCA) – connected to certifier Debio from Norway.
- Control Union (CUC) – affiliated with SKAL (Netherlands)
- Soil Association Certification Ltd – operates in South Africa
- SGS in South Africa

If a product is labelled as being Certified Organic, it authenticates that the growing and manufacturing of the product adheres to worldwide standards based on the principles and practices of organic food production. Potential members must apply stating their organic farming practices. If these are considered in line with the standards set then an inspection will occur, and if after inspection, OSA or AFRISCO are satisfied they will approve the producer as organic. AFRISCO applies mainly as an organic certification for African export to international markets whereas OSA is concerned with certifying organic produce for South African producers and supplying them with support and information.

ANNEX II: **COMPARISON OF US AND EU STANDARDS**

(Using the NOP US organic standard (2001) and the new EC 834/2007 and supporting documents)

	US STANDARD	EU STANDARD
Both share similar	Third Party Certification	
	Audit Trails	
	Annual Inspections	
	Accreditation	
	Materials Lists	
	Defined Conversion Periods	
	Sustainable Farm Plan	
Labelling Requirements:		
Organic	At least 95% must be organic	Only products containing more than 95% may be labelled as organic
Made with	70% must be organic	Prohibits any mention of organic on the labelling
Below 70%	Permits identification of organic ingredients on information panel on products over 50% organic	Prohibits any mention of organic on the labelling
Percent declarations	Not mandatory	May be mandatory in certain member states
In Transition / Conversion period	Not allowed	Not allowed
Cultural and Political Differences		
Cultural	One language for all member states and similar cultures	Made up of many nations with various languages and differing cultures
Political	Federal laws supersede state laws	Any of the 15 sovereign member states may grant exceptions to any of the rules and regulations
Materials and Materials Process		
Materials	Natural materials are allowed unless prohibited – synthetics are prohibited unless allowed	Are listed in positive lists. That is, use of inputs is prohibited with the exception of those explicitly listed on positive lists.
Cropping standards		
Conversion periods	Mandatory 3 years, no exceptions	Under old system, it could be interpreted as being shorter. Under new rules to be

		introduced, retrospective recognition of conversion is regulated, and loopholes will be fixed.
Manure restrictions	Requires minimum periods prior to harvest - must be composted within 120 days of harvest	Has a load limit on manure applications for organic cropping operations – manure from organic farms preferred and load limits on a per acre basis required.
Other Areas		
Market access	Has federal authority and will have access to all states	Does not guarantee access to all sovereign states. Has improved with changes to regulations. Non EU certification bodies can prove their equivalent status.
Processing	Observes detailed handling regulations	Processing regulations are referenced in the EU inspection regulations, and certifier verifies organic compliance
Accreditation	Federal authority dictates accreditation and USDA accredits certifiers	Accreditation is by designated accreditation bodies and peer review – each member state is responsible for ensuring that certifiers operate according to the inspection requirements as laid out in the regulation.

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ARTICULER LES QUALITES ENVIRONNEMENTALE ET GUSTATIVE DES VINS

Geneviève Teil
INRA SAD APT-PRAGMA
16 rue Claude Bernard, 75005 Paris
genevieve.teil@agroparistech.fr

RÉSUMÉ : Le vin est l'objet d'une qualification gustative, signalée ou non par des signes de qualité, qui structure très fortement sa circulation marchande. Comment cette qualification gustative peut-elle s'articuler avec une qualification environnementale ?

Cette communication[1] décrit et analyse les trois grands régimes de qualification qui se sont développés récemment en France pour créer une différence environnementale sur les vins. Ces différentes articulations entre qualité gustative et qualité environnementale ont récemment engendré de doubles redéfinitions, parfois difficiles, des qualités environnementales et gustatives.

Côté « bio », c'est la récente crise viticole qui a amené une nouvelle réflexion sur le rôle de l'économie et la notion de goût du vin sain. Cette diversification a créé des tensions au sein du mouvement bio, mais aussi un rapprochement entre vins « de qualité » et vins bio qui ont ainsi pu surmonter en partie « la mauvaise réputation » dont ils étaient l'objet.

Les producteurs qui préconisent « un retour au terroir » ont eux aussi, de leur côté, instauré un lien entre goût et environnement et demandé une réforme des AOC pour contrer l'affadissement de la qualité de terroir due, selon eux, à des années de pratiques viticoles irréfléchies. Le projet achoppe sur deux options différentes entre producteurs et administration en matière de certification. Pour les producteurs, une certification de terroir doit plutôt s'appuyer sur une certification de l'engagement des producteurs dans la recherche du terroir et non sur une certification de résultat. Un problème similaire à celui des certifications d'agriculture raisonnée.

ABSTRACT : The wine economics are strongly structured by taste quality signs. How can another environmental qualification add to this one?

This communication[2] describes and analyzes the three qualification regimes that have develop recently in France in order to make an environmental difference matter. The out-coming link-ups of taste and environmental quality have fostered double and sometimes difficult redefinitions of both environment and taste quality.

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[1] Cette communication présente les résultats d'une étude empirique menée principalement à propos des vignobles de Languedoc Roussillon et de Pays de Loire : elle repose sur une enquête de terrain auprès de plus de 280 acteurs de l'ensemble de la filière vin, des producteurs aux consommateurs en passant par les distributeurs, critiques, et pouvoirs publics…

[2] This communication presents the results of an empirical study mostly grounding upon the Languedoc Roussillon and de Pays de Loire cases. The field study includes the interview of more than 280 actors belonging to the whole wine chain: producers, wine drinkers, retailers, wine critics, restaurants, administrations…

The recent wine crisis has led the organic wine producers to reinterpret the place of economics and the notion of “good healthy taste”. This diversification of meanings created social strain among the organic vine-growers, but also a convergence between “quality” and organic wines, which helped organic wine makers to partly overcome their “bad fame”.

Producers in favour of a come back to the “truth of terroir” have on their own side reinterpreted the links between environment and taste and asked for a reform of the AOC quality sign in order to stop the AOC quality shading-off due, according to them, to years and years of bad viticulture practices. This project stumbles on two different options defended by the administration and the producers regarding the quality certification. For the producers AOC should ground upon the certification of the commitment of the producers towards the search for terroir and not on a result test. This is quite similar to the problem faced by integrated farming certifications.

INTRODUCTION

En matière d'environnement, la vigne, culture pérenne, focalise les regards, car les vignerons font un grand usage des produits phytosanitaires. Quelques facteurs reviennent pour expliquer cette très grosse consommation : la valeur ajoutée exceptionnelle du produit agricole vin qui rend le coût des pesticides très faible en regard des gains de productivité ; trois « impasses » techniques, l'esca, la flavescence et le court noué qui empêchent les vignerons d'adopter le bio et de réduire ainsi leur consommation. Les difficultés ne manquent donc pas ; mais il faut encore en ajouter une autre. Les qualifications environnementales destinées à les surmonter doivent s'ajuster aux autres qualifications qui structurent très fortement le marché vinicole.

MATERIELS ET METHODES

Cet article s'appuie sur les résultats d'un programme de recherche financé par le Ministère de l'Écologie, du Logement et de l'Aménagement du Territoire. (MELAT), « *Les vins sans pesticides ? une analyse de la prescription à la consommation* » (Teil et al., 2007) (Teil et al., à paraître). Il avait pour objet de montrer comment les acteurs parviennent à faire valoir une qualité environnementale de leurs vins et en particulier comment ils l'articulent ou non à leurs prétentions qualitatives. L'enquête de terrain a porté plus particulièrement sur les vins de Languedoc Roussillon et des Pays de Loire où, comme en Alsace, les questions environnementales semblaient plus débattues que dans le Bordelais, la Champagne ou la Bourgogne, par exemple. Elle a permis la constitution d'un large corpus de plus de 230 entretiens menés auprès de plus de 280 acteurs, producteurs, directeurs de coopératives, revendeurs, acheteurs, critiques, restaurateurs, pouvoirs publics, chercheurs et techniciens, employés de firmes phytosanitaires…

Ce programme a mis en évidence trois grands régimes marchands *faisant valoir* une qualité environnementale des vins : la viticulture raisonnée, les agrobiologies et les vins de terroir. Ils forment des collectifs qui promeuvent le changement des pratiques et peuvent servir de relais aux pouvoirs publics, s'ils sont en mesure de se développer.

Les trois sont relativement indépendants, voire même en concurrence : faire du raisonné ou du bio, c'est souvent faire un choix exclusif entre deux conceptions concurrentes de l'environnement, mais aussi de la qualité des vins et du rôle de l'économie. Néanmoins, ces régimes marchands peuvent aussi partiellement se fondre ; ainsi des vins bio se retrouvent aujourd'hui dans les circuits marchands de certains vins de terroir. Ces régimes marchands ne sont donc pas des classes mais recouvrent des collectifs variables de producteurs, de distributeurs, de critiques, d'acheteurs et de vins qui dépendent des interprétations complexes et de détail que chacun peut faire de la « qualité environnementale du vin ».

RESULTATS ET DISCUSSION

Cet article commence par rapporter brièvement le cas des agrobiologies et les controverses qui ont animé ce collectif au sujet de l'économie, puis « l'impasse » commerciale qu'elle a dû affronter au sujet de la « qualité des vins ». Il décrit ensuite le mouvement alternatif de la viticulture raisonnée qui a tenté de mettre en place une autre façon de protéger l'environnement dans les vignobles, sans mettre en cause la qualité gustative des vins. Mais ce sont finalement les vins en quête d'authenticité gustative qui ont transformé le paysage : dénonçant la dérive économique et technologique de la qualité des vins, ils prônent un retour au terroir et leur protection grâce à des pratiques respectueuses de l'environnement. Ils ont ainsi contribué à briser l'association négative entre ces pratiques et la qualité gustative des vins.

Les qualifications agrobiologiques des vins

Dans les années 1980, les viticulteurs qui se disent bio dénoncent tous ou presque, l'économie comme cause de la mauvaise qualité de la production alimentaire. L'intensification de la production qu'elle induit est mauvaise pour l'environnement, la santé, le goût… Pour eux, le bio est une qualité agronomique, une autre façon de cultiver qui refuse les techniques agricoles productivistes : la spécialisation à outrance, les engrais et produits de traitement de synthèse. C'est aussi une autre façon de penser l'agriculture qui se méfie des effets que l'économie peut avoir sur la production agricole, car c'est l'incessante recherche de productivité qui a amené la baisse qualitative ou les aberrations agronomiques et socio-économiques - la désertification des campagnes - qu'ils dénoncent. Pour s'en prémunir, ils cherchent donc à mettre en place une organisation marchande alternative où les notions de qualité des produits, d'équilibre socio-économique, de respect de l'environnement, des consommateurs et partenaires marchands, sont remis au cœur des préoccupations des acteurs.

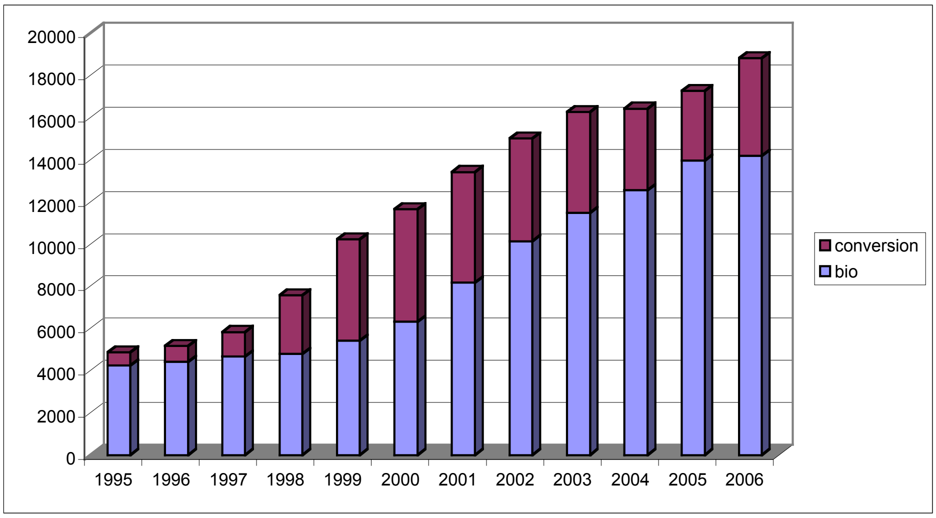
Ils militent pour obtenir la reconnaissance de leur différence sous la forme d'un label public apposé sur les produits issus de l'agriculture biologique et contrôlé par des organismes indépendants, afin que le résultat de leur action, les produits biologiques, soient reconnus par le plus grand nombre comme étant des produits différents des autres. Tous les bio ne voient pas cette initiative d'un bon œil. Une poignée de militants craignent en effet que cette reconnaissance officielle ne transforme la qualité bio en une simple recette « récupérée » par « l'agroéconomie productiviste ». Malgré cette opposition - assez peu nombreuse, semble-t-il - le décret européen du label AB est publié en 1991. Il porte exclusivement sur le volet « agronomique » et prohibe l'ensemble des produits de synthèse dans les pratiques agricoles.

La création du label AB va de pair avec une « standardisation » de la qualité bio : 9 des 11 cahiers des charges privés qui préexistaient au label disparaissent. Seulement deux lui résistent difficilement : Nature et Progrès et Déméter pour ce qui concerne la viticulture.

En matière vinicole, l'appellation « vin bio » n'est pas autorisée non plus que l'apposition du label AB sur les bouteilles de vin, seule la mention « vin de raisins issus de l'agriculture biologique l'est, à cause, notamment, de l'ajout de soufre dans les vins pour leur conservation, produit non autorisé dans les produits bio.

La croissance de la viticulture bio

Les statistiques montrent néanmoins que la viticulture sous label « AB » se développe, de nouveaux vignerons qui se convertissent. Les agrobiologistes réclamaient aussi des pouvoirs publics qu'ils prennent en charge les frais de contrôle au nom du soutien à apporter au développement de cette agriculture alternative. Cette demande qui n'aboutit pas ; en revanche ils obtiennent quelques années plus tard la création d'aides à la conversion.



Les surfaces en vigne en mode de production biologique en France Source Agence bio : 2006

Le bio se développe mais très – trop – lentement pour ses militants. Pour quelques vignerons totalement minoritaires dans les années 80-90, cette lenteur est due à ce que le label AB a adopté une « stratégie de niche », un confinement du bio à des réseaux d'échange économiques et une clientèle bio qui empêche son développement. Cette situation ne peut qu'amener une saturation des marchés qui sera encore aggravée par les aides à la conversion. Pour développer le bio, il faut selon eux lutter contre ce confinement, et donc ne pas craindre les marchés ou se protéger de la rationalité économique ou des techniques marketing, mais les utiliser. Ils optent, contre l'esprit bio le plus répandu, pour une rationalisation et une croissance de leur production qui permet d'augmenter les volumes, d'alimenter les grands réseaux de distribution nationaux ou internationaux.

L'opposition qu'ils rencontrent les amène à dénoncer ce qu'ils appellent l'esprit « sectaire » de la bio. Bien sûr les relations sont tendues au début, dans les années 1985-1995, entre partisans et adversaires de l'économie, mais ces partisans sont très peu nombreux, du moins au début.

L'impossible qualité bio du vin

La grande majorité des producteurs de vin bio exportent de 50 à 100% de leur production et alimentent les marchés du nord de l'Europe ou des Etats Unis, du Canada et de l'Asie, un marché convoité pour sa « demande », ses marges commerciales et son allègement de la tâche de commercialisation. Car sur le marché français, les vins bio ne se vendent pas en dehors des magasins « bio » qui n'écoulent que de très faibles quantités : lorsqu'ils ne sont pas vignerons, les clients bio ne sont pas de grands consommateurs de vin. A la fin des années 90, après la « crise de la vache folle », la grande distribution s'intéresse au bio. Mais le vin issu de l'agriculture biologique a du mal à trouver sa place : ce n'est ni un produit « santé » ou diététique, comme la plupart des produits bio alors. Au milieu des autres vins, il ne se vend pas : sa différence environnementale n'apparaît pas clairement, car pour la plupart des acheteurs de vins, le vin est « déjà » un produit naturel. La notion de « vin bio » apparaît au mieux comme un pléonasme, au pire comme un vin de qualité gustative douteuse. Les amateurs de vin semblent penser qu'à se priver des moyens conventionnels pour faire des vins de qualité, les vignerons bio ne peuvent maîtriser la qualité et mettent sur la marché des produits chers et plutôt moins bons que les autres. Hors de la distribution spécialisée, le label

AB est inutile, voire préjudiciable. Les quelques vignerons bio qui sont engagés dans la différenciation qualitative gustative de leurs vins ne l'affichent pas et distribuent leurs vins dans les circuits vinicoles ordinaires.

De même les vignerons bio « industriels » ou « business », comme on les appelle parfois, qui cherchent à utiliser les ressorts de l'économie marchande pour diffuser leurs produits, à l'export mais aussi sur le marché national, notamment en dehors des circuits bio, commercialisent leurs produits aux conditions de vente des vins ordinaires, non bio. Le bio est un « plus » gratuit pour le consommateur. Ils ne font pas valoir la différence par les prix, ne font pas de publicité, ils cherchent d'abord à rendre la qualité bio accessible pour qu'elle se fasse connaître et diffuse partout. Ils contournent ainsi la difficulté de l'apriori négatif des consommateurs envers les vins bio.

À la fin des années 1990, la réunification allemande et des tensions internationales notamment au sujet du nucléaire, rendent l'exportation de vins français un peu plus difficile ; l'export connaît en 2000 un fort recul sur les vins de table. L'érosion des exportations est plus lente, mais finit aussi par toucher les vins d'AOC. À partir de 2002, la commercialisation des vins devient de plus en plus difficile. Cette « crise » amène une nouvelle vague de conversions : ce sont des vignerons pour lesquels la qualification AB représente clairement une opportunité de marché pour une production qu'ils peinent à écouler. Quoiqu'ils puissent se sentir victimes de la crise, ils ne jettent pas nécessairement la pierre à l'économie. La qualité bio est pour eux avant tout une certification environnementale qui donne accès à des marchés « à l'abri de la crise ». De même que les « anti-niche bio » ou « bio-business », ils n'appuient la qualité bio sur aucun rejet de l'économie. Mais, à la différence des précédents, ils veulent « valoriser » leur qualification, soit parce que leur conversion leur « coûte cher » en baisse de rendements ou en investissement, soit parce qu'ils pensent que le bio est un surplus de qualité qui doit, pour faire sens ou être crédible, s'accompagner d'un surcoût. Leurs vins bio sont donc plus chers que les autres vins conventionnels.

Ces stratégies « économiques » poursuivies par les nouveaux convertis sont pointées du doigt par les partisans du bio éco-alternatif qui appellent ces nouveaux arrivants, des « chasseurs de primes ». Pour eux ce sont des opportunistes qui ne sont pas concernés par les problèmes environnementaux. Leurs stratégies avant tout commerciales ne peuvent que miner la capacité de différenciation du label AB en le tirant vers une interprétation de l'agrobiologie minimaliste, non réfléchie, voire frauduleuse compte tenu des difficultés techniques et commerciales qu'ils rencontrent. Ces derniers rétorquent qu'ils ne peuvent pas contribuer à une usure du label par une quelconque pression exercée sur les contraintes, parce qu'ils ont d'autant plus besoin d'un label fort et crédible que la situation commerciale se tend !

En 2003 cependant, La Fédération Nationale Interprofessionnelle des Vins de l'Agriculture Biologique et les deux associations Déméter et Nature et Progrès déposent des chartes privées du vin bio[3] qui limitent les ajouts de soufre et plus généralement les tous les additifs autorisés en vinification. Les deux associations Déméter et Nature et Progrès renforcent aussi les volets socio-économiques de leurs chartes privées qui insistent sur la nécessité de mettre en place d'autres logiques et solidarités économiques :

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[3] En 2005, l'apposition du label AB sur les bouteilles est autorisée.

> « Face au biologique intensif et indifférent aux problèmes de la désertification des campagnes, des relations Nord-Sud, des coûts énergétiques, et plus globalement des problèmes écologiques majeurs, il est nécessaire de promouvoir une agriculture biologique paysanne privilégiant les circuits courts de distribution (marchés, coopératives, etc.), une solidarité. Lorsqu'ils sont indispensables, les circuits plus longs doivent s'appuyer sur une solidarité urbains-ruraux et des échanges équitables susceptibles d'inverser les logiques marchandes destructrices pour la paysannerie et l'environnement.
>
> °La biodiversité de tous les organismes vivants est en voie de destruction, pas seulement à cause des menaces que font courir les OGM, mais aussi à cause du système économique et financier dominant qui condamne également tous les paysans de la planète. » (Nature et progrès, 2003) : 1-2

Quinze ans après le décret européen instituant la reconnaissance de la qualité biologique, deux grandes interprétations de la qualité bio coexistent tout en divergeant fortement quant aux relations qu'il faut entretenir avec les structures économiques. Les bio dits « industriels » ou « business » veulent faire du marché un outil de leur diffusion ; ils s'opposent aux bio « éco-alternatifs » pour lesquels ce recours aux organisations marchandes conventionnelles signe la perte de signification à plus ou moins long terme du bio, car elle soumet la qualité bio à des calculs de rentabilité qui ne peuvent qu'aboutir à l'érosion de sa différence. Mais aucune des deux ne réussit à faire valoir la qualité bio sur le marché des vins en France. Le vin bio est un « marché » à part et la qualification bio préjudiciable à la défense d'une qualité gustative des vins.

Le plus de la viticulture raisonnée et la faillite de son élargissement

Parallèlement à la qualification bio se développe la viticulture raisonnée. Contrairement au bio, elle ne cherche pas à faire l'impasse sur la chimie de synthèse, mais à en réfléchir les usages. Ces produits sont reconnus nocifs, pour les viticulteurs comme pour l'environnement et les promoteurs de la viticulture raisonnée cherchent donc à en diminuer drastiquement l'usage, et écarter les produits les plus toxiques au profit d'autres qui le sont moins, en adoptant notamment des techniques de lutte intégrée ou de gestion des faunes auxiliaires et de la biodiversité. Vers 1990, des vignerons du Beaujolais qui exportent beaucoup vers le japon notamment fondent Terra Vitis, dans le but de faire connaître et promouvoir cette autre façon de cultiver la vigne en respectant l'environnement.

Le collectif est initialement constitué de « techniciens », des vignerons qui cherchent ensemble à faire évoluer leurs techniques en partageant leurs expériences. Or les vignerons exportateurs, cette démarche est assez bien accueillie par leurs exportateurs ; parfois aussi, ils le font « à titre privé », par respect du consommateur, et ne cherchent pas à faire valoir cette information sur leurs bouteilles. La viticulture raisonnée fait des émules, de nombreuses associations locales promeuvent cette autre façon de respecter l'environnement dans les signes qui se regroupent derrière Terra Vitis devenue une fédération. Avec l'aide d'institut techniques, elle encadre la mise au point de cahiers des charges locaux qui s'appuient sur un noyau de pratiques commun à tous les vignobles auquel se rajoutent des contraintes plus sévères et propres à chaque vignoble. Ce cahier des charges fait apparaître un seuil différentiel entre les agricultures conventionnelle et raisonnée ; il est remis à jour périodiquement pour suivre l'évolution des produits phytosanitaires disponibles et durcir progressivement ses contraintes. En 2001, Terra Vitis dépose un logo avec ces cahiers des charge pour aider la reconnaissance publique de sa démarche.

Le logo Terra Vitis ne permet pas de meilleures marges, mais octroie souvent une préférence à l'achat à l'export. Présentée comme une lutte contre le gaspillage et les traitements inutiles, cette qualification environnementale ne génère donc pas les mêmes doutes que l’agriculture bio auprès du public des amateurs de vins. Ce « plus environnemental » amène de nouveaux adhérents dans les associations qui doivent composer en le souci d’avance technique des pionniers et la volonté de faire reconnaître et valoriser un cahier des charges stabilisé sur les marchés.

Mais ce n’est pas tant le grossissement du collectif qui fragilise le plus la viticulture raisonnée que son élargissement à l’ensemble de l’agriculture.

En effet, Terra Vitis est un regroupement d’initiatives régionales où le souci environnemental a été pensé de façon très intégrée à des écosystèmes, des conditions agro-climatiques aux variations délimitées. Le regroupement des associations a conduit à la mise au point d’un cahier des charges commun à tous les vignobles moins contraignant mais ensuite enrichi de nouvelles obligations particulières à chaque vignoble. La mise au point d’un cahier des charges commun non seulement à toute la France, mais aussi à l’ensemble de l’agriculture amène un noyau commun de pratiques qui se différencient assez peu des pratiques de l’agriculture conventionnelle.

Cependant, les concepteurs de la démarche en font un avantage. Un cahier des charges peu exigent permet d’envisager une qualification en masse des agriculteurs et d’éviter que se crée un différentiel entre une agriculture « propre », raisonnée, et une agriculture « sale », conventionnelle. La qualification agriculture raisonnée est conçue comme une procédure de transformation minimale des pratiques, mais qui doit, comme Terra Vitis, se réviser en permanence pour alimenter une « boucle de progrès ». L’Agriculture raisonnée est en quelque sorte une qualification subliminale, qui passe presque inaperçue pour l’agriculteur, au moins dans sa comptabilité. Ne pas vider un fond de cuve au fossé ne représente aucun coût, tout comme faire attention aux doses recommander et les respecter. Certaines de ces pratiques, comme l’abandon des traitements systématiques occasionnent même des économies. Faire attention ne coûte rien au départ, mais peut avoir de grandes conséquences, car une fois que l’agriculteur commence à faire attention, à réfléchir et se préoccuper d’environnement, c’est l’ensemble de ses pratiques qu’il sera ainsi être amené à réviser, à son rythme, et selon les spécificités de son exploitation. En revanche, il faut s’assurer qu’il s’engage bien dans une réflexion environnementale, et la qualification AR épouse à cet effet la structure des normes ISO 14000, des normes qui ne portent ni sur le résultat, ni sur les pratiques, mais sur l’engagement des personnes à réfléchir au respect de l’environnement et en déduire des modalités de transformation de leurs pratiques. Le contrôle de la qualification porte donc sur l’explicitation de cet engagement à travers l’élaboration de diagnostics et de plans de progrès dont les résultats sont analysés par l’agriculteur qui se qualifie au vu de ses résultats et qui le réoriente et le relance, générant ainsi la boucle de progrès attendue. L’agriculture raisonnée doit générer une dynamique : peu importe la taille des petits pas accomplis, ce qui importe c’est d’avancer et de ne jamais s’arrêter.

À sa sortie en 2002, elle est jugée comme n’importe quel signe de qualité, c'est-à-dire comme une garantie de résultat ou de moyens. Or, elle ne garantit initialement pas grand chose puisque l’esprit de la qualification est précisément de ne pas faire trop de différence pour que l’adhésion de l’ensemble des agriculteurs se fasse de façon quasiment naturelle, sans effort. La qualification agriculture raisonnée est alors dénoncée comme supercherie, une accusation qui rallie tous les suffrages, depuis les agrobiologistes pour lesquels l’agriculture raisonnée ne rompt ni avec « la grande industrie chimique » ni avec « l’industrialisation de l’agriculture » jusqu’aux agriculteurs qui rejettent l’accusation de polluer les campagnes. De plus, afin de devenir le standard de base de toute l'agriculture, la qualification Agriculture raisonnée s'est approprié le nom d'« agriculture raisonnée » et contraint les autres cahiers des charges, en particulier ceux de viticulture raisonnée, à en passer par la qualification, pourtant bien moins exigeante que le cahier des charges Terra Vitis, pour avoir le droit de se réclamer de l'agriculture raisonnée. Elle entraine alors dans sa chute une désaffection au sein même de Terra Vitis : les adhérents jugent en effet que c'est leur compétence technique même qui est déniée par cette qualification qui repose pourtant sur du vent. La qualification nationale devient ainsi un pantin inventé par les firmes phytosanitaires pour lutter à peu de frais contre leur réputation de pollueur.

Il semble que l'agriculture raisonnée ne se soit pas encore remise de ces événements malgré les efforts déployés par l'association FARRE et les accords passés avec Terra Vitis pour diminuer les effets de la double qualification demandée à ses adhérents. Mais l'agriculture raisonnée continue à vivre et se développer comme volet des dispositifs de certification de qualité industrielles, mais aussi dans un troisième régime d'action marchande, celui qui s'est développé autour de la notion de « retour à la qualité de terroir ».

Les vins de qualité et la protection des terroirs

Depuis quelques années, des vignerons dénoncent une double dérive de la « qualité de terroir » au sein des vins d'AOC français. Ces militants de la qualité de terroir accusent d'une part « l'abandon de la qualité de terroir » par les vignerons qui préfèrent ajuster la qualité de leurs vins à la demande internationale ou encore aux critiques les plus réputés et d'autre part la « logique productiviste » et ses ajouts massifs d'intrants étrangers au terroir qui en masquent l'expression. Pour lutter contre ces dérives ils se livrent à un dépouillement et une réinvention de la vitiviniculture pour retrouver le véritable goût de leurs terroirs[4]. Changeant leurs pratiques, le goût de leurs vins change, ce qui est interprété par les producteurs de ces vins comme un retour de l'expression de terroir dans leurs vins.

Pourtant depuis quelques années, certains de ces vins sont refusés à l'agrément des AOC : les jurys de dégustation y pointent un manque de typicité. Suivant la procédure, ce jugement est assorti d'un ensemble de corrections à apporter aux vins pour les faire rentrer dans le type requis afin de réussir la « session de rattrapage » proposée à tous les vignerons. Bien entendu, pour les auteurs de ces vins, c'est l'appréciation de la typicité qui est déformée par les mauvaises habitudes ou les compromissions des jurés et non les vins par les bonnes pratiques respectueuses des terroirs. Et les recommandations pour « arranger » les vins sont un comble qui achève de signer l'égarement de ces jurys, ignorants en matière de terroir mais experts en maquillage et fabrication de goûts à la demande.

Ce débat sur le terroir dans les AOC a amené l'INAO à envisager une réforme des règlements des AOC viticoles afin de renforcer la place du terroir dans la qualité des vins d'AOC. Les contraintes de production ont été revues ; la dégustation d'agrément également. Mais sa révision achoppe sur la question des critères gustatifs permettant de certifier la

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[4] Parmi les pratiques respectueuses des terroirs, les agrobiologies et la biodynamie – mais aussi l'agriculture raisonnée – tiennent une place de choix, car elles ont appris à limiter le recours à de nombreux intrants très souvent accusés de masquer le terroir. La biodynamie est certainement la théorie agronomique qui pousse au plus loin cette limitation cadrée de l'intervention du vigneron dans ses vignes et connaît donc un réel succès parmi les promoteurs de cette recherche du terroir.

présence du terroir. Pour l'administration, française ou européenne, un test gustatif est indispensable pour justifier un étiquetage discriminant sur les bouteilles. Mais les discussions achoppent sur la question des critères du goût de terroir. Trois points de vue différents s'expriment à ce sujet : les sciences du sol et agronomiques, les sciences du goût et les producteurs défenseurs militants du terroir.

Les premiers ont engagé de nombreuses recherches pour définir et caractériser les différents terroirs viticoles (Bohmrich, 1996; Morlat, 1998; Saxton, 2002; Fischer et Bauer, 2006; White et al., 2007). Mais leurs résultats sont décevants. Les tentatives de définition du terroir aboutissent à une liste toujours plus longue de facteurs à prendre en compte (Turner et Creasy, 2003; van Leeuwen et al., 2004; Deloire et al., 2005), tandis que celle des traits gustatifs caractéristiques s'amenuise. Et les pessimistes s'accordent à considérer le terroir comme une construction culturelle ou sociale (Gade, 2009), une « production humaine » (Berard et Marchenay, 2000) (Demossier, 2000) (Gade, 2004 ; Crenn et Techoueyres, 2007) et non un fait de la nature (Deloire et al., 2008).

Les sciences du goût se sont de leur côté penchées sur une classification discriminante des différents goûts des vins en fonction de leur terroir d'origine. Cette seconde stratégie a elle aussi échoué et amené une conclusion un peu différente. Ce n'est pas le terroir mais le goût lui-même qui est en cause : la recherche de correspondances entre des vins et des goûts a pointé une « irréductible » variété des goûts ce qui amène ces chercheurs à conclure au caractère résolument idiosyncrasique et incommensurable du goût (Mac Léod, 2008). Peu importe donc que le terroir soit une notion bien ou mal fondée, une construction sociale ou un fait naturel, puisque le goût lui-même amène une divergence totale des perceptions.

L'idée d'un test gustatif certifiant la présence du terroir est donc pour ces scientifiques doublement infondée. Elle l'est aussi pour les vignerons militants du terroir, mais pour d'autres raisons. Pour eux, la notion de terroir n'est aucunement infondée : le changement de pratiques qu'ils ont amorcé amène d'ailleurs un changement de goûts considérable qui les confirme dans leur démarche de réinvention de la viticulture. Mais l'idée de tester sa présence du terroir à l'aide de quelques indicateurs est trop réductrice. Des années de mauvaises pratiques ont induit une lente dérive du goût et la perte de la notion de terroir. Retrouver le goût du terroir suppose une quête dont il n'admettent pas qu'on puisse l'orienter ou la limiter *a priori* à l'aide de critères qui s'avèreront peut-être caduques demain.

Faute de test, la réforme semble suspendue. Mais elle ne saurait le rester trop longtemps sans mettre en péril l'ensemble de la législation déjà existante des AOC : les AOC étaient jusque-là dénoncées de l'extérieur, par des partisans d'une mise en marché appuyée sur la « demande » qui accusaient les AOC de n'être qu'une barrière marchande ; elles le sont aujourd'hui de l'intérieur, par les défenseurs de la qualité de terroir eux-mêmes. Il est donc urgent de savoir ce qu'est « le terroir ».

Le terroir a-t-il une réalité ?

Pour les agronomes, le terroir a une réalité s'il peut être « réduit » à une description scientifique théorique résistante qui inventorie l'ensemble des différentes contributions au terroir, ses facteurs, et rend compte de la diversité ordonnée des terroirs empiriques. C'est de l'impossibilité de mener à bien ce travail que les agronomes concluent que la notion de terroir est infondée, qu'elle n'a pas de réalité ou du moins de réalité « objective », car elle peut aussi être issue de « l'imagination » ou résulter de l'activité sociale, collective des acteurs.

Les sciences du goût procèdent autrement, non pas par inventaire des facteurs du goût, mais en tentant de construire un lien discriminant entre des produits de terroir et des représentations, ici gustatives, de ces produits. Si certains éléments ou caractéristiques des différentes représentations des vins de terroir permettent de les discriminer, c’est qu’ils proviennent d’une caractéristique particulière dans les supports de ces représentations, l’expression du terroir dans les vins. Ce travail de mise en relation des différentes manifestations du terroir exige de limiter les sources de variations qui ne seraient pas propres aux vins et viendraient polluer les représentations. Pour y parvenir, ces scientifiques recourent exclusivement à la technique de la dégustation à l’aveugle qui permet, selon eux, d’avoir une représentation « objective » du goût du vin, c'est-à-dire non influencée par le dégustateur.

Les vignerons ne procèdent pas de cette façon. Le terroir est une quête, son goût est un résultat dont l’évaluation demande aussi de prendre en considération la démarche vitivinicole et les pratiques qu’elle a suscitées. Pris individuellement, les vignerons peuvent avoir chacun leur idée du terroir, en général très attachée à leurs parcelles. Pris collectivement, la notion de terroir admet une variété d’acceptions. Les vignerons y font un tri, ils jugent chacun si la notion de terroir défendue par le vigneron ou présente dans le vin est acceptable ou non. Ce tri collectif juge, compare et hiérarchise mollement les différentes interprétations et expressions de terroir.

Le terroir n’admet donc pas qu’une unique acceptation et les vignerons sont ouverts aux propositions de leurs collègues. Quand ils jugent un vin, il leur est donc indispensable de connaître le projet du vigneron, son idée du terroir, mais aussi ses pratiques afin de confronter ce qu’ils ressentent aux intentions du vigneron qui a fait le vin, mais aussi à leurs propres attentes. Ils ne dégustent donc pas à l’aveugle, car sans connaître « l’intention » du vin, sans le comparer à d’autres réalisations similaires, il est impossible pour eux de donner un avis pertinent. Mais ils ne s’en tiennent pas à la dégustation en clair et recourent à la technique aveugle dès qu’ils sentent que leurs attentes peuvent devenir des « préjugés ». Ils ne s’en remettent pas non plus à un vin, ni une séance et multiplient les occasions de dégustation et les comparaisons. Ils ne s’isolent pas et se confrontent aux opinions des autres, vignerons, mais aussi critiques vinicoles, revendeurs, sommeliers, et tous les amateurs de vins de terroir.

La notion de terroir se trouve ainsi distribuée dans une multiplicité de jugements qui analysent le résultat, la présence du terroir, mais en la rapportant toujours à des moyens employés à cet effet, et à une intention ou une interprétation particulière de vigneron. Chaque expression de terroir est ainsi toujours partielle, elle forme une facette de ce que peut être le terroir ; elle est partiale, elle est liée au projet, aux pratiques et aux parcelles d’un vigneron ; elle est aussi provisoire, car elle sera bientôt enrichie de très nombreux autres tentatives d’exprimer le terroir dans des vins.

Le terroir est donc un objet distribué au sein d’un collectif critique qui l’analyse comme une production. Ce collectif *critique* instaure une procédure d’évaluation de l’expression du terroir très différence des procédures grâce auxquelles la science tente de saisir les objets : il ne procède pas par réduction des différentes expressions de terroir à un noyau commun stabilisé, mais par extension en incluant un nombre toujours croissant d’expressions concrètes de terroir.

Le terroir est toujours le résultat d’un processus de production et chaque membre du collectif a toujours une vision particulière, limitée du terroir. Pour autant, la notion de terroir qui en résulte n’est pas illusoire, mais irrémédiablement distribuée, temporaire et collective.

Les conséquences de ce fonctionnement différent sont importantes. Le test que les administrations appellent de leurs vœux est impossible : le contrôle critique mené par ce collectif ne peut être reproduit à partir de juges « représentatifs », dans un lieu standardisé et dans un délai déterminé. Le test est toujours trop réducteur et simpliste pour les vignerons en quête de terroir.

Une procédure alternative au test ?

Mais existe-t-il des procédures de qualification ou de normalisation qui permettent de garantir la présence d'un objet aux contours nécessairement indéfinis parce qu'il est sans cesse reproduit, réévalué et repensé ?

On a coutume d'associer les normes à des garanties de résultat, voire de moyens, appuyées sur des tests qui permettent de garantir la présence de l'objet recherché. Pourtant les normes environnementales comme l'ISO 14000 ou la qualification agriculture raisonnée dont il a été question ci-dessus, procèdent autrement. Elles ne s'appuient pas sur une définition de l'objet, ni sur un inventaire des pratiques nécessaires et au contraire sur l'engagement de la personne qui se qualifie envers un but à poursuivre ou une certaine attention à apporter à un objet. Or, c'est bien le contrôle de cet engagement qui permet de repenser une garantie du terroir qui respecte la quête du terroir menée par ces vignerons et leur travail de recherche des meilleures pratiques permettant de l'exprimer. Et il ne faudrait surtout pas juger les garanties d'engagement comme des pseudo garanties, des signes creux ne garantissant rien. Elles permettent au contraire d'éviter la rigidité des normes et de les adapter à la variété des situations concrètes, d'instaurer des dynamiques réflexives qui permettent de lutter contre les copies notamment, mais aussi contre l'érosion des standards qualitatifs.

Le terroir moteur du bio

Mais les vignerons de terroir n'ont pas fait que demander une réforme des AOC ; ils ont aussi contribué à l'émergence d'une nouvelle réflexion parmi les vignerons bio.

La réflexion sur le développement du bio, les difficultés commerciales rencontrées par des vignerons bio depuis 2003-2004 et les développement de nouveaux circuits de commercialisation de vins respectueux des terroirs ont amené des producteurs à revenir sur la question de cette « mauvaise réputation » des vins bio. Pour eux, cette mauvaise réputation ou cette incompatibilité qu'ils observent entre leur qualification environnementale et leurs prétentions à faire un produit de qualité est totalement « aberrante ». Le bio permet au contraire, mieux que les techniques conventionnelles d'arriver à des produits plus sains, produits de façon moins intensive, plus artisanale et plus naturelle et donc meilleurs au goût. Leur double préoccupation qualitative rencontre l'intérêt récent des adeptes du renouveau de la qualité de terroir. Pour ces derniers le bio n'est pas un but en soi, mais le moyen de retrouver une qualité authentiquement de terroir. Un vin bio n'est pas nécessairement bon, mais pour être bon, il est important de respecter les terroirs et donc de recourir à des pratiques qui respectent l'environnement. Ils combattent donc à la fois l'idée que le vin serait « à l'évidence un produit naturel » ou que les pratiques bio seraient nécessairement à l'origine de déficiences qualitatives puisque les meilleurs producteurs les ont adoptées. Si la « mauvaise réputation » des vins bio semble devenir progressivement un mauvais souvenir pour ces producteurs qui affichent dorénavant leurs pratiques sur leurs bouteilles, ils doivent néanmoins, comme les producteurs de terroir faire « valider » leurs prétentions qualitatives par la critique viticole qui met les préoccupations environnementales au second rang, derrière le goût, et surtout, qui accepte de considérer aussi bien des adeptes d'une viticulture très raisonnée, que du bio ou de la biodynamie. Dans ce nouvel espace marchand qui retravaille la notion de qualité de terroir, la cohabitation n'est donc pas toujours aisée entre défenseurs de l'environnement et du terroir qui divergent sur les priorités qu'ils se donnent, comme sur l'inventaire des bons moyens pour y parvenir. Mais la viticulture bio peut enfin espérer attirer de nouveaux clients parmi les amateurs de vin, faire valoir ses qualités et bousculer les murs dans lesquels elle se sentait à l'étroit.

CONCLUSIONS

L'agriculture raisonnée « en panne », c'est le bio qui semble redevenu le moteur du changement des pratiques viticoles, grâce à la remise en cause des AOC comme garanties de la qualité des terroir des vins qui a permis de faire valider les pratiques bio en tant que moyen de la production de vins de bonne qualité gustative. La réforme des règlements d'AOC, instaure une qualification des exploitations à partir de l'examen des pratiques propres à respecter les terroirs mises en œuvre dans les exploitations. Actuellement dans l'impasse, il est possible qu'elle ne débouche pas ; mais les deux qualités gustative et environnementale ont été articulées, ce qui devrait faciliter l'adoption de pratiques respectueuses par les vignerons, que ce soit pour ceux qui se lanceraient dans des stratégies de terroir, avec les agrobiologies ou des stratégies plus « industrielles ».

Cet exposé rapide a tenté de montrer que l'articulation entre les qualités gustative et environnementale ne va pas de soi. Mais pour comprendre les difficultés de cette articulation, son blocage puis son ouverture, il faut élargir le compte rendu aux discussions et craintes des acteurs envers l'économie. Les différences ne « valent » pas toutes seules ; les étiquetage ne sont pas performatifs. Il faut faire valoir les différences, et donc les démontrer, les justifier, les vérifier, ce qui exige de ans cesse lutter contre les sources de contestation ou d'amoindrissement de la différence à faire reconnaître. Or, l'attention accordée à la « menace économique », de même que l'inventaire des meilleures façons d'y parer instauraient des divergences et controverses dans les trois régimes marchands étudiés.

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Verbrauchereinstellungen zu Bioweinen in Deutschland

D. Hoffmann, G. Szolnoki
Forschungsanstalt Geisenheim - Von-Lade-Str. 1 - 65366 Geisenheim
E-Mail: d.hoffmann@fa-gm.de; gszolnoki@yahoo.de

ZUSAMMENFASSUNG

Die Verbraucherbefragung zu der Einschätzung von biogesiegelten Weinen hat gezeigt, dass sowohl bei Getränken wie auch bei Wein Bioprodukte nur von einem relativ kleinen Kreis von Verbrauchern (weniger als 4 % der Weintrinker) präferiert werden. Dies macht deutlich, dass die allgemeine Bio-Orientierung für Frischprodukte verschiedener Art bei den Getränken und hier speziell bei Wein in bei weitem nicht vergleichbaren Umfang vorkommt. Daraus muss der Schluss gezogen werden, dass biohergestellte Weine als klassische Nischenprodukte anzusehen sind. Aufgrund des überdurchschnittlichen Konsumvolumens der Bioweine präferierenden Verbraucher ist aber in den nächsten Jahren noch ein Wachstum möglich. Der vierprozentige Marktanteil scheint nach allen Marktdaten bei Weitem noch nicht erreicht zu sein. Allerdings ist auch nicht mit einer wesentlichen Erweiterung der Angebotsdichte in den Regalen der verschiedenen Handelstypen zu rechnen. Nach unseren Hochrechnungen ist bei einem vierprozentigen Marktanteil mit einem Gesamtmarktvolumen von ca. 50 – 60 Millionen Liter Biowein in Deutschland zu rechnen, die gegenwärtig höchstens zu 50 % ausgeschöpft sind. Insofern hat diese Nische noch ein Wachstumspotenzial von ca. 100 % für die nächsten Jahre vor sich, ohne dass dadurch eine wesentliche Veränderung des gesamten Weinsortiments erfolgt.

1 EINLEITUNG

Im Zusammenhang mit der Diskussion über den globalen Klimawandel und die enge Vernetzung der Welt in allen wirtschaftlichen und gesellschaftlichen Bereichen nimmt auch die Diskussion und das Engagement um biologisch und/oder organisch erzeugte Nahrungsmittel und Getränke ebenso zu, wie Fair Trade und CO_2-Footprintanalysen eine immer stärkere Beachtung im internationalen Handel finden. In Deutschland wie auch in anderen europäischen Ländern hat vor allem nach der Einführung des Bio-Siegels der EU - und damit die für den Verbraucher sehr verständliche Nutzung des Kurzbegriffs ‚Bio‘ in einer besonderen Produktkennzeichnung das Angebot an biologisch erzeugten Produkten in allen Lebensmittel- und Getränkekategorien zugenommen. Vor dem Hintergrund dieser allgemeinen Entwicklungen, die vor allem zu einer schnellen Verbreitung von ‚Bio‘ -gekennzeichneten und –erzeugten Produkten in allen Vertriebslinien des Handels führten, wirft sich die Frage auf, welche Bedeutung die Kennzeichnung und Herstellung von Weinen nach spezifischen Regeln der biologischen / organischen Erzeugung beim Verbraucher hat. Nachdem in den 90er Jahren eigenständige Vertriebslinien, wie z. B. das Handelsunternehmen Alnatura entstanden sind, haben seit Anfang 2000 alle Einkaufsstätten bis zu den Discountern mit dem Angebot von biologisch erzeugten Produkten und auch mit biologisch erzeugten Weinen begonnen.

Ziel der hier vorgestellten Studie war die Verbreitung und die speziellen Einstellungen deutscher Weinkonsumenten zu biologisch erzeugten Weinen auf der Basis einer repräsentativen Verbraucherbefragung zu erfassen. Dazu wurde ein spezieller Fragenkatalog erarbeitet, der im Rahmen einer Mehrthemenumfrage auf der Basis einer repräsentativen Stichprobe für die erwachsene Bevölkerung und ihrer sozialen Struktur im November 2009 durch zufällig ausgewählte Personen beantwortet wurde. Die daraus gewonnenen Ergebnisse werden hier vorgestellt.

2 METHODIK

Im Rahmen einer Mehrthemenumfrage zu Wein, Sekt und anderen Getränken wurden im November 2009 auf der Basis einer zufällig ausgewählten repräsentativen Stichprobe von 1.960 Personen, repräsentativ für die soziodemografische Struktur der Bevölkerung ab 16 Jahren in Deutschland, mittels eines vollstrukturierten Fragebogens verschiedene Fragestellungen zur Nutzung, zum Einkauf und zur Bewertung von Weinen aus biologischer Erzeugung und mit ‚Bio-Siegel' – Kennzeichnung versehene Weine erhoben. Die einzelnen Fragestellungen werden im Zusammenhang mit der Darstellung der Ergebnisse erläutert. Die Auswahl der Testpersonen erfolgte auf der Basis einer Quotenstichprobe aus einer Grundgesamtheit von 63,2 Millionen Personen, die durch 501 Interviewer befragt wurden. Die Felderhebung zu dieser Studie hat die Gesellschaft für Konsumforschung (GfK) in Nürnberg durchgeführt. Die Auswertung erfolgte durch das Fachgebiet Betriebswirtschaft und Marktforschung der Forschungsanstalt Geisenheim mittels SPSS Software.

Wenn im Folgenden von Bioweinen als Kurztitel gesprochen wird, so sind darunter im Sinne der Verbrauchereinschätzung Weine aus biologisch erzeugten Trauben zu verstehen. Da bisher keine allgemeingültigen Regeln für die spezifische Bioweinherstellung existieren, kann der Begriff Biowein als Oberbegriff für die aus biologisch erzeugten Trauben im Sinne der vereinfachten Abgrenzung und des Sprachgebrauchs der Verbraucher verwendet werden.

3 ERGEBNISSE

3.1 Konsumstruktur Wein in Deutschland

Bevor auf die speziellen Ergebnisse zur Bekanntheit, Präferenz und den Einstellungen zu biologisch erzeugten Weinen näher eingegangen wird, folgt eine Darstellung der allgemeinen Konsumstruktur von Wein in Deutschland.

Zunächst wird auf die Konsumhäufigkeit näher eingegangen. In der Tabelle 1 finden sie die Ergebnisse der Befragung auf der Basis der 1.940 Personen umfassenden Gesamtstichprobe, aus der hervorgeht, dass immerhin 41 % der Befragten zu diesem Zeitpunkt und im vorausgegangenen Jahr keinen Wein getrunken haben. Damit verengen sich der relevante Konsumentenkreis und die Reichweite auf 59 % der erwachsenen Bevölkerung. Die Konsumhäufigkeit ‚mehrmals pro Woche' oder ‚einmal pro Woche' nehmen mit 8 % und 9 % einen relativ kleinen Anteil an der gesamten Stichprobe ein. Damit konzentriert sich der wesentliche Teil des Konsums von Wein in Deutschland auf einen relativ kleinen Kreis von erwachsenen Verbrauchern.

Um die Bedeutung dieser Zielgruppe hervorzuheben, wurden den einzelnen Intensitätsgruppen - wie in der Tabelle 1 dargestellt – jeweils geschätzte Konsummengen pro Jahr zugeordnet und hochgerechnet auf ein Gesamtvolumen von ca. 13,25 Millionen hl Wein als Haushaltskonsum in Deutschland. Auf der Basis dieser erweiterten Schätzung kann die Relevanz der intensiv konsumierenden Verbraucher für den Gesamtmarkt dargestellt werden. Daraus wird deutlich, dass sich immerhin 43 % des gesamten Weinvolumens in der relativ kleinen Zielgruppe der wöchentlich mehr oder weniger regelmäßig konsumierenden

Weintrinker, die nur 8 % der erwachsenen Bevölkerung ausmachen, konzentriert. Die zweite Intensitätsstufe mit ‚einmal wöchentlich' erreicht immerhin mit ihren 9 % der erwachsenen

Tabelle 1: Konsumhäufigkeit und –volumen bei Weintrinkern in Deutschland (2009)

Konsumhäufigkeit	Befragte	Prozent	repräsentativ für Personen	Konsum in l pro Jahr	Konsum der Gruppe in l pro Jahr	Anteil der Konsum-menge
Mehrmals pro Woche	148	8%	4.797.655	120	575.718.650	43%
Einmal pro Woche	166	9%	5.398.898	60	323.933.872	24%
Zwei- bis dreimal pro Monat	259	13%	8.425.551	30	252.766.538	19%
Einmal pro Monat	185	10%	6.010.215	14	84.143.005	6%
Seltener als einmal pro Monat	389	20%	12.643.218	7,0	88.502.525	7%
Nie	793	41%	25.775.463	0	0	0%
Gesamt	**1940**	**100%**	**63.051.000**		**1.325.064.589**	**100%**

Quelle: eigene Erhebung

Bevölkerung 24 % des gesamten Haushaltkonsumvolumens an Wein. Beide zusammen erzielen immerhin 69 % des in Deutschland in Haushalten getrunkenen Weines, wenn man zunächst von dem geschätzten Gesamtvolumen von 13,25 Millionen hl Hausverbrauch ausgeht.

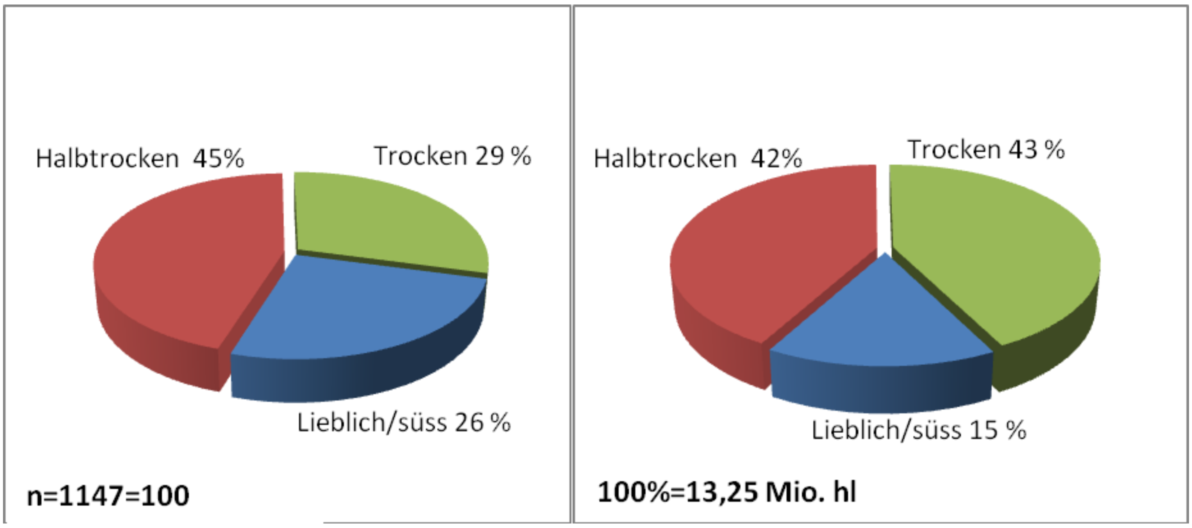
Der ermittelte Gesamtverbrauch von 13,2 Millionen hl ist als eine zurückhaltende Schätzung einzustufen, die lediglich dazu dient, die Relevanz der verschiedenen Intensitätsgruppen zu erfassen und sie bei den späteren Auswertungen näher zu berücksichtigen. Geht man bei der Auswertung nach der Anzahl der befragten Personen, so wird die Relevanz der relativ kleinen Verbrauchersegmente mit hoher Konsumintensität nicht ausreichend für die Gewichtung des gesamten Marktes berücksichtigt. Deswegen werden im Folgenden zahlreiche Auswertungen auf der Basis der mit dem Konsumvolumen gewichteten Urteile vorgenommen, um die gesamte Marktrelevanz der Befragungsergebnisse deutlicher hervorzuheben.

Besonders deutlich wird die Bedeutung der Mengengewichtung an dem Befragungsergebnis zur präferierten Geschmacksrichtung in Abb. 1 a und b im Vergleich dargestellt.

Danach erreichen die Zielgruppe der die trockene Geschmacksrichtung präferierenden Konsumenten nur 29 % während immerhin 26 % der Befragten lieblich/ süße Weine bevorzugen. Hinterlegt man die einzelnen Urteile mit dem jeweiligen Konsumvolumen, so wird die Relevanz der die trockene Geschmacksrichtung präferierenden Verbraucher mit 43 % des Marktvolumens gegenüber den lieblich und süß präferierenden Verbrauchern mit 15 % des Marktvolumens besonders deutlich. Dazwischen liegt die Zielgruppe der die halbtrockene Geschmacksrichtung bevorzugenden Verbraucher mit 42 % des Volumens, die weitgehend dem Personenanteil mit 45 % entspricht. Aufgrund dieser Ergebnisse wird deutlich, dass die die trockene Geschmacksrichtung bevorzugenden Verbraucher deutlich überdurchschnittliche Konsumintensitäten bei Wein realisieren und damit für den Markt eine höhere Relevanz haben, als dies ausschließlich die Auswertung nach einzelnen Personen wiedergeben würde.

Abb. 1: Verteilung der Präferenz der Geschmacksrichtungen bei Weinkonsumenten

a) nach Befragten **b) nach Konsumvolumen**

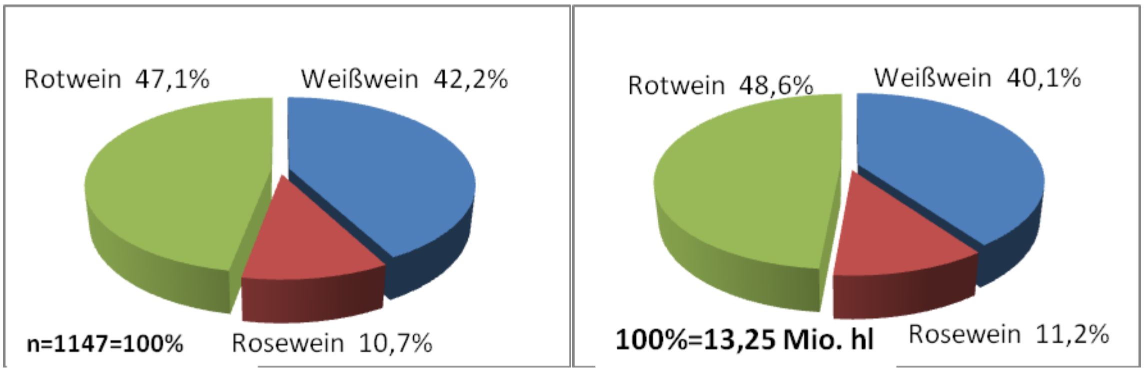


Quelle: eigene Erhebung

Bei der Befragung nach der präferierten Weinfarbe (Abb. 2) wird deutlich, dass die Konsumintensität keinen wesentlichen Einfluss ausübt, da immerhin 47 % der Befragten mit einem Volumenanteil von 48,6 % den Rotwein präferieren und 42,2 % der Befragten mit

Abb. 2: Verteilung der Präferenz nach Weinfarben bei Weinkonsumenten

a) nach Befragten **b) nach Konsumvolumen**

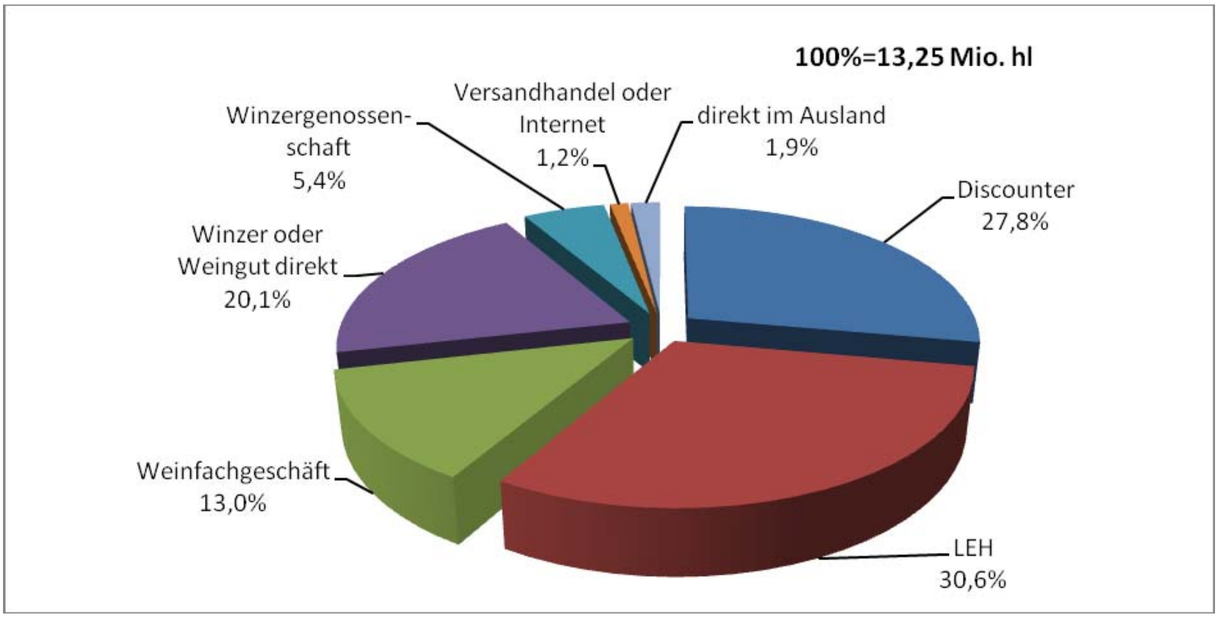


Quelle: eigene Erhebung

einem Volumenanteil von 40,1 % den Weißwein vorziehen. Sieht man von geringfügigen Schätzfehlern ab, so zeigt diese Auswertung keine wesentlichen Ergebnisdifferenzen ob nach Personen oder Volumenanteilen ausgewertet wird. Daraus wird deutlich, dass nicht in allen Fragestellungen das Konsumniveau einen wesentlichen Einfluss auf das Ergebnis hat.

Die Struktur nach Einkaufsstätten geht aus der Abb. 3 hervor. Hier wurde ebenfalls die Auswertungsvariante mit der Gewichtung des individuellen Konsumvolumens bevorzugt, um vor allem den Unterschied zwischen Intensiv- und Seltenkonsumenten für die gesamten Marktstrukturen zu erfassen. Danach wird deutlich, welch bedeutende Position nach Selbsteinschätzung der Verbraucher als Einkaufsstätten vor allem der Weineinkauf direkt im Weingut, bei der Winzergenossenschaft oder im Weinfachgeschäft haben. Die Relevanz der Discounter mit 28 % des erfassten Weinverbrauchsvolumens geht hier gegenüber anderen statistischen Erhebungen wie z. B. dem GfK Haushaltspanel (2010) deutlich zurück. Zwar erreicht der Lebensmittelhandel mit LEH und Discount zusammen knapp 60 % des Hauskonsums und haben damit zumindest volumenmäßig eine bedeutende Stellung in der Versorgung der Haushalte mit Wein. Gleichzeitig wird aber aus diesen Ergebnissen auch deutlich, dass in Deutschland eine sehr breite Einkaufsstättenstruktur von den Verbrauchern genutzt wird und damit eine differenziertere Analyse des Gesamtmarktes nach den unterschiedlichen Zielgruppen in den verschiedenen Einkaufsstätten sinnvoll ist. Im Zusammenhang mit den späteren Auswertungen für den Einkauf von Bioweinen wird auf diesen Aspekt näher eingegangen.

Abb. 3: Volumenanteil der Einkaufsstätten (gewichtet mit dem individ. Verbrauch)



Quelle: eigene Erhebung

3.2 Intensität des Einkaufs von Bioprodukten allgemein

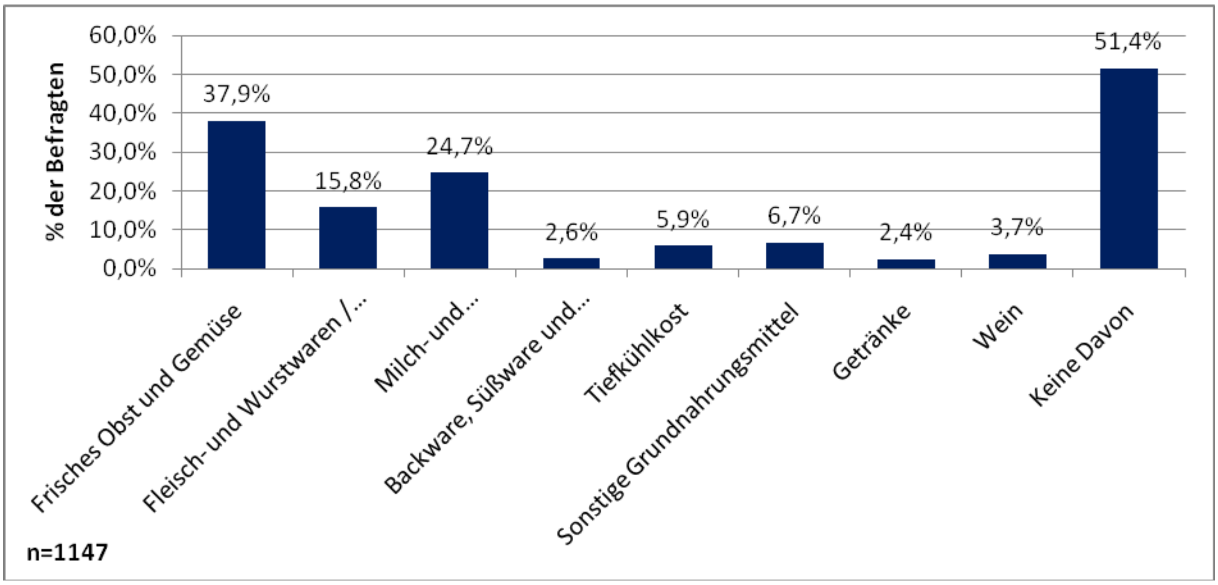
Um die Bedeutung und Stellung von Getränken und Wein im Bewusstsein der Verbraucher bei Bioprodukten im Allgemeinen genauer einordnen zu können, wurden nach dem Kauf von Produkten ausgezeichnet mit Bio-Siegeln in einer relativ breiten Palette verschiedener Produktarten gefragt. Wie in der Abb. 4 dargestellt, zeigt sich, dass die biogesiegelten Produkte vor allem im Bereich von frischem Obst und Gemüse, Milch und Molkereiprodukten eine hohe Verbraucherbeachtung finden. Ebenso erreichen Fleisch- und Wurstwaren mit immerhin 16 % der präferiert biogesiegelten Produkte einen relativ hohen Marktanteil. Gleichzeitig zeigt sich aber auch, dass immerhin 50 % der Befragten keine große Beachtung biogesiegelten Nahrungsmitteln und Getränken schenken.

Getränke erreichen lediglich ein Präferenzniveau von 2,4 % und Wein erreicht ein Präferenzniveau von 3,7 % der befragten Weinkonsumenten. Dabei ist zu beachten, dass diese

Auswertung sich lediglich auf die engere Zielgruppe der weinkonsumierenden Verbraucher bezieht, wie mit der Fallzahl (n = 1147) angegeben ist. Damit wird deutlich, dass biogesiegelte Weine einen klassischen Nischenmarkt darstellen und nicht im Fokus der allgemeinen Bioorientierung der Verbraucher liegen. Hier stehen eindeutig Frischeprodukte wie Obst, Gemüse, Fleisch, Wurst und Milch- und Molkereiprodukte im Vordergrund, die eine fünf- bis zehnfach höhere Beachtung bei den Verbrauchern finden.

Die Nutzung der entsprechenden Einkaufsstätten für den Einkauf von Bioprodukten gibt einen Hinweis auf mögliche Abweichungen vom Durchschnitt der allgemeinen Einkaufsstättennutzung.

Abb. 4: Anteil der regelmäßig gekauften Produkte mit Bio-Siegel (nach Befragten)



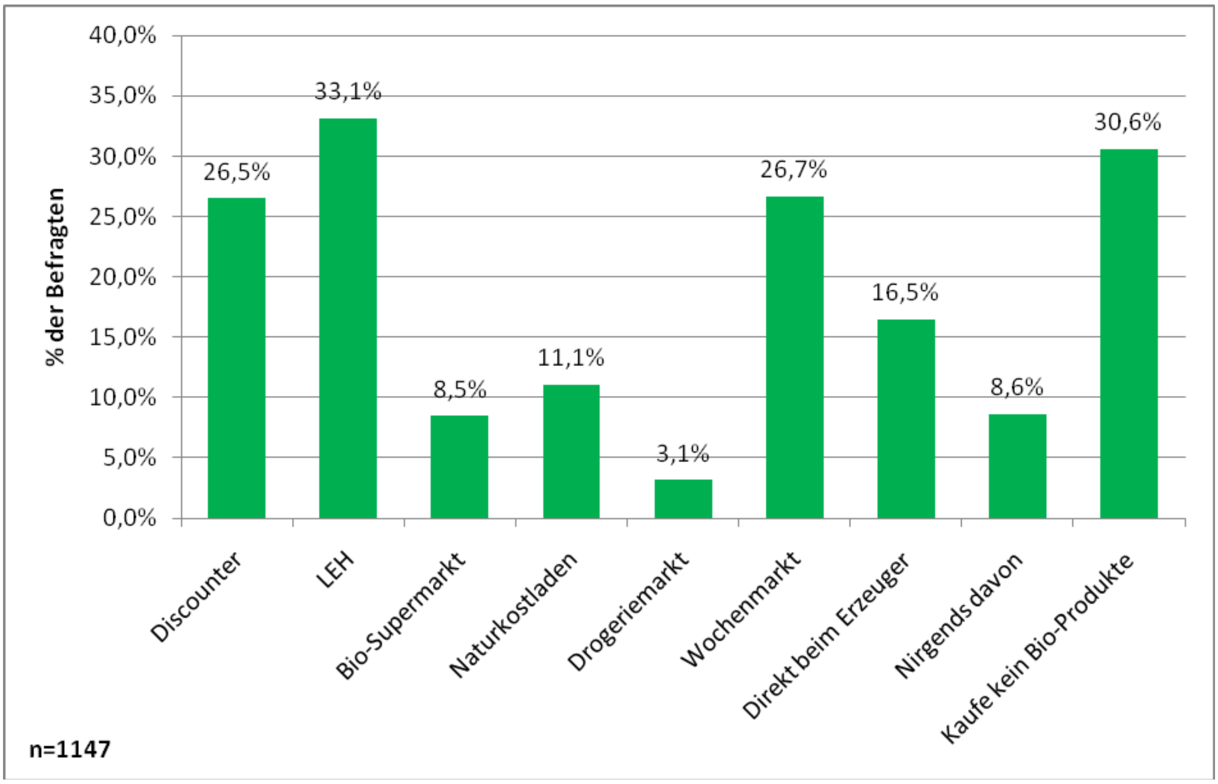
Quelle: eigene Erhebung

Daraus wird deutlich, dass wie in der Abb. 5 dargestellt, als spezifische Einkaufsstätten für Bioprodukte vor allem der Wochenmarkt und direkt beim Erzeuger eine hervorragende und gegenüber dem allgemeinen Einkaufsinteresse bedeutende Stellung genießen. Gleichzeitig wird aber auch deutlich, dass Discounter und LEH (Lebensmitteleinzelhandel) immerhin mit 26,5 % und 33 % der befragten Verbraucher als Einkaufsstätten für Bioprodukte akzeptiert sind. Der Spezialhandel für Bioprodukte mit Bio-Supermärkten und Naturkostläden liegt zwischen den beiden Gruppen und erreicht jeweils gerade um 10 % der Verbraucherpräferenz.

Diese Ergebnisse verdeutlichen, dass mittlerweile in der Gesellschaft allgemein nahezu alle Einkaufsstätten für das Angebot und den Einkauf von Bioprodukten akzeptiert sind. Zweifelsohne gibt es dabei jeweils auf der individuellen Verbraucherbasis starke Abweichungen, wie später in der Präferenz für den Einkauf von biologisch hergestellten Weinen ersichtlich wird.

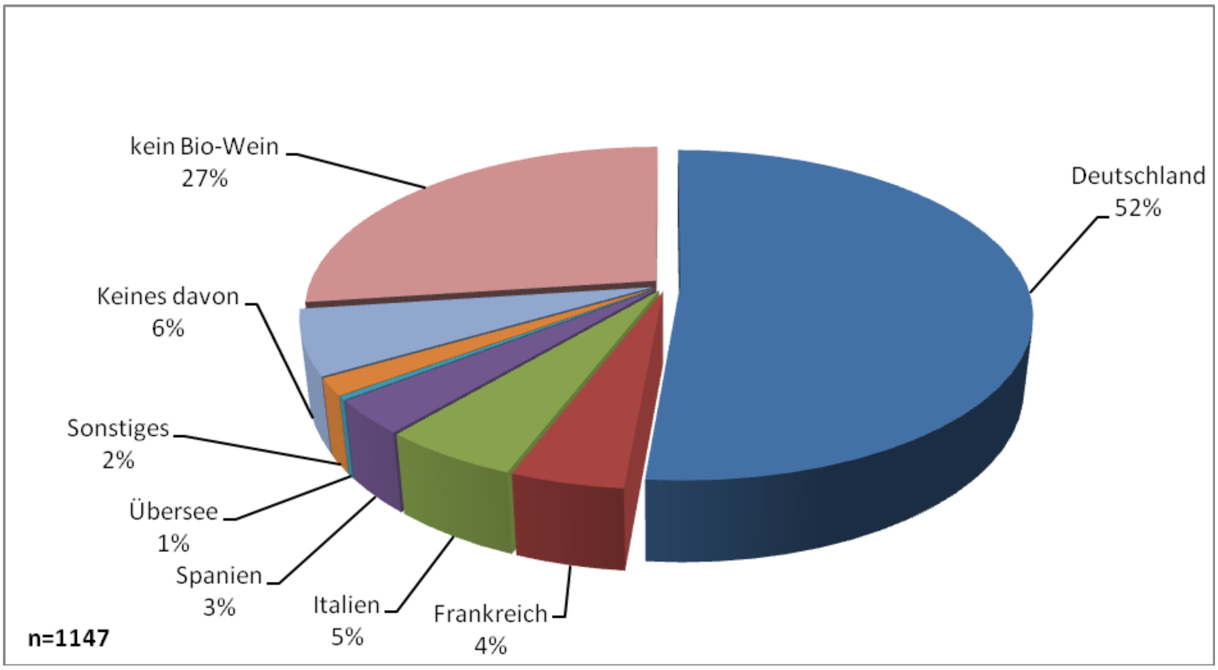
Bei der differenzierten Auswertung nach Männern und Frauen zeigt sich, dass Frauen tendenziell eine höhere Präferenz zu Bioprodukte allgemein haben als Männer.

Abb. 5: Nutzung der Einkaufsstätten für Bio-Produkte (nach Befragten)



Quelle: eigene Erhebung

Abb. 6: Bevorzugte Herkunft bei Bio-Weinen (nach Befragten)

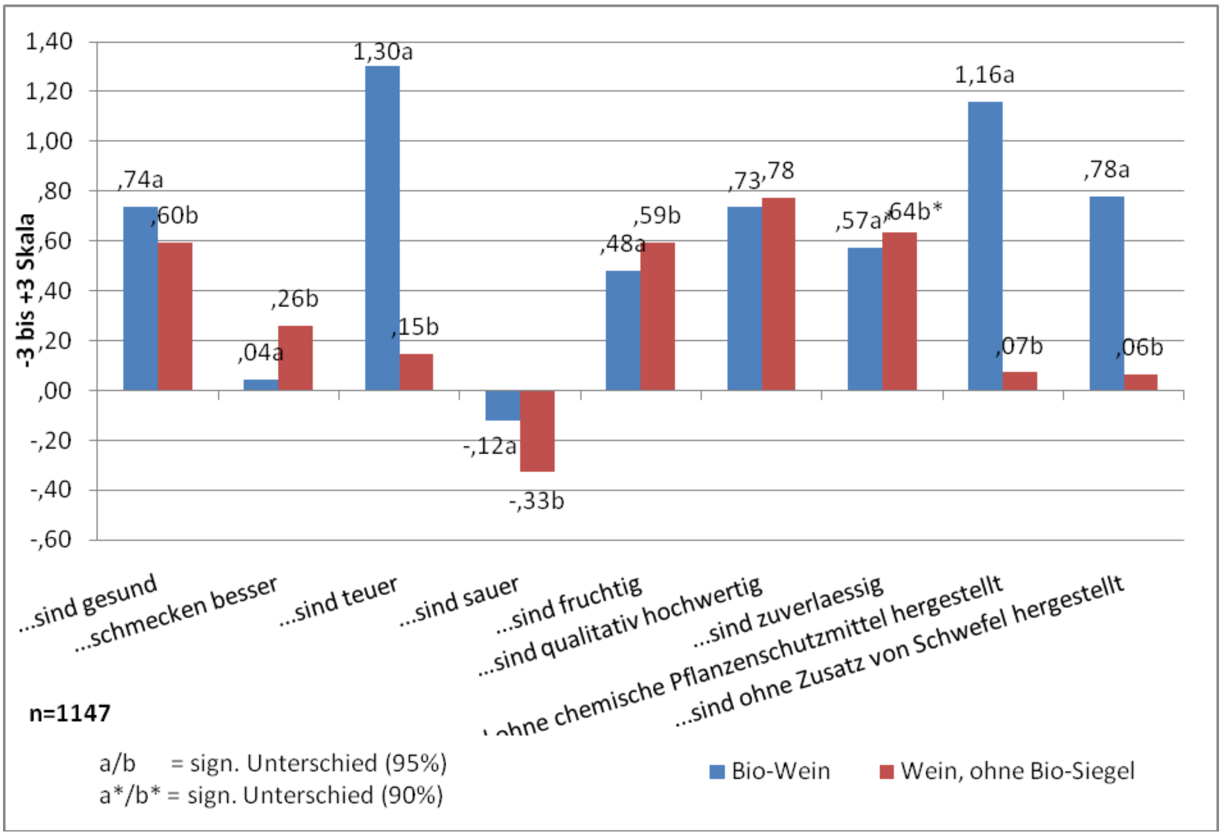


Quelle: eigene Erhebung

3.3 Präferenzen zu Bioweinen

Bei der Herkunft der in Deutschland bevorzugten Bioweine steht das Heimatland Deutschland mit 52 % der Präferenz im Vordergrund des Verbraucherinteresses (Abb. 6). Allerdings ist zu berücksichtigen, dass auch bei dieser Frage direkt 27 % der befragten Weinkonsumenten angaben, keinen Biowein zu bevorzugen. Hier wird deutlich, dass gerade bei Bioprodukten und in diesem speziellen Fall auch bei Wein die engere regionale Herkunft im Umfeld der Konsumenten eine deutlich stärkere Rolle spielt, und damit der internationale Handel tendenziell zunehmende Schwierigkeiten erhält. Vergleicht man diese Daten mit dem relativ großen Import von Biowein aus anderen europäischen Ländern, beispielsweise über den Großhandelsspezialisten Riegel in Süddeutschland, so zeigt sich ein Unterschied zwischen der geäußerten Präferenz für Deutschland und der Marktrealität. Es ist davon auszugehen, dass der Anteil der importierten Bioweine in den Regalen des Handels deutlich über die 50%-Marke hinausgeht und damit die hier geäußerte Präferenz eher einer Wunschvorstellung entspricht und von den Marktrealitäten abweicht. Dies ist ein Hoffnungsschimmer für Anbieter aus den verschiedenen Ländern um den deutschen Weinmarkt herum.

Abb. 7: Imagedimensionen* von Bio-Weinen und Weinen ohne Bio-Siegel



Quelle: eigene Erhebung,

*bewertet auf einer Ratingskala von -3 (stimme nicht zu) bis +3 (stimme zu)

Die spezifischen Imagedimensionen von Bioweinen gehen aus den Ergebnissen dargestellt in der Abb. 7 hervor. Dabei wird in der Darstellung zwischen Weinen mit und ohne Bio-Siegel differenziert. Danach wird ersichtlich, dass die Bioweine nur in wenigen Dimensionen sich markant von denjenigen Weinen unterscheiden, die kein Biosiegel tragen. So haben die Wein-Käufer beispielsweise die Auffassung, dass Bioweine deutlich teurer sind, sie ohne chemische Pflanzenschutzmittel hergestellt werden und die Weinherstellung ohne Zusatz von Schwefel stattfindet. In den anderen Dimensionen bezüglich Gesundheit, Geschmack, Fruchtigkeit, etc. werden Bio-Weine weitgehend gleich mit ihren Weinen ohne spezielle Bio-Siegel eingestuft.

Von besonderem Interesse ist immer wieder die Frage hinsichtlich der Zahlungsbereitschaft für biogesiegelte Weine. Immerhin waren 46 % der Verbraucher der Meinung, dass Bioweine bis zu 25 % teurer sein könnten, als vergleichbare, nicht biogesiegelte Weine. Allerdings sagen auch immerhin 23 %, dass sie keine höhere Zahlungsbereitschaft für biogesiegelte Weine haben und besitzen 18 % dazu keine Meinung. Eine hohe Zahlungsbereitschaft, die über die 50 %-Marke hinausgeht, existiert nur bei einem ganz kleinen Kreis von Verbrauchern. Dies zeigt, dass auch biogesiegelte Weine einer scharfen Preisbewertung der Verbraucher unterliegen.

Die spezifische Auswertung der befragten Personen in zwei Segmenten zwischen denjenigen, die biogesiegelte Weine präferieren und denjenigen, die keine biogesiegelten Weine präferieren, brachte nur in einigen wenigen Aspekten markante Unterschiede. So zeigt sich dass die Bioweintrinker letztendlich über ein geringfügig höheres Nettoeinkommen verfügen, dass sie generell in deutlich größerem Umfang Bioprodukte allgemein und ganz speziell Wein einkaufen und tendenziell ein etwas überdurchschnittliches Weinkonsumvolumen realisieren. Bei Geschmacksrichtungen und Herkünften zeigen sich keine besonderen Abweichungen. Besonders deutlich sind die Abweichungen bei den präferierten Einkaufsstätten für Bioprodukte allgemein, da bei den mit Biosiegeln präferierten Produkten der Spezialhandel - wie Biosupermärkte und Naturkostläden - stärker beachtet wird. Insbesondere lehnen Bioweintrinker die Einkaufsstätten Discount und Drogeriemarkt für Bioprodukte weitgehend ab.

Can Organic Wine Be A Profitable Differentiation Strategy?

A. Stasi[1a], *A. Seccia*[2], *A. Lopolito*[1b], *G. Nardone*[1c]

[1]Università degli Studi di Foggia
Via Napoli 25, 71100, Foggia, Italy
[1a]a.stasi@unifg.it; [1b]a.lopolito@unifg.it; [1c]g.nardone@unifg.it
[2]Università degli Studi di Bari
Via Amendola 165/A, 70100, Bari, Italy
seccia@agr.uniba.it

1. Abstract

Organic production in wine industry in the large firms concerns commonly a production line. Seeing organic wine as a product portfolio differentiation strategy for the firm we answered to the question of whether organic differentiation is a profitable industrial strategy. In order to do so we derived supply information from demand and we tested a supply differentiation conduct model. Results highlight that, although organic is not as profitable as other wine categories, it could still be a valid strategy for maximizing profits. Incentives to keep environmental sustainability could lead firms not to move from organic to other type of differentiation.

La produzione di vini biologici nell'industria del vino riguarda esclusivamente linee di produzioni, raramente l'intera azienda. Se, quindi, questa è una strategia industriale di differenziazione del portafoglio prodotti, la domanda a cui abbiamo risposto riguarda appunto la profittabilità di tale strategia. Per fare ciò ci siamo derivai le informazioni sull'offerta a partire dalla domanda e abbiamo testao il modello di condotta dell'offerta. I risultati mettono in luce is fatto che il vino biologico come strategia di differenziazione non genera profitti tanto alti quando altre tipologie di vino. Ciononostante, risulta ancora una strategia valida per massimizzare i profitti. Degli incentivi per mantenere la sostenibilitaà ambientale come strategia sarebbero necessari affinchè le aziende non si dirigano verso altre tipologie di differenziazione.

2. Introduction

Organic production has been recognized worldwide as a sustainable strategy because of its reduced impact on environment and the ability to assign to agriculture a longer term productivity compared to conventional cultivation practices (Rigby and Caceres, 2001; Lampkin, 1994; Henning et al., 1991; York, 1991).

The increasing consumers' sensitiveness towards environment related issues such as global warming, water-soil pollution and health related aspects, over the last decade, turned into economic research producing a plethora of analyses on consumers' preferences towards environmental friendly productions, finding large consensus a relatively high consumers' willingness to pay towards organic products. Yiridoe et al. (2005) concluded, however, that consumers show skepticism and uncertainty towards organic labeled food products, although the corresponding demand price elasticity is relatively small, these results were found also by Thompson (1998). On the other hand, Magnusson et al. (2001) concluded that price plays a major role in determining the market share of organics; in fact price differential between organic and conventional limits their purchase. Gil et al. (2000) found that Spanish consumers identify organic attributes with perishable products such as fresh fruit and vegetables, and for those they are willing to pay a premium.

Literature on profitability of organic farming, on the other hand, is rather limited, and looks at production by measuring directly costs and net returns. Lansink et al. (2002) found that Finnish organic farming is more efficient than conventional agriculture, although less productive. Nieberg and Offerman (2003) concluded that in Europe, organic production sustainability is strictly connected to the realization of higher farm-gate prices, and that profits in organic and conventional farms are similar because of higher costs when producing organic. Nonetheless, existing scientific contributions have neglected the role of organic productions as a firms' differentiation strategy. Producing organic wine within the product line, in fact, could differentiate the winery, give it a reputational return and increase its profits. Economic sustainability of organic wine productions that could be intended in terms of firm, product and product line profitability, in fact, is a key issue for having a long term perspective on the presence organic wine production in the Italian wine market and predicting whether more firms will invest towards this direction.

The objective of the paper is to verify whether producing organic wine is a profitable strategy for the main Italian wine firms. The hypothesis we intend to test, therefore, is that the adoption of such a portfolio strategy could result into an effective differentiation tool and, as consequence, determine an increase of the price-cost-margin (PCM).

The paper investigates the consumer demand for organic wine as part of a broader wine product category by combining the distance metric (DM) method developed by Pinkse Slade and Brett (2002) with LA/EASI demand system developed by Lewbell and Pendakur (2008) and Pendakur (2008). The model consists of a brand level consumer demand relative to the main Italian wine firms differentiating their production by including organic wine into their portfolio, foreign wine sold into Italy and the rest of the wine market. Estimates, then, are used jointly to pricing rule models of firm conduct in order to depict PCM without observing actual costs.

Demand is modeled as function of product characteristics and heterogeneous consumers' preferences. The model is applied to a scanner database of the wine purchase in the Italian retail market. Data concern a representative sample of about 6,000 Italian consumers and consists of the record of their purchase over the period 2002-2005. The panel structure of the data allows controlling for quality attributes and brand intercepts and retrieving the full substitution matrix. The estimated demand system is used to compute PCM implied by the differentiation strategy. High PCM would be due to high consumers' willingness to pay not to lack of competition, given also the actual structure of the sector, as described in Section 2. Therefore, the presence of market power in this industry is due to the firms' ability to maintain a portfolio of differentiated products.

The exercise relies of the ability to consistently estimate demand. Estimation deals with: (1) consumers/households taste heterogeneity, (2) the large number of own-price and cross-price elasticities given the large number of Italian wines sold through the retailing sector; both difficulties were encountered also in Neio (2001). The first difficulty has been overcome by referring to the EASI implicit Marshallian Demand developed by Lewbel and Pendakur (2008), which can easily incorporate unobserved preference heterogeneity, differently to the other demand system estimation procedures. The second difficulty has been overcome by assembling the Distance Metric technique, hereafter DM (Pinkse, Slade and Brett, 2002), with the EASI implicit Marshallian Demand System; with the result of a consistent reduction of parameters dimensionality and estimation ease.

The rest of the paper is organized as follows. Section 2 gives a short description of the industry. In section 3 the empirical model is described. Section 4 describes the data and the estimation procedure. Results of the demand model are presented in section 5. Section 6 concludes and outlines.

2.1 *The Italian wine industry and Organic wine production*

Italy produces about 55 million hl and offers about 400 wine varieties on the market (Seccia et al., 2009). The number of producers operating in the sector is enormous; the last census, in fact, reported about 73,000 wineries (ISTAT, 2008). As consequence there is a high level of differentiation but the sector results competitive from the structural point of view, as also found in Stasi et al. (2009).

Concerning the organic wine production, after a decade of growing interest into the cultivation of vines with organic techniques, after the approval of the regulation of 1992, nowadays we are registering a consistent reduction of the cultivated areas. The historical maximum has been of 44’000 ha in 2001 while last data released by the Ministry of Agriculture and Fisheries report a 31,000 ha of total area dedicated to organic vines (www.ilvinobilogico.it).

Talking into comparative terms, however, Italy results the most important producer of organic wine (www.ifoam.org), in front of France and Spain. This classification refers to the area cultivated for the production of organic grapes.

Concerning the consumption of organic wine, 0.51% of wine shopping regards organic wine. Average price and its standard deviation do not differ significantly from conventional to organic wine. Same findings concern other quality attributes such as GI, color and packaging (our elaboration from AcNielsen data).

3. THEORY AND METHOD

The approach adopted here consists of considering a model of supply conduct where the price decision depends on brand-level demand, which depends upon product characteristics and consumers’ preferences. Demand parameters are estimated and used to compute the PCM.

3.1 *Supply*

The hypothesis is that a firm *n* behaves maximizing its profits by jointly setting the prices of the Y_n set of wines it produces:

$$\max_{p_i} \pi_n = \sum_{j \in Y_n} q_j(p_j - c_j) - F_j \tag{1}$$

Where *c* refers to the marginal costs of the *j* product, *p* to the price, *q* to the quantity, and *F* to the fixed costs. Assuming that prices are the outcome of a Bertrand-Nash equilibrium, the optimization process leads to setting a vector of FOC:

$$p_j - c_j = \Omega^{-1} \mathrm{q_j}(\mathrm{p_j}) \tag{2}$$

Where Ω^{-1} corresponds to the product of the ownership matrix and the Jacobian of the demand with respect to prices. By consequence of equation (2) the PCM is defined as follows:

$$\frac{p_j - c_j}{p_j} = -\left[\eta_{jj} + \sum_{j \neq k} \eta_{jk}\Omega_{jk}\frac{p_j}{p_k}\right]^{-1} \tag{3}$$

Using the estimates of the demand parameters, we can obtain estimates of PCM without observing the actual costs. Various types of PCM can be estimated, in this paper we evaluate the conduct model based on product differentiation, and hence, the mark-up is due only to the differentiation strategy of firm *n*.

3.2 *Demand*

The previous section highlights the fact that PCM estimates rely on the ability to consistently estimate the own- and cross-price elasticities. As pointed out in Section 1, a difficult task is to consider consumers' taste heterogeneity and large number of closely related products. In this analysis the methodological approach uses the EASI implicit Marshallian demand developed by Lewbel and Pendakur (2008).

The Exact Affine Stone Index (EASI) demand system developed by Lewbel and Pendakur (LP hereafter) has the desirable feature of incorporating unobserved heterogeneity in a theoretically consistent way, allowing the measures of consumer welfare to be influenced by such heterogeneity.

One particular functional form proposed by LP for the share equations in (4) is

$$\mathbf{w} = \sum_{r=1}^{R}\mathbf{b}_r y^r + \mathbf{Cz} + \mathbf{Dz}y + \sum_{l=0}^{L} z_l \mathbf{A}_l \mathbf{p} + \mathbf{Bp}y + \boldsymbol{\varepsilon} \tag{7}$$

Where, for the linear approximation, implicit utility is defined as

$$y^0 = x - \sum_i p_i w^0_i \tag{8}$$

and x denotes the logarithm of total nominal expenditure and w^0 refers to a base value of the share. The vectors $\mathbf{b}_r$ and matrices $\mathbf{C}, \mathbf{D}, \mathbf{A}_l$, and $\mathbf{B}$ are parameters to be estimated.

Compensated semi-elasticities are easily calculated as follows

$$\begin{aligned} \partial w_i / \partial p_j &= \sum_{\ell=0}^{L} z_\ell a_{\ell ij} + b_{ij} y \qquad \forall i, j \\ \partial w_i / \partial y &= \sum_{r=1}^{R} r b_{ir} y^{r-1} + \sum_{\ell=0}^{L} d_{i\ell} z_\ell + \sum_{j=1}^{J} b_{ij} p_j \qquad \forall i \\ \partial w_i / \partial z_\ell &= c_{i\ell} + d_{i\ell} y + \sum_{j=1}^{J} a_{\ell ij} p_j \qquad \forall i, \ell \end{aligned} \tag{10}$$

Semi-elasticities are converted into normal elasticities by dividing for the corresponding budget share.

In order to solve the dimensionality issue, we adopted the Distance Metric (DM) approach developed by Pinkse, Slade and Brett (2002), which consists of imposing cross-price parameters to be function of the distance in attribute space between product *j* and product *k*. The approach adopted in this paper follows also what suggested in Rojas (2008), Rojas and Peterson (2008) and Bonnanno (2009), which applied DM to LA/AIDS, with the difference of using LA/EASI theoretical derivation.

The DM adaptation of LA/EASI consists of incorporating attribute distance variables, δ^D into the model. Let Z^D be an indicator variable such that $z^D_{jl} = 1$ if product *j* shows characteristic *l*; 0 otherwise[1], so that δ^D:

$$\delta^D_{jk} = 1 \text{ if } |z^D_{jl} - z^D_{kl}| = 0;\ 1 \text{ otherwise} \tag{11}$$

Using the attribute distance variables, cross-price terms in the LA/EASI are reformulated as follows:

$$\sum_l z_l Ap = a_{jj}p_j + \sum_{k\neq j}\lambda_j \sum_l \delta^D_{jk}\, p_k \tag{12}$$

Where λ is a parameter to be estimated. Following the work of Pinkse and Slade (2004), Rojas (2008), Rojas and Peterson (2008) and Bonanno (2009), brand intercepts are interacted with own price elements, intercept and expenditure coefficients. In this way, only one equation can be estimated with the result of a considerable reduction of parameters and equations to be estimated[2].

3.3 *Data and Estimation*

The data employed are drawn from the ACNielsen Italian HomeScan panel. The particular sample of 6,701 households includes unit prices and quantities purchased weekly of all types of wine over the two-year period from December 2002 to December 2004. Purchases of wine away from home are not included. Time invariant socio-demographic variables for each household are also included with the panel on wine purchases.

The approach adopted allows exploiting the panel nature of the data. In fact, our data present more than 32,000 observation concerning wine sold in bottles of 0.75 liter purchases. On the other hand, the panel is highly unbalanced due to not all households purchasing each week.

Attributes considered are 4 brands corresponding to those owning the larger market share and producing also organic wine, color, GI or table wine, organic. Foreign wine and a last category including the rest of the market have also been considered. The resulting combinations are 48 products or market combinations.

Equation (11) has been estimated by Minimum Distance estimator using cost variables taken from ISTAT tables, to account for the endogeneity. The variables used as instrument: farm level grape price and farm level wine price, labor and energy cost indexes.

4. RESULTS

Given the specification in eq. (11) wine attributes include: brand, color, GI and Organic dummies and distance parameters. Socio-demographic shifters were also considered in the model.

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[1] Continuous attributes could also be included in the model by applying the Euclidean distance metric as indicated in Rojas (2008) and Bonanno (2009). In this paper, on the other hand, quality attributes are considered to be discrete

[2] A limitation of this approach is the fact that Engel curves flexibility is restricted to linearity because only one equation is estimated, while EASI demand systems allow Engel relationships to be polynomial of order 1 less the number of equations (Pendakur, 2008).

4.1 *Estimated Parameters and Elasticities*

Semi-elasticities calculated at base prices, imposed to be the median, correspond to DM-LA/EASI parameters or a combination of them based on the attributes, or attribute-distance, considered. Therefore, dividing those by the corresponding share we obtain the elasticities, which are presented in table 1. Brands name cannot be presented because of contractual restraints with the AcNielsen, however, they will be indicated with alphabetical letters. PCM are calculated as in eq. (3) assuming a conduct model based on pure product differentiation strategy, in which the ownership matrix corresponds to an identity matrix times the Jacobian of the demand with respect to price. The present hypothesis corresponds to a Bertrand-Nash equilibrium.

In order to make reading and interpretation easier, given the high number of elasticities and PCM, they have been calculated at each characteristic and reported in Table 1. Those measures indicate that, *ceteris paribus*, differentiating through GI and/or Organic contributes to a lower PCM.

Therefore, one possible explanation of the lower PCM for GI and Organic, which turns into lower profitability compared to the competing categories, is that there are lower volumes on the market, hence, consumers are not aquatinted with those products and prefer the traditional product sold under a specific brand. Moreover, skepticism could be another source of lower profitability for the GI and Organic differentiation strategy, and/or those products are infrequent purchases, while "less differentiated" wine satisfy a more habitual consumption with the consequence of lower demand elasticity, therefore a higher PCM.

At this point, a natural question one may pose is: why do firms would prefer to include organic in their product line when this differentiation strategy is not more profitable than conventional products? In reality, mark-up or PCM are still positive, thus adding an additional product to the line which is differentiated because it is organic might increase profits more than trying to increase the sales of the conventional product. In fact, in order to obtain a profit increase by selling out more of the conventional wine final price should be reduced, with no sure positive outcomes in terms of profits. Adding a differentiated product to the portfolio, on the other hand, would increase supply profits with more ease.

Significant differences among brands are not present, nor differences among main brands and other Italian wine brands, except for slightly higher profits for brand A, B and D. On the other hand foreign wines sold into Italy generate high price sensitiveness, result of Italian consumers' skepticism towards non-domestic products.

Table 1 – Compensated own-price elasticities Lerner index and price-cost margin in euro at median price.

Characteristic	***Elasticity***	***St. Error***	***PCM***	***EURO***
red	-8.440	0.942	0.135	0.541
white	-8.433	0.971	0.128	0.513
table	-7.183	0.946	0.155	0.621
GI	-9.690	0.967	0.108	0.433
A	-6.940	0.755	0.155	0.618
B	-8.250	1.612	0.127	0.508
C	-9.740	0.927	0.106	0.425
D	-5.874	1.016	0.194	0.776

other	-9.592	0.620	0.108	0.432
foreign	-10.224	0.809	0.101	0.403
conventional	-7.334	0.704	0.151	0.603
organic	-9.540	1.209	0.113	0.451

5. CONCLUSIONS

Elasticities calculated for both organic and conventional productions at each brand allow testing the level of profitability over the hypotheses of Bertrand-Nash equilibrium. Price-Cost Margin measures, therefore, are estimated so that the profitability of organic wine for the main Italian wine brands is depicted.

Results show that organic wine does generate increase in profits. On the other hand, the scarce ability of this differentiation strategy to generate lower consumers' price sensitiveness and allow firms to push prices above marginal costs results as a strategy less profitable than conventional products. Those results imply firms finding convenient to differentiate their product line by adding organic wines. Contrarily, the fact that organic is not as profitable as table wines could lead firms to find other differentiation strategies that could lead to better positioning and higher profits, such as producing table wines.

On the other hand, other conduct model could be tested in further researches such as the product line strategy, in order to understand whether the inclusion of organic wines into the firm portfolio would be a profitable strategy.

Policy implications could concern incentives to producers in order to drag the agricultural production towards more sustainable practices from both environmental and economic point of view.

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Alkoholfreier Wein? Erfahrungen in Deutschland

Hans H. Hieronimi
Rechtsanwalt, Roonstr. 6, 56068 Koblenz, Deutschland, rhh@ra-hieronimi.de

ABSTRACT

In the wake of international anti-alcohol-campaigns, the growing awareness of health and fitness and the call for renouncement of alcohol in the road traffic the alcohol free and alcohol reduced bevarages enjoy a favorable trend. Therefore also the winesector is giving more and more attentiveness to the production and marketing of those beverages. In Germany "alcohol free wine" is a wine-product with a tradition of 100 years produced by dealcoholisation of wine. With section 47 of its Weinverordnung (Wine Regulation) in Germany exists a legal provision, governing the production and description of "alcohol free wine", "alcohol reduced wine", "sparkling beverage on the basis of alcohol free wine" and "sparkling beverage on the basis of alcohol reduced wine". The details of these regulations will be presented. The use of the terms "alcohol free wine" and "alcohol reduced wine" is differently appraised within various states. A harmonisation of the definitions of the generic names is necessary in order to facilitate the trade of these "new" wine-products.

RÉSUMÉ

Les diverses campagnes anti-alcool et une prise de conscience accrue de l'importance de la santé et de l'alimentation, ainsi que les nombreuses discussions en faveur d'un taux zéro au volant font que les vins sans alcool ou à teneur en alcool réduite sont de plus en plus à la mode. En Allemagne, le "vin sans alcool" est un produit vitivinicole traditionnel obtenu par désalcoolisation du vin, et ce, depuis plus de 100 ans. Avec l'article 47 du Weinverordnung (règlement sur les vins) l'Allemagne possède une disposition régissant la fabrication et la dénomination de "Vin sans alcool", "Vin à teneur en alcool réduite", "Boisson mousseuse à base de vin sans alcool" et "Boisson mousseuse à base de vin à teneur en alcool réduite". Cette réglementation sera présentées en détail au cours de l'exposé. Les dénominations "Vin sans alcool"et " Vin à teneur en alcool réduite" sont percues différemment selon les pays dans lesquels elles sont utilisées. Afin de faciliter la commercialisation de ces "nouveaux" produits vitivinicoles, il est donc impératif de trouver des définitions harmonisées pour les différentes dénominations.

I. Einführung

Anti-Alkohol-Kampagnen, ein gestiegenes Gesundheits- und Fitness-Bewusstsein und die Forderung nach einem weitgehenden Alkoholverzicht im Straßenverkehr haben das Konsumverhalten der Verbraucher durchgreifend verändert. Der Absatz von alkoholischen Getränken geht weltweit zurück. Demgegenüber steigt die Nachfrage nach alkoholfreien, alkoholreduzierten und Light-Getränken national und international immer mehr an.

Auf diesen Konsumtrend müssen die Hersteller alkoholischer Getränke reagieren. Es ist eine Markanpassung erforderlich, damit durch ein zusätzliches Angebot leichter, alkoholreduzierter und alkoholfreier Getränke verlorene Marktanteile zurück gewonnen werden können.

Den Bierherstellern ist auf diesem Wege in den letzten Jahrzehnten bereits der Marktdurchbruch gelungen. Nach anfangs schleppenden, sich später erfreulich entwickelnden

Absatzerfolgen hat alkoholfreies Bier zurzeit einen Marktanteil von über 3% erlangt. Neben dem alkoholfreien Bier haben auch Leicht-Biere und kalorien- sowie alkoholreduzierte Biere an Bedeutung gewonnen.

Auch auf dem Weinsektor wird der Herstellung alkoholfreier und alkoholreduzierter Getränke immer größere Aufmerksamkeit geschenkt.

In Deutschland hat die Herstellung von alkoholfreiem Wein seit über 100 Jahren Tradition. Die Kellerei Jung aus Rüdesheim besitzt seit 1908 ein Patent zur Herstellung von alkoholfreiem Wein. Sie hat seit dieser Zeit vor allem für den Export und einen geringen Inlandsmarkt alkoholfreie Weine erzeugt. Mitte der 80iger Jahre des vergangenen Jahrhunderts entstand in Deutschland ein fast boomartiges Interesse an der Erzeugung von Weinen mit niedrigem Alkoholgehalt. Seit dem befassen sich auch andere deutsche Wein- und Sekthersteller mit der Produktion und Vermarktung alkoholfreier und alkoholreduzierter Weine und daraus hergestellter schäumender Getränke.

II. Material und Methoden

Für die Arbeit wurden juristische und getränkewissenschaftliche Publikationen zum Thema „Alkoholfreier Wein“ aus den letzten Jahrzehnten verwendet. Eingeflossen sind auch praktische Erfahrungen des Verfassers aus seiner Beratungstätigkeit für Produzenten und Vermarkter von alkoholfreiem Wein.

III. Ergebnisse und Diskussion

1. Rechtlicher Rahmen und Definitionen

Nur in Deutschland (und in Österreich) existieren gesetzliche Vorschriften, welche die Herstellung und Bezeichnung alkoholfreier und alkoholreduzierter (in Österreich: alkoholarmer) Weine regeln: § 47 Weinverordnung vom 9. Mai 1995 i. d. F. der Bekanntmachung vom 21. April 2009 (§§ 2, 15, 26 Österreichisches Weingesetz 1999). Die übrigen Weinbau treibenden Länder innerhalb und außerhalb der EU kennen vergleichbare gesetzliche Bestimmungen nicht.

Die in § 47 Weinverordnung geregelten Getränke stellen kein „Erzeugnis“ im Sinne des nationalen Weingesetzes oder der EU – Weinmarktordnung dar. Sie verkörpern rechtlich ein Zwittergebilde an der Schnittstelle zwischen Weinrecht und Lebensmittelrecht.§ 47 Weinverordnung enthält keine abschließende Regelung über Herstellung und Bezeichnung von alkoholfreiem und alkoholreduziertem Wein. Ergänzende Vorschriften finden sich in den lebensmittelrechtlichen Bestimmungen der Zusatzstoffzulassungs-Verordnung und der Lebensmittelkennzeichnungs-Verordnung.

Das deutsche Weinrecht enthält keine Definition des Begriffs „Alkoholfreier Wein“. Es bestimmt nur, unter welcher Voraussetzung bestimmte Verkehrsbezeichnungen zur Beschreibung des alkoholfreien Weins und daraus hergestellter Getränke erlaubt sind. Hierzu werden neben dem Ausgangsprodukt Wein bestimmte Grenzwerte des vorhandenen Alkoholgehalts und einzelne Herstellungsverfahren als begriffsnotwendig genannt.

Rechtshistorisch war umstritten, wie ein durch nachträgliche Alkoholreduzierung von Wein hergestelltes Getränk rechtlich einzustufen sei. Nach richtiger Auffassung wurde es nicht als Wein angesehen, obschon es eine alkoholische Gärung durchgemacht hatte. Die einhellige Meinung hielt es aber für zulässig, ein solches Getränk als „Alkoholfreien Wein“ in Verkehr zu bringen.

Als „Alkoholfrei“ wird nach deutscher Rechtsprechung ein Getränk angesehen, dessen Alkoholgehalt so gering ist, dass er auf alkoholempfindliche Menschen (Kinder, Kranke) keinen feststellbaren Einfluss mehr ausübt oder befürchten lässt, wobei in der Praxis die Grenze bei 5 g/l gezogen wurde.

Erstmals im Jahre 1971 gestattete die deutsche Weinverordnung Herstellung und Inverkehrbringen von alkoholfreiem Wein unter Beschränkungen, jedoch zugleich auf Weiterverarbeitungserzeugnisse erstreckt. Diese Vorschrift wurde speziell zu dem Zweck geschaffen, damit der seit siebzig Jahren in Deutschland hergestellte und vermarktete alkoholfreie Wein auch weiterhin unter dieser Bezeichnung in den Verkehr gelangen konnte.

Gleichwohl sollte die Bezeichnung „Alkoholfreier Wein“ durch EG-Recht verboten werden, weil der Begriff „Alkoholfreier Wein“ nicht der Legaldefinition des Begriffes „Wein“ in der EU-Weinmarktordnung entsprach. Denn hiernach ist Wein „das Erzeugnis, das ausschließlich durch vollständige oder teilweise alkoholische Gärung der frischen, auch eingemaischten Weintrauben oder des Traubenmostes gewonnen wird“. Auf Drängen der Bundesrepublik Deutschland ist dann die Ausnahmeermächtigung geschaffen worden, welche es den Mitgliedsstaaten gestattet, den Begriff „Wein“ zuzulassen, wenn er „Teil eines zusammengesetzten Ausdrucks“ ist. Von dieser Ermächtigung hat Deutschland durch § 47 Weinverordnung Gebrauch gemacht.

Durch eine Änderung der deutschen Weinverordnung aus dem Jahre 1986 ist der Begriff „Alkoholfreier Wein“ durch den Begriff „Entalkoholisierter Wein“ ersetzt worden. Diese Regelung folgte der damaligen Rechtssetzung im Lebensmittelrecht, die Hinweise auf das Nichtvorhandensein eines Stoffes nur noch dann zuließ, wenn dieser Stoff im Lebensmittel tatsächlich nicht mehr vorhanden war.

Durch eine weitere Änderung der nationalen Weinverordnung im Jahre 1993 wurde die Verkehrsbezeichnung „Entalkoholisierter Wein“ wieder gegen die Bezeichnung „Alkoholfreier Wein“ ausgetauscht. Der Gesetzgeber folgte hierbei der Verkehrsauffassung bei Bier, welche die Angabe „alkoholfrei“ bei einem Alkoholgehalt bis zu 0,5 % vol. erlaubte. Die aus Wein hergestellten Getränke sollten bezeichnungsrechtlich nicht anders behandelt werden als Bier.

Neben der früheren Verkehrsbezeichnung “Entalkoholisierter Wein“ ließ das deutsche Weinrecht seit 1990 den Begriff „Teilentalkoholisierter Wein“ zu mit einem Alkoholgehalt von weniger als 2 % vol. Gesetzgeberischer Grund waren zunehmende Absatzschwierigkeiten auf dem Weinmarkt vor dem Hintergrund der allgemeinen „Light-Welle“, welche die Bedenken wegen einer Verwischung der notwendigen Grenzen zwischen Wein und anderen Erzeugnissen zurückstellen ließ.

Durch eine Änderung der Weinverordnung im Jahre 1993 ist die Verkehrsbezeichnung „Teilentalkoholisierter Wein“ durch die Bezeichnung „Alkoholreduzierter Wein“ ersetzt worden. Gleichzeitig wurde aus Gründen der Geschmacksverbesserung der zulässige Alkoholgehalt auf 4 % vol erhöht. Der Gesetzgeber erfüllte mit dieser Regelung Forderungen der Weinwirtschaft nach einer Verkehrsbezeichnung, die nicht nur geschmacklich, sondern auch sprachlich besser als die komplizierte Angabe „Teilentalkoholisierter Wein“ den Marktbedürfnissen entsprach.

Durch Vergärung oder unter Zusatz von Kohlensäure können aus alkoholfreiem Wein oder alkoholreduziertem Wein „schäumende Getränke“ hergestellt werden. Das Inverkehrbringen solcher Getränke war schon im Jahre 1971 erlaubt. Als Verkehrsbezeichnung war früher lediglich die Angabe „Aus entalkoholisiertem Wein hergestellt“ zulässig.

Durch die Änderung der Weinverordnung im Jahre 1993 sind die Verkehrsbezeichnungen „Schäumendes Getränk aus alkoholfreiem Wein“ und „Schäumendes Getränk aus alkoholreduziertem Wein“ eingeführt worden. Der Mindest- und Höchstalkoholgehalt dieser schäumenden Getränke entspricht demjenigen der jeweiligen Ausgangsprodukte alkoholfreier bzw. alkoholreduzierter Wein. Ein bestimmter Kohlensäure-Überdruck ist nicht vorgeschrieben.

Nach deutschem Weinrecht dürfen Getränke, welche mit Erzeugnissen im Sinne des Weingesetzes verwechselt werden können, nicht verarbeitet, in den Verkehr gebracht oder eingeführt werden. § 47 Weinverordnung enthält eine Ausnahme von diesem grundsätzlichen Verbot und erlaubt die Herstellung und das Inverkehrbringen folgender Getränke:

- **„Alkoholfreier Wein“**
- **„Alkoholreduzierter Wein“**
- **„Schäumendes Getränk aus alkoholfreiem Wein“**
- **„Schäumendes Getränk aus alkoholreduzierten Wein“**

2. Herstellungsfragen

Für alkoholfreien Wein und die daraus hergestellten Getränke gilt, dass sie aus Wein und unter schonender Anwendung bestimmter Entalkoholisierungsverfahren hergestellt sein müssen.

Alkoholfreier Wein muss **aus „Wein“** hergestellt werden. Aus anderen Rohstoffen darf alkoholfreier Wein nicht hergestellt werden. Die gleiche Regelung gilt für alkoholreduzierten Wein und für schäumende Getränke aus alkoholfreiem/alkoholreduziertem Wein. Es muss sich um Wein im Sinne der Definition in Anh. XIb Nr. 1 Verordnung (EG) Nr. 1234/2007 handeln. Der Begriff ist hier weit zu verstehen. Er umfasst alle Weinarten und Weingruppen. Auch aus Drittlandwein kann in Deutschland alkoholfreier Wein hergestellt werden. Aromatisierte Getränke im Sinne der Verordnung (EWG) Nr. 1601/91 sind kein Wein, sie dürfen nicht zu alkoholfreiem Wein verarbeitet werden.

Als **Entalkoholisierungsverfahren** sind ausdrücklich „thermische Prozesse, Membran-Prozesse, bei deren Anwendung eine Volumenminderung des Weines von höchstens 25/100 eintreten darf, oder Extraktion mit flüssigem Kohlendioxyd“ zugelassen. Die Regelung enthält keine enummerative Aufzählung einzelner zugelassener Verfahren, sondern die allgemeine Beschreibung von Technologien, um die technische Entwicklung nicht einzuengen. Damit keine Verfahren angewandt werden, bei denen gewonnenes Konzentrat erst durch Rückverdünnung mit erheblichen Wassermengen wieder trinkfähig wird, ist im Interesse der Produktqualität eine Obergrenze von 25 % für die Volumenverminderung des Ausgangsweines festgelegt worden.

Zur Qualitätssicherung ist nur die **„schonende Entgeistung“** erlaubt. Nur solche Entalkoholisierungsverfahren sind zulässig, welche dem Wein zwar den Alkohol entziehen, hierbei jedoch die weinspezifischen Aromastoffe so weit wie möglich erhalten.

Moderne Entalkoholisierungsverfahren sind in der Lage, den fruchtigen und weinigen Geschmack des Ursprungsweines weitgehend zu bewahren. Qualitativ wertvolle und sortentypische Weine mit hohem Gehalt an Bukettstoffen sind zur Herstellung von geschmacklich überzeugenden alkoholfreien Weinen besonders geeignet. Allerdings liegt es in der Natur der Sache, dass alkoholfreier Wein organoleptisch Unterschiede zu Wein haben muss. Es fehlt nun einmal der Alkohol, welcher bei Wein ein wichtiger Geschmacksbilder und –träger ist.

Bei der Herstellung des Ausgangsproduktes Wein sind sämtliche nach dem Gemeinschaftsrecht und dem nationale Recht zugelassenen önologischen Verfahren erlaubt.

Hinsichtlich der zulässigen önologischen Behandlung des Alkoholfreien Weines und der daraus hergestellten Erzeugnisse gelten folgende Besonderheiten:

Zur **Konservierung** ist der Zusatz von Dimethyldicarbonat (DMDC) zu alkoholfreiem Wein erlaubt, nicht zu alkoholreduziertem Wein etc. Die Höchstmenge beträgt 250 mg/l, eine Deklaration des Zusatzes ist nicht erforderlich. **Sorbinsäure** darf zu keinem der Erzeugnisse direkt zugegeben werden, kann jedoch im verwendeten Ausgangswein bereits enthalten sein (carring over). In diesem Fall muss der Gehalt gekennzeichnet werden.

Schwefeldioxid darf dem alkoholfreien Wein bis zu einem Gesamt-Gehalt von 200 mg/l zugesetzt werden. Nicht erlaubt ist es, alkoholreduziertem Wein sowie den schäumenden Getränken aus alkoholfreiem Wein bzw. alkoholreduziertem Wein Schwefeldioxid zuzusetzen. Diese dürfen jedoch den Schwefeldioxid-Gehalt ihrer Ausgangsstoffe aufweisen. Höchstmengen sind, außer für die jeweiligen Ausgangsstoffe für die Endprodukte nicht eigens festgelegt. Wie bei allen Getränken ist die Angabe „enthält Sulfite“ oder alternativ „enthält Schwefeldioxid“ vorgeschrieben, wenn der Schwefeldioxid-Gehalt des Fertigproduktes mehr als 10 mg/l beträgt.

Wasser darf bei alkoholfreiem Wein und daraus hergestellten Getränken weder zum Volumenausgleich noch zur Säureverminderung zugesetzt werden. Das Verbot gilt nicht für das Zusetzen von verfahrenstechnologisch notwendigem Fremdwasser. Bei dem für die Entalkoholisierung zugelassenen Membranverfahren werden dem zu entalkoholisierenden Wein zwar erhebliche Mengen von sogenanntem Prozesswasser zugefügt. Dieses Wasser wird jedoch wieder entfernt und ist im Endprodukt nicht mehr vorhanden.

Streitig ist die Zulässigkeit einer **Aromatisierung** von alkoholfreiem Wein und daraus hergestellten Getränke. Die Zulassung von Aromen ist problematisch, weil hiervon auch das Zusetzen von Weinaromen erfasst wird. Nach Ansicht des Verfassers muss das Zusetzen von

Aromen als zulässig angesehen werden, weil § 47 Weinverordnung bezüglich der Zutaten keine abschließende Regelung enthält. Zur Vermeidung einer Irreführung der Verbraucher ist allerdings das Zusetzen von Aromen in der Etikettierung deutlich kenntlich zu machen. Die Rückführung von weineigenen Aromen ist erlaubt und wesentlicher Bestandteil der Entalkoholisierungsverfahren.

Zur **Süßung** von alkoholfreiem Wein und daraus hergestellten Getränken war zunächst nur das Zusetzen von Traubenmost und rektifiziertem Traubenmostkonzentrat zulässig. Ob auch Saccharose zur Süßung erlaubt war, war zunächst streitig, ist jedoch auf Drängen der Weinwirtschaft ausdrücklich zugelassen worden. Denn die Süßung mit Saccharose hat sich zur Geschmacksverbesserung als notwendig erwiesen.

Für die Herstellung von alkoholreduziertem Wein gelten spezielle, in der Weinverordnung geregelte Sonderbestimmungen, welche das Vermischen von Wein mit alkoholfreiem Wein erlauben.

Für schäumende Getränke aus alkoholfreiem/alkoholreduziertem Wein ist zur Schaumbildung die Gärung oder Karbonisierung gestattet. Ein bestimmter Kohlensäure – Überdruck ist nicht vorgeschrieben.

3. Bezeichnungen

§ 47 Weinverordnung enthält die Verpflichtung zur Angabe der Verkehrsbezeichnungen **„Alkoholfreier Wein“, „Alkoholreduzierter Wein“, „Schäumendes Getränk aus alkoholfreiem Wein“** und **„Schäumendes Getränk aus alkoholreduziertem Wein“**. Hinsichtlich der übrigen Pflichtangaben in der Etikettierung gelten die allgemeinen Kennzeichenvorschriften des Lebensmittelrechts.

Bezeichnungen wie „entalkoholisierter Wein“ oder „alkoholfreier Sekt/Schaumwein“ sind unzulässig, weil bei alkoholreduzierten Getränken aus Wein nur die oben genannten, in der Weinverordnung geregelten Verkehrsbezeichnungen zugelassen sind.

Fakultativ darf der Name einer einzigen **Rebsorte** und der Name eines **bestimmten Anbaugebietes** angegeben werden, wenn das Erzeugnis hierfür typisch ist.

Schäumende Getränke aus alkoholfreiem Wein dürfen in der Schaumweinflasche vermarktet werden. Ab einem Alkoholgehalt von mehr als 1,2 % vol. sind in der Schaumweinflasche abgefüllte alkoholreduzierte Getränke in Deutschland schaumweinsteuerpflichtig.

IV. Märkte

Als Konsumenten von alkoholreduzierten Weinen ist jeder angesprochen, der gerne Wein trinkt, aber aus irgendeinem Grund auf Alkohol verzichten möchte oder muss. Wegen der restriktiven Alkoholgesetzgebung und des Trends der Konsumenten zu einer gesundheitsbewussten Ernährung ist zurzeit ein verstärktes Interesse an alkoholfreien Weinen festzustellen.

Aktuell wird Alkoholfreier Wein weltweit von sieben Produzenten hergestellt, davon drei in Deutschland. Im Jahre 1991 sind in Deutschland ca. 3,5 Mio., im Jahre 1992 ca. 4,3 Mio., im Jahre 1993 ca. 6,1 Mio. 0,75-Liter-Flaschen vermarktet worden. In den Folgejahren stagnierte der Absatz bei ca. 5 Mio. Flaschen. In den letzten Jahren ist die Produktion wieder angestiegen. Zurzeit beträgt die Produktion in Deutschland ca. 17 Mio. Liter jährlich. Auch in den USA werden alkoholfreie Weine in größeren Mengen hergestellt, genaue Zahlen sind nicht bekannt.

Bei einem Absatzvolumen von 17 Millionen Litern jährlich allein in Deutschland ist der Markt der Alkoholfreien Weine auch für die Weinerzeuger attraktiv.

Skandinavien, Benelux, Deutschland, England, Japan, Nord-Amerika und Russland sind gut entwickelte Absatzmärkte. Der arabische Markt ist nicht bedeutend. Denn das Wort „Wein“ ist dort– selbst in Verbindung mit dem Begriff „Alkoholfrei“ – aus religiösen Gründen nicht akzeptiert.

Überwiegend werden „Alkoholfreie Weine“ und „Schäumende Getränke aus alkoholfreiem Wein“ vermarktet. Schäumende Getränke nach § 47 Weinverordnung sind geschmacklich besonders ansprechend, weil die Kohlensäure geschmackliche Nachteile auszugleichen vermag. Dementsprechend positiv ist die Akzeptanz dieser Produkte auf dem Markt.

Für alkoholreduzierten Wein besteht im In- und Ausland bislang keine Nachfrage. Im Zweifel entscheidet sich der Konsument für alkoholfreien Wein.

V. Schlussfolgerungen

Vor dem Hintergrund der internationalen Anti-Alkoholkampagne, einem gestiegenen Gesundheits- und Fitnessbewusstsein und der Forderung nach Alkoholverzicht im Straßenverkehr sind die Marktchancen für alkoholfreien Wein als positiv zu bezeichnen. Auf dem Weinsektor wird der Herstellung und Vermarktung dieser Getränke immer größere Aufmerksamkeit geschenkt. Um den Handel mit diesen neuen Wein-Erzeugnissen zu erleichtern, sind einheitliche Definitionen der Verkehrsbezeichnungen erforderlich. Bei der Festlegung solcher Definitionen können die in Deutschland gemachten Erfahrungen bei der Herstellung, Bezeichnung und Vermarktung von Alkoholfreiem Wein und den daraus hergestellten Produkten hilfreich sein.

Auf der Grundlage dieser Erfahrungen sind folgende Definitionen sachgerecht:

1. **„Alkoholfreier Wein“** ist ein durch Alkoholisierung von Wein hergestelltes Getränk, welches weniger als 0,5 % vol Alkohol enthält.

2. **„Alkoholreduzierter Wein“** ist ein durch teilweise Entalkoholisierung von Wein oder durch Vermischen von alkoholfreiem Wein mit Wein hergestelltes Getränk, welches mindestens 0,5 % vol und weniger als 4 % vol Alkohol enthält.

3. **„Schäumendes Getränk aus alkoholfreiem Wein“** ist ein durch Vergärung von alkoholfreiem Wein oder durch Zusatz von Kohlensäure hergestelltes Getränk, welches weniger als 0,5% vol Alkohol enthält.

4. „Schäumendes Getränk aus alkoholreduziertem Wein“ ist ein durch Vergärung von alkoholreduzierten Wein oder durch Zusatz von Kohlensäure hergestelltes Getränk, welches mehr als 0.5 % vol und weniger als 4 % vol Alkohol enthält.

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ATTITUDINE DEI CONSUMATORI VERSO GLI ALIMENTI OGM: UNA RASSEGNA DELLA LETTERATURA APPLICATIVA

33° CONGRESSO MONDIALE DELLA VIGNA E DEL VINO

G. Benedetto
Università degli Studi di Sassari
Via E. De Nicola, Sassari, Italia
gbenedet@uniss.it

RIASSUNTO
Numerose sono le questioni che hanno animato il dibattito che ruota attorno alla domanda e all'offerta di alimenti GM (dalla definizione, alle norme regolatrici in materia di coltivazione, trasformazione e vendita, etichettatura e così via..) con l'obiettivo principale di garantire la sicurezza alimentare. Soffermando l'attenzione sulla domanda, assai ampio è il numero di studi che si sono occupati di raccogliere le opinioni e le attitudini dei consumatori verso gli alimenti GM all'interno di diversi paesi, o aggregazione di questi attraverso analisi qualitative e quantitative. In questo lavoro, si è proceduto ad una disamina dei risultati ottenuti negli studi che, negli ultimi dieci anni, si sono occupati di indagare sulla relazione tra consumatori e alimenti GM, focalizzando l'attenzione sulle differenziazioni per tipologia di prodotto alimentare, per categoria di consumatori, per appartenenza geografica. L'analisi dei risultati ha consentito di individuare nuovi scenari di analisi ed aspetti lasciati scoperti da studi precedenti.

ABSTRACT
There are numerous issues which have driven the debate revolves around supply and demand GM food (by definition, rules regulations on cultivation, processing and sale, labeling, etc. ..) with the main objective of ensure food safety. Focusing on demand for GM food, it is observed that numerous studies have dealt with people's opinions and attitudes of consumers towards GM foods in different countries, or through aggregation of these, through quantitative and qualitative analysis. In this work, we carried out an analysis of the results obtained in the studies, in the last ten years, have been involved in investigating the relationship between consumers and GM foods, focusing on differences by type of food, for category of consumers, and geographical location. Analysis of the results has enabled the identification of new scenarios and analysis issues left uncovered by previous studies.

INTRODUZIONE La letteratura esaminata, che ha preso in considerazione un arco temporale di dieci anni (dal 1999 ad oggi), consente di distinguere due grandi gruppi di studi: le analisi *qualitative*, volte alla misurazione della percezione dei consumatori e dell'opinione pubblica relativamente agli alimenti GM all'interno di differenti paesi, in cui le metodologie di indagine utilizzate si basano sulla somministrazione di questionari ad un campione più o meno ampio di popolazione; il principale risultato di queste ricerche porta ad individuare l'accettazione o il rifiuto di alimenti GM, ed a graduarne il livello in funzione delle caratteristiche socio-economiche e demografiche, ad individuare le ragioni che giustificano il diverso atteggiamento nei confronti di queste produzioni; le analisi *quantitative*, studiate per qualificare il processo di acquisto del consumatore e le preferenze per i cibi GM, all'interno di differenti paesi e per diverse categorie di alimenti; in questo caso vengono utilizzati strumenti di analisi statistica come cluster analysis, contingent valuation (volta a quantificare la Disponibilità A Pagare (DAP) o ad Accettare (DAA) una somma di moneta per evitare l'acquisto di alimenti GM o a titolo di risarcimento), modelli multiattributo (conjoint analysis). L'osservazione congiunta dei risultati consente di costruire: un quadro ragionato del livello di accettazione dei cibi GM; il processo di acquisto; le possibili linee di ricerca future.

MATERIALI E METODI Prima di procedere all'esame dei risultati ottenuti pare doveroso sottolineare che questa rassegna è tutt'altro che esaustiva, che si tratta di contributi scientifici che sono nella maggior parte statici, poiché fanno riferimento al solo anno di indagine e non consentono pertanto di valutare il fenomeno in termini evolutivi; utilizzano dati diversi; le domande vengono formulate in maniera differente; il contesto e le possibili specificità del campione di intervistati cambia, per cui anche il confronto potrebbe essere azzardato e portare a deduzioni troppo affrettate. Tenendo a mente questi limiti, andiamo a presentare i risultati degli studi analizzati per fornire una visione del grado di opposizione o accettazione da parte dei consumatori di alimenti GM, della relativa variazione in funzione della localizzazione geografica e delle caratteristiche socio-economiche, delle principali determinanti che guidano il processo di acquisto.

RISULTATI E DISCUSSIONE La dimensione geografica. La prima grande ripartizione vede tra loro contrapposti Stati Uniti ed Unione Europea, che guidano rispettivamente la classifica dei favorevoli e contrari. Negli Stati Uniti (IFIC, 1997, 1999, 2000, 2001, 2002); (Gaskell, Bauer, Durant, & Allum, 1999); Angus Reid Group (2000); Pew (2002), gli americani intervistati tendono a considerare gli alimenti GM utili per la società, moralmente accettabili e privi di rischi per la salute (Eurobarometer, 2005; IFIC, 2008). *Survey* più recenti, tuttavia, mostrano che il grado di accettazione è andato diminuendo nel corso del tempo a causa del fatto che le esportazioni statunitensi si sono ridotte in misura drammatica da quando i consumatori europei hanno iniziato a non acquistare gli alimenti GM (Botelho-Kurtz, 2008). Un'altro importante mercato di produzione e propensione al consumo di alimenti GM è rappresentato dalla Cina *(Bonny, 2003).* I risultati di un'indagine condotta a Pechino rivela che i consumatori cinesi considerano favorevolmente l'immissione sul mercato di prodotti quali il riso e l'olio di soia GM (*Quan Li et al., 2002*). Inoltre è notizia recente (Vania, 2008) che un gruppo di ricercatori dell'Università di Yangling, ha realizzato in laboratorio un super-vino usando varietà di uve geneticamente modificate, sei volte più ricche di *resveratrolo*. Anche il Brasile rientra in questo gruppo di paesi ed occupa il terzo posto per estensione della superficie destinata alla coltivazione di varietà GM (soia, mais, semi di colza, patate e cotone) a livello mondiale; circa l'80-90% dell'olio di soia prodotto nel mondo deriva da colture GM che richiedono ridotte quantità di erbicidi. Tuttavia, negli anni anche in Brasile si è costituito un movimento simile a quello che si è sviluppato in paesi contrari all'introduzione delle tecnologie genetiche in agricoltura, pertanto sono state condotte delle indagini pilota per verificare la percezione pubblica delle biotecnologie all'interno del paese (Oda, Soares, 2000). Stessa inversione di tendenza è stata registrata in Giappone, dove fino al 2000 i cittadini mostravano un atteggiamento favorevole, poi mutato nel corso dei successivi cinque anni (Nishiura et al, 2005) per effetto dell'aumento della percezione del rischio sulla salute; mentre il mondo scientifico risulta ancora favorevole allo sviluppo della ricerca, che deve però essere accompagnata da una corretta campagna di informazione attraverso i mass media.

In tutti i paesi che appartengono all'UE, Argentina (Mucci, Hough, 2003), Nuova Zelanda (Campbell et al, 2000; Gamble, Gunson, 2002), Singapore (Subrahmanyan and Cheng, 2000), Australia (Lockie t al, 2005) esiste un movimento di forte opposizione alla diffusione di alimenti GM. La maggior parte delle ricerche effettuate all'interno dell'UE hanno rilevato che il livello di opposizione si è mantenuto pressoché costante nel corso del tempo. Non si può trattare la materia senza fare preciso riferimento alle indagini svolte da Eurobarometer (1991, 1993, 1996, 1999, 2002, 2005, 2008), organizzate e supervisionate dalla Commissione Europea, che hanno interessato un campione di circa 16.000 persone, fornendo interessanti risultati sulle attitudini e opinioni in merito alle biotecnologie, in generale, ed agli alimenti GM oggetto specifico del presente studio, che permettono di ragionare anche in termini di confronto in quanto è stato utilizzato lo stesso questionario nei diversi paesi dell'UE. Come primo risultato si può riportare che nonostante l'avversione generale rilevata, è possibile graduare questa ostilità all'interno dell'UE dividendo i cittadini europei tra meno favorevoli (Francia, Grecia, Austria, Lussemburgo); e più favorevoli (Spagna, Olanda, Regno Unito, Belgio e Danimarca). Infatti nel corso degli anni (1999 e 2005) tutti i 15 paesi dell'UE, ad eccezione dell'Austria, hanno manifestato un trend crescente in valutazione positiva delle biotecnologie: questo indice di ottimismo copre un range che va da un massimo di 75 in Spagna ad un minimo di 19 in Grecia, mostrando una forte divergenza di opinioni tra paesi; lo stesso si verifica all'interno dei 10 nuovi stati membri dove tale indice assume un valore pari a 81 a Malta e 47 in Slovenia. Nella maggior parte dei paesi poveri (Asia e Medio Oriente) non sono presenti colture atte alla produzione di alimenti GM o di mangimi destinati all'alimentazione animale, perché le autorità governative non consentono questo tipo di coltivazione a titolo precauzionale, di sicurezza per la salute e per l'ambiente, così come per evitare limitazioni all'importazione nei paesi ad alto reddito, come Europa e Giappone, che presentano normative in materia di etichettatura e tracciabilità per tutti i derivati da alimenti GM ed i mangimi (Paalberg*, 2002*).

Il processo di acquisto degli alimenti GM. La sintesi sistematica della letteratura ha consentito di costruire anche il comportamento di acquisto dei consumatori di alimenti GM. Si tratta della schematizzazione di un processo che consente di arrivare ad individuare, a monte, le determinanti del processo di accettazione o rifiuto dei cibi GM, che verranno qui di seguito esaminate.

Il ruolo dell'informazione. Le ricerche hanno dimostrato che i consumatori apprendono le informazioni principalmente attraverso i mass media, e dai quotidiani in particolare (Hallman, Metcalf, 1995; Hoban, 1998; Priest, 2002; Eurobarometer, 2008); i quotidiani sono anche una delle principali aree di discussione dove le questioni controverse balzano all'attenzione non solo dei cittadini, ma anche dei gruppi di interesse e dei politici (Nisbet, Brossard, Kroepsch*, 2003*). Uno studio recente (Bothelo-Kurtz, *2008*) suggerisce che l'attenzione dei media (negli Usa e Regno Unito) si va concentrando in misura pronunciata su eventi, spesso, negativi relativi agli alimenti GM, senza riportare informazioni circa gli effetti positivi registrati dall'industria alimentare, soprattutto per quanto attiene lo sviluppo di nuova ricerca e tecnologia. La stessa difficoltà di comunicare, in misura bilanciata, i benefici ed i potenziali rischi associati all'uso degli OGM è responsabile del declino di ottimismo nei confronti di queste produzioni che ha caratterizzato il trend decrescente nei paesi dell'UE tra il 1991 ed il 1999 (Eurobarometer*, 2005*).Questo rapporto mostra, infatti, una crescita nel supporto pubblico relativo all'introduzione delle biotecnologie e applicazioni alle scienze umane, ad eccezione dei cibi associati ad OGM. Come suggerito da Bredhal (1999) questo non significa necessariamente che le attitudini del consumatore verso i cibi GM diverranno positive, a seguito di una informazione più puntuale, perché questo dipende da come l'informazione

stessa viene percepita, ma, a parità di condizioni, probabilmente più stabili.
La fiducia. Sempre secondo le indagini svolte da Eurobarometer (1999) le differenze mostrate nei confronti degli alimenti GM nell'opinione pubblica statunitense ed europea sarebbe da addebitare alla fiducia riposta nei confronti delle procedure di regolamentazione, della letteratura scientifica, e della rassegna stampa; in alcuni paesi europei, le agenzie ufficiali e di governo sono le meno accreditate tra le diverse fonti di informazione, ancora meno delle fonti di informazione provenienti dall'industria (da Moses, 1999 fino ad oggi). Mentre sulla base dell'indagine effettuata nei diversi paesi europei la fonte di informazione più attendibile in merito al tema delle biotecnologie sarebbe rappresentata dall'associazione consumatori, seguita dalle associazioni ambientaliste, dalle scuole ed università, dalle autorità pubbliche e dall'industria (Barling et al, *1999*). Le associazioni di consumatori nel Regno Unito sono generalmente le più accreditate, e sono viste come le maggiormente informate e proattive, avendo come principale obiettivo il benessere sociale (Frewer et al, *1999*) ed essendo meno legate ad interessi commerciali (Green et al, *2003*).
La ricerca qualitativa svolta in Belgio, Germania, Francia, Italia, Paesi Bassi, Norvegia, Svezia e Regno Unito (Moses, 1999) ha rilevato che un obiettivo primario delle organizzazioni dei consumatori è proprio quello di garantire che i consumatori siano informati e possano effettuare le proprie scelte in maniera consapevole. Secondo le indagini di Eurobarometer (2005), tuttavia, la fiducia nelle istituzioni che a vario titolo si occupano di biotecnologie (università, ricercatori, industrie) è andata crescendo in Europa nel corso del tempo. Inoltre, gli studi indicano che una più elevata percentuale di intervistati vorrebbe che la regolamentazione inerente le biotecnologie venisse predisposta dall'UE piuttosto che dai singoli governi nazionali.
La conoscenza del prodotto e processo. La conoscenza dello specifico prodotto/processo è cruciale nella determinazione delle attitudini dei consumatori. Si caratterizza per una conoscenza oggettiva, derivante dalla possibilità di riconoscere i cibi GM (etichettatura del prodotto.....); ed una conoscenza soggettiva, che si riferisce a ciò che i consumatori pensano dei cibi GM (opinioni vs informazioni, attributi individuali/valori...). Le evidenze empiriche (Eurobarometer, 2005) mostrano una crescita nel processo di conoscenza delle biotecnologie e della genetica applicata fin dal 2002, sebbene questa sia ancora assai ridotta. Tuttavia, fin dal 2001, il 95% degli europei intervistati vorrebbero effettuare le proprie scelte di acquisto dei cibi GM con piena consapevolezza; l'86% di questi desiderano avere informazioni *"per sapere di più di questo tipo di cibo prima di mangiarlo*"; i risultati delle ricerche indicano, inoltre, che la conoscenza percepita delle tecnologie di produzione può giocare un ruolo significante per la formazione delle attitudini dei consumatori ed, in ultima istanza, per la disponibilità ad accettare i cibi GM (Bredhal*, 1999).*
Gli attributi individuali. Le analisi condotte (Eurobarometer, vari anni) hanno evidenziato che la struttura socio-demografica della popolazione è strettamente correlata con l'attitudine verso i cibi GM; in particolare ad un elevato livello di istruzione si associa il riconoscimento dei benefici introdotti con gli OGM, mentre un basso livello di istruzione si combina con una elevata percezione dei rischi. Allo stesso modo l'età, il livello di istruzione, l'etnicità, il livello di reddito sono variabili strettamente connesse con le attitudini dei consumatori. Il minore livello di rifiuto si rileva tra studenti, giovani, redditi elevati: in particolare gli studenti ed i giovani sono i meno ostili nei confronti degli OGM (risultato questo che tuttavia non è correlato con un maggiore livello di conoscenza); le donne sono più ostili degli uomini, così come gli individui a basso reddito, i vecchi e le casalinghe.
I valori individuali. Il set di valori di un consumatore deriva: a) dall'attitudine nei confronti dell'ambiente, della tecnologia, cultura e così via; b) dalla percezione del prodotto (gli

alimenti GM sono associati ad un mercato monopolistico - valore negativo); ad altri due valori negativi che sono rappresentati dall'incertezza e dalle ripercussioni sulla salute, mentre i metodi di produzione convenzionali vengono associati a valori positivi come salute e sicurezza alimentare. La maggior parte degli europei ritengono che i cibi GM siano dannosi; tuttavia si tratta ancora di una questione aperta per la maggior parte di essi. Varia molto da paese a paese in ragione degli aspetti culturali, delle differenze emerse nei dibattiti pubblici, degli interventi di governo, della storia dell'economia dello sviluppo e della situazione industriale (Springer et al., *2002*).

La percezione dei rischi. Per l'opinione pubblica, gli OGM sono percepiti soprattutto come poco utili, non naturali e rischiosi (Eurobarometer, diversi anni); in Italia, Danimarca, Germania e Regno Unito, l'adozione delle modificazioni genetiche è associata alla mancanza di naturalità e bassa affidabilità dei prodotti ottenuti, indipendentemente dal fatto che i materiali GM siano tracciabili nel prodotto (Bredhal, 1999; Eurobarometer, *2005*); la percezione del rischio esercita un'influenza assai maggiore della percezione dei vantaggi (Balasubramanian*, 2001*), per cui una delle cause dell'opposizione degli europei nei confronti degli OGM è associata al fatto che i relativi vantaggi nella produzione di alimenti sono spesso considerati deboli o inesistenti, mentre i relativi rischi sono considerati sostanziali (Bonny*, 2003*). Sia le analisi qualitative che quelle quantitative, hanno dimostrato che diversi fattori socio-culturali sono alla base della percezione del rischio associata all'uso di alimenti GM (Finucane, Holup*, 2005*). I consumatori italiani sarebbero maggiormente avversi al rischio, manifestando una minore propensione all'acquisto di prodotti alimentari GM rispetto ai consumatori statunitensi. Sarebbe invece simile l'importanza attribuita, dai consumatori dei due paesi, al controllo delle produzioni GM da parte delle istituzioni pubbliche e quindi il grado di fiducia nei loro confronti è fondamentale per favorire la predisposizione all'acquisto di alimenti GM (Harrison*, 2004*).

Il caso del vino GM. Studi recenti (Plahuta et al, 2007) mostrano una risposta dei consumatori simile a quella registrata nei confronti degli altri alimenti. In Slovenia la disponibilità ad accettare i cibi GM è guidata soprattutto dalla loro percezione dei rischi, dei benefici, della sicurezza legata a queste tecnologie; enologi e dettaglianti forniscono opinioni qualche volta strettamente affini a quelle dei consumatori. Negli ultimi anni sono stati fatti progressi considerevoli nello sviluppo di ceppi di lieviti vinicoli, la ricerca va avanti coprendo aspetti qualitativi legati alle differenti fasi del processo produttivo, lo studio delle preferenze del consumatore rispetto a vini che abbiano caratteri particolari (come i vini ad alto contenuto di resveratrolo, Hurlé *et al*, 2008), il calcolo della possibile riduzione dei costi ottenibile come risultato dell'introduzione di GMO all'interno della produzione di uva e tecnologia vinicola (Plahuta *et al*, 2006), analizzando i rischi per la salute e l'ambiente ponendo a confronto differenti pratiche viticole ed enologiche (Plahuta e Raspor, 2007), analizzando cambiamenti, opportunità e benefici potenziali delle biotecnologie in ambito vinicolo (Pretorius e Hoj, 2005). Nonostante ciò a tutt'oggi l'industria vinicola non utilizza i lieviti vinicoli transgenici per produrre vino su scala mondiale e l'introduzione di nuovi ceppi di lievito per l'industria enologica si basa ancora quasi esclusivamente sulla variabilità genetica naturale attraverso l'isolamento e la selezione di ceppi naturali (Cebollero *et al*, 2007). Il movimento di forte opposizione nei confronti dell'utilizzo delle biotecnologie all'interno del processo di produzione del vino (consumatori, ambientalisti, leggi e regole restrittive approvate a livello internazionale) inducono, infatti, a pensare che sarebbe un *suicidio* commerciale immettere sul mercato il vino GM (Pretorius, 2005, p. 87). Alcuni autori (Cebollero *et al*, 2007) ritengono che le ragioni per le quali il vino GM non arriva ai consumatori sono essenzialmente le stesse che impediscono la diffusione nel mercato degli altri cibi GM e fra queste vengono incluse le lunghe e costose procedure amministrative, almeno in EU, così come la sfiducia dei consumatori e l'opposizione degli attivisti. Nel caso del vino si aggiungono problemi legati alle specificità del mercato vinicolo, fra cui le regolamentazioni internazionali (OIV), nazionali e locali (denominazioni di origine); ancora le tecnologie GM costituiscono una minaccia per l'immagine tradizionale del prodotto, che rappresenta invece un elemento di forza per produttori e regioni di provenienza; per questa serie di ragioni si ritiene che i tempi per la diffusione del vino GM non siano ancora maturi (Cebollero *et al*, 2007). Si ritiene (Pretorius, 2005; Pretorius and Hoj, 2005; Cebollero *et al*, 2007), peraltro, che l'introduzione delle tecnologie di modificazione genetica nella produzione di uva e vino sia inevitabile nei prossimi anni, soprattutto nei paesi del Nuovo Mondo, ritenendo arduo predire quanto tempo dovrà trascorrere prima che i consumatori possano accettare gli enormi benefici potenziali offerti dalle biotecnologie in questo settore, citando come riflessione il caso che si presentò oltre un secolo fa, quando il suggerimento di Pasteur fu sottoposto ad un lungo periodo di pubbliche discussioni e ci vollero venti anni prima che questo benefico processo venisse accettato a livello mondiale (Pretorius, 2005).

CONCLUSIONI La ricerca rende evidente che una sostanziale porzione della popolazione tende a evitare l'uso dei cibi GM, soprattutto all'interno dell'UE. Numerose sono le questioni cui ricerche future possono dare risposta, prima fra tutte una valutazione approfondita dei fattori indicati prima che portano al rifiuto o all'accettazione dei cibi GM; l'individuazione delle strategie attraverso cui superare la resistenza all'accettazione dei cibi GM, come programmi di etichettatura che trasmettano informazioni ai consumatori mettendoli in condizione di formulare le proprie scelte di acquisto in misura consapevole; indagare in misura sistematica sul livello di accettazione/rifiuto all'acquisto di vino GM da parte dei consumatori dei paesi che appartengono all'OIV, partendo appunto dalla definizione di vino GM attualmente in discussione.

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Studies of *Di (N-Butyl) Phthalate* in Viticulture Products and its Influence on the Quality

M. Katsitadze [1], JSC "Sarajishvili", # 4 Sarajishvili, 0153, Tbilisi, Georgia

D. Abzianidze [2], "Sarajishvili", # 4 Sarajishvili, 0153, Tbilisi, Georgia

M. Khositashvili [3], Institute of Horticulture, Viticulture and Oenology, # 6 Marshal Gelovani ave., 0159, Tbilisi, Georgia

T. Kortava [4], Institute of Horticulture, Viticulture and Oenology, # 6 Marshal Gelovani ave., 0159 Tbilisi, Georgia

T. Kituashvili [5], "Askaneli brothers" ltd, 110[a] Kakheti highway, 1298 Tbilisi, Georgia

V. Gorgisheli [6], Institute of Horticulture, Viticulture and Oenology, # 6 Marshal Gelovani ave., 0159 Tbilisi, Georgia

ABSTRACT

Toxic substances - phthalates get to the grapes processing products from polymeric materials (from pesticides, tare-ware, machine - equipment, etc.). The plasticizers used in polymeric materials provoke human organism pollution; their toxicity is caused by action of phthalates disintegration products. The Research objective was establishment of phthalates composition in wine materials and in a brandy and possible ways of their ingress in it. The researches have established that, in the specified products, there is a migration of toxic substances worsening the quality of production and making it inedible.

Les substances toxiques dans les matières de vin et dans la Brinze se trouvent depuis des poisons chimiques, d tares d'emballage, machines –installations etc. Les agents de plastifications polymères, c'est-à-dire les agents amollissants provoquent la pollution de l'organisme, dont la toxicité est conditionnée par l'action des produits de décomposition des phtalates composants ces agents. L'objectif de l'examen consistait à la définition de la constitution des phtalates dans les matières du vin de la Brinze et dans les alcools et l'établissement des voies éventuelles de leur entrée dans les susdites matières. Il a été examiné que quelqu'un component de matériau du vin et d'alcool de la Brinze entre chez les matériaux polymers, ils maîtrisent la matière hors, quoi deterioration le degré production et se passe la nourriture mauvaise.

INTRODUCTION

Migration of the toxic substances, entering into their formulation, from the polymeric materials widely used in the food industry with the products being in contract takes place. Use of the specified products for food causes pollution of an organism, which has became a problem of a century by this time. Plasticizers belong to the group of such substances, i.e. softeners. They unite nearly 2500 kinds. Among them, it is necessary to note derivative of phthalic acid – ethers, which belong to toxic substances of the 2nd and 3rd class. Phthalates have been using for several tens of years, because of their numerous useful chemical properties. They are found in all spheres. Their toxicity is caused by action of phthalates disintegration products in an organism.

On the assumption of previously mentioned, a foodstuff may become completely inedible, unhealthy and toxic food for human health (Tyurin, 1964: Tavadze, 1968; Stankevich etc., 1980). Therefore, the questions concerning formation and migration of toxic substances in winemaking must be revised.

In 2003, European Parliament considered existence of toxic substances fixed in foodstuffs and developed a normative document concerning food safety and planed measures for their minimization (Hahn, 2003).

Wave of worldwide technological progress over the last years provokes an enrichment of foodstuffs with unhealthy chemical substances. Toxic substances get into foodstuffs, particularly into wine materials and brandy, from pesticides, tare-ware, machine - equipment, etc.

On the assumption of aforesaid, for the purpose of food safety, Institute of gardening, viticulture and wine-making, based on experiments set up on the base of JSC “David Sarajishvili and Eniseli”, has studied existence of toxic substances – phthalates in brandy wine materials and spirits and their influence on the quality of product.

Almost all European countries, with the purpose of human health support, have developed hygienic regulations, the purpose of which is ascertainment of bounds of chemical substances content isolated from polymers contacting with foodstuffs. Therefore, under such documents, toxicological examination of drinks on composition of the chemical substances used in formulation of polymers manufacture is necessary.

In March, 2005, the hygienic regulation of the project of the order of Ministry of Labour, Health and Social Security "On the approval of admissible quantity of the chemical substances isolated from polymers and other materials, being in contact with foodstuffs" defined admissible norms of chemical substances in materials of the principal kinds contacting with foodstuffs, according to which, a certain quantity of some phthalates are admissible, and some are absolutely inadmissible in foodstuffs.

MATERIALS AND METHODS

We have planned a research objective to define possible ways of ingress of dibutyl phthalate into wine and other alcoholic drinks. For this purpose, it became necessary to develop some technological parameters (module (1:2,5), temperature and duration) of dibutyl phthalate liberated from test samples and model solutions. The research provided the following matters:

1. Qualitative analysis of dibutyl phthalate in model solutions, wine materials, brandy spirits and brandy.
2. Determination of quantity of dibutyl phthalate in brandy spirits and brandy.
3. Analysis of the ways of ingress of dibutyl phthalate into the test samples.

The area being in contact (wine materials and brandy) makes migration of a complex of chemical substances from polymeric materials. Therefore, for the purpose of their action studying, it is necessary to liberate toxic substances with model solution, for which module establishment it is necessary to take the shattered parts of rubber tubes and polymers containing dibutyl phthalate. For preparation of a model solution, the liquid is added to them in such module that mutual ration of weight of the taken materials and added samples to be 1: 1,5; 1: 2,0; 1: 2,5; 1: 3 and 1:10. As a result of research, we have chosen the module 1:2,5 as an optimum alternative.

RESULTS AND DISCUSSION

For the establishment of possible ways of dibutyl phthalate ingress from the polymeric materials used in winemaking, we divided the sample of a wine material, where the pieces of rubber and polyvinylchloride (admissible for a contact with foodstuffs) were put, into six parts and processed thermally at a temperature of 10, 15, 60, 65, 120 and 125°C. Temperature regimes were taken from technological processes of thermal processing of wine materials (Valuiko, 2001), the samples of wine materials, where the tubes of polyvinylchloride and a unity of mix of rubber and polyvinylchloride were put, were similarly processed. Also, water and brandy spirit, in which there were same polymeric materials, were used as a model solution. Samples were placed in the thermostat for a different time (from 10 to 30 days). In the received samples, the quantity of dibutyl phthalate was determined by spectrophotometric method (see table 1).

Table 1

Influence of temperature regime on dibutyl phthalate composition

in the model solution of water

#	Temperature, ^{0}C	Quantity of dibutyl phthalate, mg/dm^3					
		Polyvinylchloride		Rubber		Pvc + rubber	
		By sp.	Average	By sp.	Average	By sp.	Average
1	10	0,65	0,665	0,68	0,716	0,72	0,73
2	15	0,68		0,70		0,75	
3	60	1,25	1,26	1,28	1,31	1,33	1,35
4	65	1,27		1,34		1,37	
5	120	2,72	2,725	2,70	2,725	3,00	3,05

In the samples taken for the analysis, as a model solution of which we used distilled water, brandy wine material and brandy spirit, according to the temperature regime, we determined the quantity of dibutyl phthalate and arithmetic mean of received indicators by spectrophotometric method, by means of which we calculated the velocity coefficient of migration. The research shows that the factor of speed of the migration of dibutyl phthalate from polymers grows together with temperature increase.

As follows from the table, together with temperature increase, the quantity of dibutyl phthalate has grown from all polymeric materials in the model area. Dibutyl phthalate intensively passes into a liquid from the very beginning. Afterwards, its quantity in the model solution increases, but not intensively. As follows, the variety of polymers in different quantity enriches the model solution with dibutyl phthalates. Similar dependence is appreciable in the model solutions of wine materials and brandy spirits in connection with quantity growth of dibutyl phthalates, with the distinction that in the model area of brandy spirit, in comparison with other areas, the quantity of dibutyl phthalate increased.

Both temperature increase and aggression of areas of model solutions (spirit and acid) are the reason of increase of dibutyl phthalates quantity in the model solutions of brandy wine materials and brandy spirits. Hence, it may be noted that some components of wine materials and brandy spirit enter into a reaction with polymeric materials (with covering varnishes and other materials) and absorb external substances that worsens quality of production and makes it inedible.

Destruction of polymers begins after a certain time. By this time, the most actual is the question studying polymers' solidity dependence on duration of time. Destruction of polymers under the influence of temperature and mechanical influence intensifies accompanied by chemical processes.

The experiment shows that power of aggressive areas of the model solutions increases the process of destruction of the same polymers and the more the temperature increases, the more process intensifies.

All samples presented to the tasting commission, except those samples, in which dibutyl phthalate was given in the form of signs, were characterized by a similar slight smell of dibutyl phthalic model solution. Strength of a smell was caused by a complex of components, which was gripped by dibutyl phthalate during migration and enriches a liquid with other volatile components, of which the formula of polymeric materials consists.

Those samples of brandy spirit and brandy, in which phthalates had been quantitatively fixed, were characterized by specific slight smell of a mix of polymers of the model solution. In those samples, where in the form of signs dibutyl phthalates had been given on organoleptic properties (phthalates less than 5 mg/dm^3), it was impossible to feel their smell.

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Some Biological Activities of Vine Stilbens and New Georgian Grape Products with a Functional Designation

M. Bezhuashvili, T. Kobaidze, M. Kokhtashvili, M. Meskhi, Z. Sturua
Institute of Horticulture, Viticulture and Oenology of Georgia
#6, Marshal Gelovani St., 0159, Tbilisi, Georgia
mbezhuashvili@yahoo.com

ABSTRACT

The following have been isolated from the one-year shoots of *Rkatsiteli* vine species: trans-resveratrol, ε-viniferin, two Tetramers of resveratrol (1991-1994). Some biological activities of individual stilbens and stilben-containing new Georgian products of a functional designation have been identified. In particular, the antioxidant activity has been identified in human blood serum as the degree of inhibition of the formation of malondialdehydes by experiments *in vitro*. trans-resveratrol has shown the stimulating effect on the intensity of propagation of wine yeast (Sacch.vini-Kakhuri 42; Sacch.chodati-Teliani 79). The phyto-alexin property of trans-resveratrol has been proved by the antagonist action against Agrobaqterium tumefaciensis causing the vine cancer.

Key words: trans-resveratrol, ε-viniferin, Tetramers of resveratrol, Biological activity.

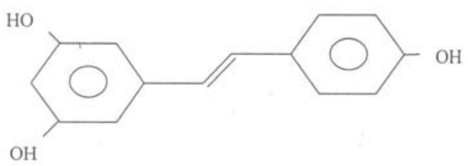
RESUME

Il a été distingué et identifié de la branche annuelle du cépage de vigne de Rkatsitéli le trans le trans-resvératrol, la ε-viniférine, deux tétramètres de resvératrol (1991 - 1994). Quelques activités biologiques des stilbènes individuels et de nouveaux produits géorgiens les contenant et ayant une importance fonctionnelle ont été découvertes. Notamment une activité antioxydant est découverte dans le sérum du sang humain sous forme du taux d'inhibition de l'apparition de Malondialdehyde dans les essaies "in vitro". Un effet stimulant de trans-resvératrol a été découvert sur l'intensité de la multiplication des levures du vin (Sacch.vini-Kakhuri 42; Sacch.chodati-Teliani 79). Un caractère phytoalexinique de trans-resvératrol est prouvé par son effet antagonique par rapport à Agrobaqterium tumefaciensis, causant le cancer de la vigne.

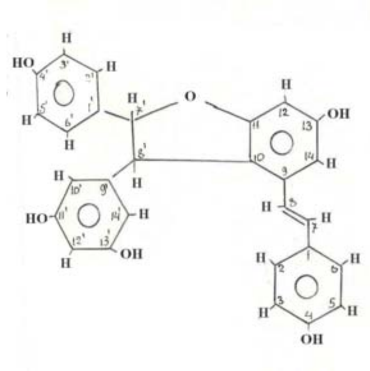
Mots clés : Trans-resvératrol, ε-viniférine, tétramètres de resvératrol, activité biologique.

INTRODUCTION. Among the biologically active natural phenol compounds, a group of stilbens is worth special attention. Their monomer representative is resveratrol, and its derivatives - dimmers, trimers, tetramers and their glycosides greatly determine the stilbene spectrum. An intense study of stilbens, as that of biologically active natural substances of vine and wine started in connection to the search of basics of the "French paradox" phenomenon. Numerous studies identified the content of stilbens in the vine species growing in different countries and wine produced with them and role of stilbens in forming the curative and nutritive value of red wines. The versatile biological activity of monomer stilben – resveratrol, is identified by its antioxidant (Blond et al., 1995), bactericidal (Docherty et al., 2007), phyto-alexin (Langcake, Pryce, 1977), antithrombotic (Bertelli et al., 1995), anti-cancer (Yang et al., 1997) and other effects. The presence of biologically active resveratrol in red wines, together with other phenol compounds, greatly makes contributes to their curative properties for cardiovascular, atherosclerosis, cancerous and other diseases (Kondo et al., 1994; Pace-Asciak et al., 1995; Klatsky et al., 1997; Szmitko, Verma, 2005; Micallef et al., 2007; Balestrieri et al., 2008).

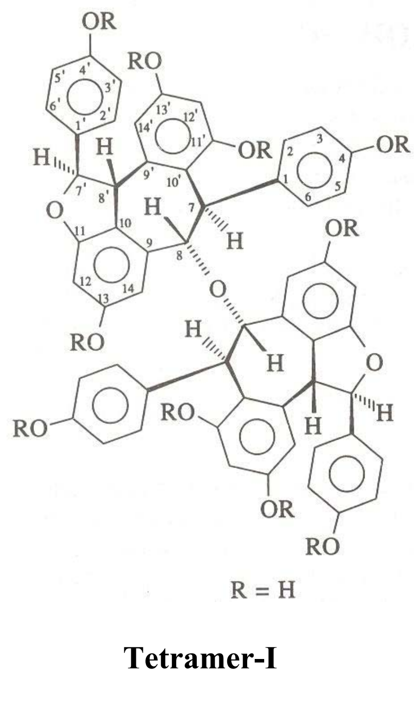
We started to study stilbens in 1991, by individually isolating and identifying trans-resveratrol (Bezhuashvili et al. 1991), ε-viniferin (Bezhuashvili, 1994), two Tetramer stilbens (Bezhuashvili et al., 1997) from the one-year shoots of *Rkatsiteli* (*Vitis vinifera* L.) vine species, which is the principal technical species growing in Georgia. By continuing the studies, we studied trans-resveratrol in several red grape vine species growing in Georgia and red wines produced with them. At the same time, we developed the technologies to produce new products of a functional designation enriched with stilbens. One of the fundamental fragments of the mentioned studies is to determine the biological activity of individual substances and production containing the given substances. As for the trend of "Wine and health", we are carrying out intense research by studying stilbens in red Georgian wines specifically.



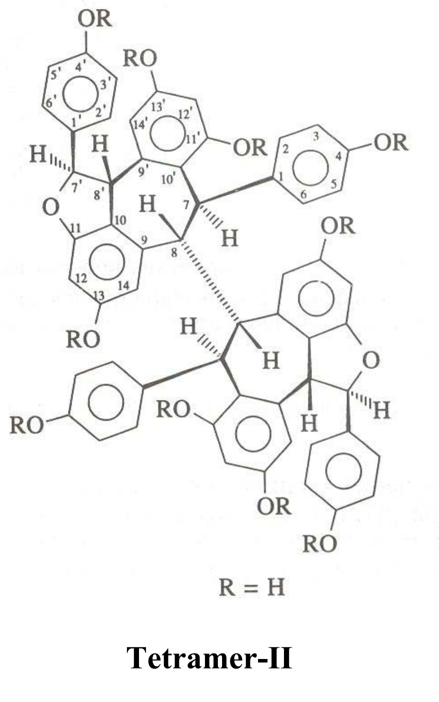
Trans-resveratrol



ε-viniferin



Tetramer-I



Tetramer-II

MATERIALS AND METHODS. The objects of our study were: individual stilbens gained from the vine, wine phenol acids and flavonoids, concentrate containing natural stilbens produced by new technologies, alcoholic drink enriched with natural stilbens, red wine "Mkurnali" with the curative and preventive designation; special red dessert wine, grape seeds extract and alcoholic drink balsam "Hamo".

We established the antioxidant activity in human blood serum as the degree of inhibition of the formation of malondialdehydes by experiments "*in vitro*" (Andreeva et al., 1988). We established the impact of trans-resveratrol on the intensity of propagation of wine yeasts during the alcoholic fermentation for the pure cultures of Sacch.vini-Kakhuri 42 and Sacch.chodati-Teliani 79 strains. We conducted alcoholic fermentation in Reeder synthetic area and by adding trans-resveratrol to the grape must and without it. We calculated yeasts with a microscope. We proved the phyto-alexin property of trans-resveratrol against Agrobaqterium tumefaciensis causing the vine cancer, which we imported to the solid areas treated with the trans-resveratrol solutions of a different concentration. We defined the action area in cm (Egorov, 1965). Aiming at identifying the content of trans-resveratrol, we extracted the samples in advance with ethyl acetate and carried out qualitative and quantitative analyses for the gained fractions. 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. The total amount of phenol substances was determined by us spectrophotometrically by using Folin-Chokalteu reagent (Sader,Datunashvili, 1972). The determined the total amount of paints spectrophotometrically (Valuiko, 1973).

RESULTS AND DISCUSSION. The natural vine stilbens turned out to have high antioxidant activity. The antioxidant activity in the range of stilbens trans-resveratrol - ε-viniferin - Tetramer (I) - Tetramer (II), increases as follows: 105%-118%-178%-169%. The antioxidant activity of stilbens is higher than that of flavonoids. In particular, Rutin and Kaempferol, which, as compared to trans-resveratrol, contain more 4-hydroxyl groups, have much less antioxidant activity within the range of 53-67% than trans-resveratrol (105%). Individually, in stilbens, as well as in Flavonoids (Rutin, Kaempferol, Quercetin, 53%-67%-83%), the antioxidant activity is increased as the quantity of hydroxyl groups increases in the molecule (Bezhuashvili et al., 2005).

The impact of trans-resveratrol on the propagation intensity of the wine yeasts was proved in the process of alcoholic fermentation. The number of yeast cells during the fermentation in the Reeder synthetic area in the presence of trans-resveratrol much exceeds that of the reference variants. The intensity of yeast propagation is obvious in the II-V days' interval, and from the VI day, the number of cells in the fermenting area starts to diminish. The stimulating effect of trans-resveratrol on the intensity of the propagation of the yeast cells is particularly well seen for Sacch.vini-Kakhuri 42 what is even more obvious during the alcoholic fermentation of must ("Tab 1"). The cells are intensely propagated during the I-III days whereas the number of cells in the fermenting area starts to diminish from the IV day. The increase of the Sacch.vini-Kakhuri 42 cells in the Reeder area under the influence of 0.1 g/l concentration of trans-resveratrol constitutes 3200000, as compared with that of the reference group and it is 26720000 for the must (Kokhtashvili et al. 1999).

«Tab. 1» Intensity of the propagation of wine yeasts during the alcoholic fermentation of must in the presence and absence of trans-reservatrol

Yeast description	Number of cells per 1 cubic cm					
	I day of fermentation	II day of fermentation	III day of fermentation	IY day of fermentation	VII day of fermentation	VIII day of fermentation
Saach. vini – Kakhetian 42						
Reference	25200000	51920000	52400000	52800000	18720000	12560000
Experimental (+0.1 gr/l reseveratrol)	28080000	61760000	79120000	74600000	28800000	18320000
Saach. chodati – Teliani 79						
Reference	10720000	44080000	46560000	45720000	16720000	3760000

Experimental (+0.1 gr/l reseveratrol)	**12240000**	**45840000**	**47520000**	**46240000**	**35440000**	**4880000**

Trans-resveratrol (of the concentrations of 0.1 g/l and 0.01 g/l) showed an antagonistic action to Agrobaqterium tumefaciensis causing the vine cancer what was proved by 0,3 cm and 0,5 cm suppression zones, accordingly (Bezhuasvili et al. 1999).

By continuing the research works, we developed the technologies to produce new Georgian products of grape origination of a functional designation enriched with stilbens:

1. Alcoholic drink enriched with natural stilbens made with the white wine of a traditional Kakhetian type and biologically active natural stilben-containing extract. The white wine of a Kakhetian type made with *Rkatsiteli* grape species with traditional technology, is richer in phenol compounds (2,5-5,3 g/l) as compared with the wine of a European type (0,4-0,8 g/l). The mentioned phenolic acids are mainly represented by proantocyanidines (oligomeric and polymeric), phenolic acids, flavanols, flavonols, etc., each having antioxidant activity what determines the anti-oxidant property of the wine with a directly proportional ratio. The antioxidant activity of the wine phenol acids was identified in the following order: gallic >protocatechic>gentizin>caffeic>ferulic>P-coumaric>4-oxybenzoic>salicylic>syringic; 95%-84%-83%-78%-71%-46%-43%-41%-40%. A negative correlation of vanillic acid has been found. The antioxidant activity of the natural stilben-containing concentrate was fixed at 178%, with the total stilben proportion of 126%. By adding this concentrate to the Kakhetian type wine accordingly increases the antioxidant activity of the wine. See “Tab. 2”.

“Tab. 2”. Change of antioxidant activity according to the concentration of phenol compounds in self-clarifying Kakhetian wines

Place of grape harvesting (in Kakheti)	Phenol compounds, gr/l		Antioxidant activity, %	
	Initial	By adding stilben-containing concentrate	Initial	By adding stilben-containing concentrate
Akhasheni	5,0	5,7	178	228
Kardenakhi	2,3	3,0	100	126
Gurjaani	4,3	5,0	137,1	169
Tsinandali	3,5	4,2	135,7	178
Kvareli	3,9	4,6	126	160

The alcohol-content of the alcoholic drink varies between 11,5 and 13,7 vol/%; the total phenol substances amount to 3,0-5,7 g/l, including 22,0-24,3 mg/l of trans-reseveratrol, ε-viniferin is 19,4-23,5 mg/l; the antioxidant activity changes within the range of 126-228% (Bezhuashvili et al. 2005; Meskhi, 2006; Bezhuashvili et al. 2008).

2. The red wine “Mkurnali” with curative and preventive actions is made of slightly pressed, naturally fermented *Saperavi* pomace, by slow finished simmering of its must (for 16 to 18 days) and by stabilizing the gained bulk wine on the same pomace for up to 1 month (Kobaidze,Chakhunashvili, 2002). The natural wine “Mkurnali” produced with this technology has the best organoleptic features what is the result of the perfect grape species aroma maximally mobilized by its retaining on the fermented pomace. This is followed by the formation of the tones preceding by 3 or 4 months of the stabilized wine what causes up to 0,25-0,30 more points during the wine sampling and allows the early realization of the wine (in January). The slow finished fermentation of the must with high concentration of sugar (24-25%) allows gaining aromatic wine rich in phenol substances (5,0-5,7 g/l) what accordingly, improves the antioxidant activity and increases the physiological value of the wine. It should be noted that the wine is high-alcoholic (13-14 vol. %) what is 0,3-0,5 vol. % higher than the reference one.

The wine was subject to the clinical and experimental examination for 2 years (2005-2006) to treat the cardiac ischemia in 57 patients (at Tbilisi cardiologic clinic “Guli” (“The Heart”)). The male patients were given 200-250 ml of wine daily, and female patients were given 150-200 ml daily. The gained results were positive and no side effects were observed. In addition to phenol substances, the wine “Mkurnali” is rich in K (1800 mg/l) what increases its curative effect to the certain extent.

3. Special red dessert wine is made by fortifying the stemless grape pomace with proper the fermented grappa alcohol extract. As compared to the reference wine, the dessert wine is enriched with biologically active components, which are the grappa residue - phenol and colouring substances. trans-resveratrol was also found in the fermented grappa of the *Saperavi*, *Otskhanuri Sapere* and *Cabernet Sauvignon* (*Vitis vinifera* L.) fermented grappa. The red special wines produced from these species by the new technology are characterized by generic features and versatile spectrum of phenol compounds what increases their antioxidant activity by 26-30% and improves their curative and nutritive value, accordingly (Kokhtashvili, 2006).

4. The grape seed extract is made of water and alcohol extract of *Saperavi* grape seed (as the wine-making residue) and natural stilben-containing vine extract (Bezhuashvili, Kobaidze, 2010). We used the seed extract and several other ingredients to make the alcoholic drink - Balsam “Hamo” (Bezhuashvili et al. 2010). It is rich in phenol compounds - procyanidines (oligomeric and polymeric) flavanols, flavonols, anthocyanins, phenol acids, stilbens. It also contains organic acids, and micro- and macro-elements. Both, the seed extract and balsam, owing to their high biological activity, are the products of a functional designation and are to be used for the given purpose.

CONCLUSIONS. Based on the accomplished scientific studies, some biological activities of trans-resveratrol, ε-viniferin, Tetramers of resveratrol were identified and the technologies to produce new Georgian products of grape origination containing biologically active natural stilbens of a functional designation were developed.

ACKNOWLEDGMENTS. We thank the following scientists for their cooperation in the studies:

Pataraia M. of the Microbiological laboratory at the Institute of Horticulture, Viticulture and Oenology of Georgia for identifying the influence of trans-resveratrol on the wine yeasts.

Bostoghanashvili M. and Malania M. of I. Kutateladze Institute of Pharmacochemistry for specifying the antioxidant activities.

Lomtatidze Z. and Mamulashvili K. of Ketskhoveli Institute of Botany of Georgia for specifying the phytoalexin activity.

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Production of Non-alcoholic and Low-alcohol Drinks with Bioactive Compounds

Authors: Zaira Shapatava [(1)], Lali Goginava [(2)], Merab Zhgenti [(3)], Marina Meliqishvili [(4)], Nazi Melanashvili [(5)]
[(1) (2) (3) (4) (5)] **Organization:** Institute of Horticulture, Viticulture and Oenology
Adress: Marshal Gelovani N6, 0159, Tbilisi, Georgia,
e-mail: zairashapatava@mail.ru

ClAIM

The objective of the present paper is using of the red wine (Saperavi) in products with dietetic, preventive and medicinal properties.

The production technology is based on the removal of alcohol in low temperature and low pressure conditions.

The products are as follows: low-alcoholic drinks – 5-7^0 alcoholic content and addition of stevioside; complete removal of alcohol and addition of stevioside; complete removal of alcohol, blending it with the matter of relatively high acidity and addition of stevioside.

The selected concentrate of stevioside and proportions for a particular drinks. The solution concentrate > 0.15%.

Das Ziel der geleisteten Arbeit war, mit der Benutzung des Rotweines (Saferavi) propilaktische, Diät- und Heilprodukte zu erzeugen.

Der Technologie liegt die Entfernung des Alkohols unter den Bedingungen der niedrigen Temperatur und des niedrigen Druckes zugrunde.

Die erhaltenen Produkte sind: Das Getränk mit wenigem Alkohol - der Gehalt von Alkohol 5-7%, der Zusatz von Steviozid; Die völlige Entfernung des Alkohols, der Zusatz von Steviozid; Die völlige Entfernung des Alkohols und das Coupagieren mit dem relativ hochsäurigen Objekt, der Zusatz von Steviosid.

Es ist die Konzentration und das Verhältnis von Steviosid zur Zubereitung der einzelnen Getränke ausgewählt. Die Konzentration soll nicht > 0,15% sein.

Theme title: New products, new technologies, new challenges
Theme subtheme title: Health impact of vitivinicultural products
Type: oral
Language: English
Title: Production of Non-alcoholic and Low-alcohol Drinks with Bioactive Compounds

INTRODUCTION

Interest in grapes is often associated with wine making. The diversity of wine grape varieties and influence of their growth conditions are continuously studied and new technologies of wine production are developed. Wine deserves special attention due to its taste and aroma as well as its bioactive substances inhibiting or slowing free radical generation. Free radicals damage cells, alter genetic material and are involved in pathogenic processes causing cardiovascular diseases, arthritis, various kinds of cancer, etc. Red wine has the highest protective effect due to the high concentration of anthocyanins [Vinson J.A., Hontz B.A. 1995; Hesfor F., Schneider K.1997; Clifford M.N.2000]. However due to the alcoholic content red wine is not recommended for patients with cardiovascular diseases, atherosclerosis and diabetes. Diabetes is so widespread worldwide that it is often called a “non-infectious epidemic disease of the 21st century”.

The World Health Organization (WHO, 2003) recommends considerable reduction of sugar consumption. Sucrose is an effective immunosuppressive substance, which affects mineral metabolism, increases the levels of glucose and insulin, provokes atherosclerosis, contributes to obesity. A total of >50 negative effects are listed [http://www.alternative-doctor.com/nutrition/sugar.htm; http://www.forum.zelek.ru /index php?showtopic=4576].

In recent years there have been some deep and significant changes in the field of nutrition physiology. The latest tendency is production of low-calorie products with high concentration of biologically active substances. At present sugar substitutes are widely used. Stevioside is especially noteworthy. It is glycoside made from *Stevia rebaudiana Berton* of Asteraceae family. Its empirical formula is $C_{38}H_{64}O_{11}$. Stevioside is a low-calorie sweetener, which normalizes blood sugar level and arterial pressure. It also has anti-hyperglycemic, insulinotropic and cancer preventive properties. [Rodionova N. C. et al, 1998; Jeppesen P.B. et all. 2002; Kostina V.V. Abelyan V.A. 2003].

The unique properties of stevioside account for its wide use in food production. The stevioside is especially effective when the raw material has high concentrations of bioactive substances.

Toxicity tests of stevia extract which have been conducted worldwide for the past thirty years have proved its safety [Yasukava K., Kitanaka S., Seo S. 2000].

The objective of this research is production of dietic products with preventive and somewhat therapeutic properties using red wine (Saperavi) and stevioside.

METODS:

The grape juice production technology is as follows: grape concentrate is added to the demineralised water [Schobinger U.2004]. With this technology grape juice can be produced at any time of the year. But this product is not recommended to a large number of consumers. It is necessary to produce sugar-free red grape juice.

Sugar-free grape juice can be produced with wine (instead of grape concentrate). After alcohol removal wine is turned into sugar-free semiprocessed material, which retains antioxidant and other useful properties.

The raw material is Saperavi wine with 13^0 alcoholic content.

Juice production technology comprises the following processes: alcohol is removed from wine through evaporation at $40\text{-}45^0$C temperature in vacuum. The duration of this process depends on the quantity of wine. Alcohol is completely removed after evaporation of $\approx$ 50% of moisture. The final product is mixed with the solution of stevioside concentrate and demineralised water (proportions of 1:1). The code of the drink is A.

The technology of low alcohol ($5\text{-}6^0$) drink production is the same as described above. In this case 20-25% of material is evaporated. Then 0.4% stevioside solution is added to the drink to reach the original amount (proportions of 4:1). The code of the drink is B.

The bioactive properties of the non-alcoholic drink can be improved by adding bilberry juice. The proportions of A drink and bilberry juice are 1:1. The code of the produced drink is C. The stevioside concentration in the final product should be no more than >0.15%.

The total concentration of anthocyanins in the aforementioned drinks was measured with photoelectric calorimeter at a wave length of 530 nm. The titratable acidity, pH, anthocyanins, flavonols, phenolic carbon acids were measured with highly-effective Varian Prostar liquid chromatograph. Concentration of mineral elements was measured with atomic absorption spectrometer (AANALIST 400).

RESULTS:

The present research is innovative for optimum use of wine. Its objective is production of non-alcoholic or low-alcoholic drinks, which preserve all the substances essential for human health and expand the use of wine in nutrition physiology.

Red wine has antioxidant effect mainly due to its concentration of anthocyanins. Therefore during the processing the main focus is on their stability.

The concentration of anthocyanins in wine is 535mg/l. In case of 50% evaporation theoretically the concentration should be reduced to 1070 mg/l, but the analysis showed that the actual concentration is 981 mg/l. After alcohol removal the total concentration of anthocyanins is slightly reduced and amounts to 8.3% (Fig.1).

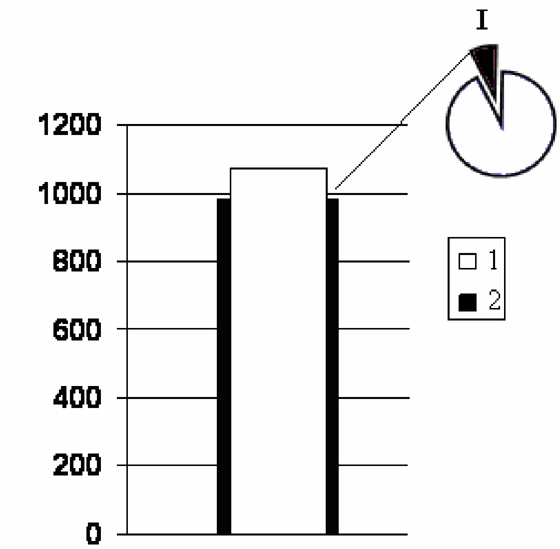

Figure 1. The Total Concentration of Anthocyanins in Concentrated Wine

1. Non-alcoholic concentrated wine (theoretical);
2. Non-alcoholic concentrated wine (actual);

I.- Loss of anthocyanins during processing.

0.2% stevioside solution is added to the drink and the substance concentrations of the product made from wine is identical to those of wine. The total concentrations of anthocyanins is 486 mg/l. >15 anthocyanins have been found in wine, mainly malvidin derivatives and peonidin derivatives as well as a small amount of cyanidin derivatives. The analysis of wine (Saperavi) conducted by the other researchers showed the similar results. [Bezhuashvili M. et al. 2009].

There is increasing interest in bilberries due to their antioxidant effect.

The contents of flavonols and phenol carbonic acids in the bilberry 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.1).

Table 1

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, which is quite high. However it is less than the concentrations stated by other researchers [Kähkönen M.P., Hopla A. I. 2001]. 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.

Addition of Bilberry juice increases the total concentration of anthocyanins and improves their capacity to inhibit various free radicals.

Mineral elements, which are found in juice also contribute to its high quality (Tabl.2), especially Zn which has high antioxidant efficiency and increases the effect of other antioxidants, as well as K, Ca, Mg and Na, which are essential for human health. The literary sources show even higher contents of these elements [Tsapalova I.E., Gubina M. D. et all, 2005], which evidences the importance of bilberry.

Table 2

Mineral Elements Concentrations in Bilberry Juice, mg/l

Mineral Element	Concentration
K	1052
Ca	127
Mg	74
Zn	1,6
Na	21
Fe	4,3

The total concentrations of anthocyanins in A and B drinks are similar to that of wine (486, 494 and 535 respectively). After addition of bilberry juice this concentration increases 3.5 times and amounts to 1770 mg/l (Fig.2). Besides, the bilberry juice increases the concentrations of flavonols and phenolic carbon acids in the drink thus improving its antioxidant action.

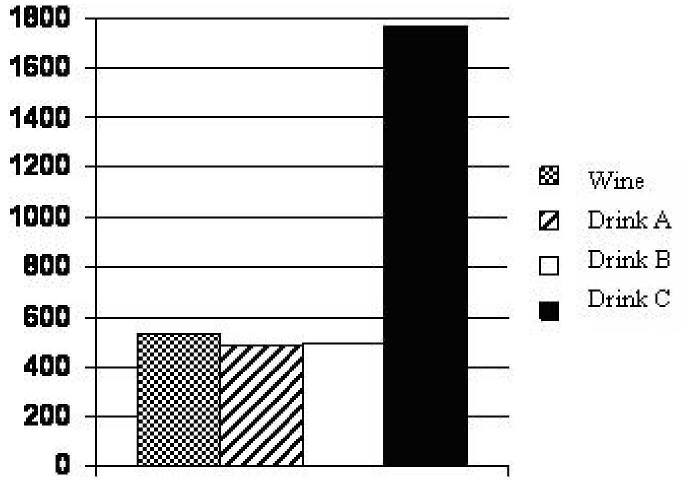

Figure 2. The Total Concentration of Anthocyanins in Wine and Its Products, mg/l

Maintaining the appropriate levels of titratable acidity and P^H in drinks are important in terms of technology, sensory qualities and nutrition physiology. The levels of titratable acidity and P^H in wine products are similar to those in wine: titratable acidity – 6.4 g/l and P^H – 3.75; in A and B drinks – 6.0g/l -3.8 and 6,2 g/l -3.8 respectively. The titratable acidity of the drink with bilberry juice is slightly high, 7,7 g /l and P^H is 3.6 (Tabl.3).

Table 3

Titratable Acidity and P^H in Wine and Its Products

Drink	Titratable Acidity gr/l	pH
Wine	6.4	3.75
Drink A	6.0	3.8
Drink B	6.2	3.8
Drink C	7.7	3.6

The research of phenolic compounds, antioxidant action and production technologies of the aforementioned products will continue and the focus will be on the production of low-calorie products.

CONCLUSIONS

Three products were received from red wine using stevioside concentrate, namely low-alcoholic $5\text{-}7^0$ drink with 0.08-0,1% stevioside concentration, non-alcoholic drink 0.1-0.12%; stevioside concentration and a mixture of non-alcoholic drink and bilberry juice with 0.1% stevioside concentration.

The stevioside concentration in the product is <0.15%.

Stevioside is resistant to thermal processing and does not affect acidity. The P^H of 0.2% solution is high, 5.7.

The total concentration of anthocyanins in low-alcoholic and non-alcoholic drinks is similar to that of wine.

The addition of bilberry juice increase antioxidant effect of the product. It also expands the range anthocyanins in the product improving its capacity to inhibit various free radicals. The flavonols and phenolic carbon acids also contribute to this improvement.

The addition of bilberry juice also increase the contents of K, Ca, Mg, Zn and Fe mineral elements.

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Patrimoine, santé, environnement : valeurs en conflit?

Françoise BRUGIERE
FranceAgriMer
12 rue Henri Rol Tanguy
TSA20002
93555 Montreuil sous bois
Francoise.brugiere@franceagrimer.fr

Résumé

Patrimoine, santé, environnement : valeurs en conflit?

À partir d'études réalisées en France, la communication fera le point sur l'importance relative des axes "tradition et patrimoine", "santé et nutrition" et "environnement" dans les arbitrages et motivations des consommateurs en matière d'alimentation en général et de vin en particulier. On s'attachera également à comprendre comment ces opinions se traduisent dans l'évolution de la demande exprimée sur le marché du vin.

Heritage, health, environment: values in conflict?

Using studies conducted in France, the communication will focus on the relative importance of "tradition and heritage", "health and nutrition" and "environment" in the motivation and decision-making of consumers regarding food in general and wine in particular. We will also focus on trying to understand how these opinions affect the evolution of demand expressed on the wine market.

Patrimonio, sicurezza, ambiente: valori in conflitto?

A partire da studi svolti in Francia, la comunicazione farà il punto sull'importanza relativa degli assi "tradizione e patrimonio", "salute e nutrizione" e "ambiente" nei pareri e nelle motivazioni dei consumatori in materia di alimentazione in generale e di vino in particolare. Inoltre, analizzeremo il modo in cui queste opinioni si traducono nell'evoluzione della domanda espressa sul mercato del vino.

Patrimoine, santé, environnement : valeurs en conflit?

Introduction

Les consommateurs sont soumis à de nombreux flux d'informations destinés à influencer leurs choix et leurs achats. Les citoyens reçoivent également de nombreux messages et information qui influent sur leurs opinions et leurs comportements. En utilisant des études française la présente communication s'interrogera sur l'impact de ces influences sur la consommation en général et celle du vin en particulier.

Une sensibilité accrue aux questions environnementales, sociales, citoyennes. Faire de la consommation un acte signifiant au plan politique ?

Défense de l'environnement

La défense de l'environnement fait partie des valeurs « post matérialiste » importante au même titre que l'hédonisme, la réalisation de soi, la liberté individuelle, le droit des femmes, la liberté sexuelle, caractéristique des classes supérieures des pays industrialisées, celles qui sont dégagées des préoccupations matérielles de base (se nourrir, se loger, se vêtir). Néanmoins les enquêtes d'opinion récentes tendent à montrer que l'ensemble du corps social déclare accorder de l'importance à la question. Mais il reste un grand écart entre la sensibilité des individus pour l'environnement, les efforts qu'ils déclarent être prêts à faire et ce qu'ils font réellement. Néanmoins, l'enquête permanente de l'INSEE sur les conditions de vie « pratiques environnementales » révèle par exemple une progression notable de la pratique de tri des déchets : le verre est trié par 77% des ménages en 2005 (vs 64% en 1998) et le papier par 71% des ménages en 2005 vs 36% en 1998. Quand l'acte eco-citoyen entraine un cout, sa généralisation se heurte à la question du revenu : en 2005, 32% des ménages du quartile des plus pauvres utilisaient des lampes à basse consommation contre 50% pour le quartile des ménages les plus aisés, alors que si l'acte citoyen conduit à une économie immédiate, le lien avec le revenu est inversé : 86% des ménages les plus modestes contre 80% des ménages aisés déclarent faire attention à leur consommation d'électricité. Ces taux particulièrement élevés rappellent que l'adhésion à la cause de l'environnement s'imposant de plus en plus comme une norme sociale consensuelle, les enquêtes centrées sur la mesure des opinions et attitudes à l'égard de l'environnement entrainent un effet de désirabilité sociale, bien connue des sondages. Il s'agit ainsi pour l'individu interrogé de répondre en conformité avec la perception qu'il a de ce qu'est la posture la plus répandue dans le corps social sur un sujet donné. L'enquête contribue donc d'elle-même à amplifier l'écart entre ce que disent et ce que font les individus.

Dans son étude sur les consommations d'énergie dans l'habitation, le CREDOC[1] établit le constat que le système de valeurs des Français en faveur du développement durable, et en particulier la sensibilité écologique, n'ont que peu d'incidences sur les pratiques de chauffage dans les logements qui privilégient la recherche du confort mais sont plus nettement corrélés pour ce qui est de l'usage d'ampoule basse consommation[2]..

Sensibilité sociale

D'après un sondage de l'association de consommateurs CLCV[3] la moitié des Français a entendu parler de produits « solidaire » (favorisant l'emploi de personnes en difficultés et/ou marginalisées) ou de produits équitables (permettant de mieux rémunérer les producteurs des pays du sud). Cette notoriété est plus importante chez les cadres et les ménages aisés.

D'après le rapport « Fair trade in Europe 2005 » le chiffre d'affaires net global des produits bénéficiant d'un label équitable y a quintuplé en quatre ans pour atteindre près de 1,25

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[1] Centre de recherche pour l'étude et l'observation des conditions de vie

[2] « la température du logement ne dépend pas de la sensibilité écologique » A. Dujin – B. Maresca mars 2010

[3] Confédération de la consommation du logement et du cadre de vie

milliards d'euros en 2006 dont 158 millions d'€ en France. Bien qu'il reste un micro marché, le commerce équitable est l'un des secteurs économiques les plus dynamiques d'Europe avec une progression moyenne des ventes de produits labellisés de 20% par an depuis 2000, ce qui fait. On trouve des produits équitables dans plus de 55 000 supermarchés et plus de 2800 boutiques spécialisées.

En conclusion de cette partie : si la sensibilité à la nécessaire défense de l'environnement et aux valeurs de solidarité nationale ou internationale font consensus, la mesure du passage aux actes des consommateurs reste difficile. Ce passage aux actes semble corrélé avec l'aisance sociale : revenu et niveau de diplôme traduisant sans doute à la fois la difficulté de compréhension de l'information consommateur sur les qualités sociale et environnementale des produits et l'obstacle que constitue le pouvoir d'achat.

L'achat alimentaire sous influences ?

Evolution des valeurs mises en avant : santé ou plaisir

Une enquête sur les comportements alimentaires des français est régulièrement menée auprès d'un échantillon de la population par le CREDOC. La dernière date de 2007[4]. Une partie du questionnaire concerne les représentations du « bien manger.»

En 2004, lors de la précédente enquête, bien manger c'était d'abord (pour 27% des répondants) une question de gout, de bon gout voire, pour 18% des répondant, de « se régaler, se faire plaisir ». En 2007, une autre notion domine : celle de manger équilibré (pour 36% des répondants contre 22% en 2004) soutenu par « manger varié » (17%) et « manger sain » (11%). Ainsi, bien que la crainte du « risque alimentaire » ait reculé (15% des français le place en tête des risques contre 18% en 2004), la fonction alimentaire bascule du côté de l'hygiène « manger équilibré » [5].

De plus en plus de Français déclarent que la façon dont ils mangent influe sur leur état de santé (75% en 1997, 89% en 2007) et pour toutes les catégories sociodémographiques, « faire attention à son alimentation » constitue le premier moyen de prévention en matière de santé. D'abord « en évitant de prendre du poids » puis en recherchant ou en évitant certains nutriments : sont recherchés le calcium et les vitamines, sont évités les matières grasses et l'alcool (devant le sucre et le cholestérol).

Un certain sentiment de culpabilité s'est installé parmi la population puisque, en 2007, seulement 69% des français déclarent avoir une bonne alimentation contre 81% en 2003. Ce sentiment encore plus accentué chez les moins de 45 ans, augure d'une persistance de l'ordre des valeurs en faveur de l'hygiène et de la santé et au détriment du plaisir hédonique.

Les critères d'achats de produits alimentaires :

L'**origine** France est de moins en moins un critère : 69% des français la considèrent en 2007, contre 79% en 1995 avec un désintérêt plus marqué des moins de 35 ans. Il semble

[4] CCAF 2007 R. Berger – P. Hebel – F. Lehuede – F. Recours décembre 2007

[5] Par ailleurs, 34% des français ont le plus peur des accidents de la route et 17% du nucléaire.

donc que la dimension environnementale d'un achat local (du pays) ne soit pas perçue ou pas porteuse. L'incitation à l'achat d'un produit parce qu'il porte un label connait la même érosion de 81% en 1995 à 69% en 2007 sachant qu'en France, les labels sont soit associés soit identifiés à des indications géographiques, on peut y relever la même contradiction que pour l'origine France. Par contre, la consommation de « produits du terroir » progresse en 2007 (73% des français déclarent en consommer) et s'étend sociologiquement : l'élargissement de l'offre permet de toucher des populations moins favorisées. Les jeunes sont nettement moins consommateurs peut-être parce qu'ils préfèrent les produits sucrés et qu'ils n'ont pas de nostalgie gustative.

La cuisine exotique, qui est une autre version du terroir ou de l'origine progresse également et touche près de la moitié des français.

Consommation engagée

Equitable. La vente des produits équitables (surtout café et chocolat) dans la grande distribution a fortement stimulé leur pénétration : 44% des personnes interrogées par le CREDOC en 2007 déclaraient en avoir acheté contre 16% en 2003. La clientèle est sociologiquement marquée : si l'âge n'est pas discriminant, le niveau de diplôme, le revenu et le fait d'habiter une grande ville sont des facteurs favorisants.

Bio, d'après le baromètre de l'Agence bio[6], 66% des personnes interrogées en consomment (contre 54% en 2003) dont 26% au moins une fois par semaine. Cette consommation est sociologiquement déterminée : beaucoup de 50-64 ans (génération 68 !), de diplômés, de cadres,…La motivation de consommation est d'abord « pour ma santé » (95% des motivations) et « pour ma sécurité, pour manger des produits sains » (91%, stable) mais de plus en plus « pour préserver l'environnement » (85% en 2004, 94% en 2009), de moins en moins « pour le goût (87% en 2009 contre 92% en 2004).

L'achat et la consommation de vin : quelles spécificité ?

Valeurs spécifiques

Le vin occupe une place particulière dans le régime alimentaire des Français. C'est une boisson, prioritairement et usuellement consommée au cours d'un repas même si la part des vins consommés en dehors des repas a tendance à augmenter[7]. Pour une proportion de Français non négligeable (mais en diminution), le vin est absolument indispensable au repas, et pour une proportion croissante il est indissociable du « bon repas » notamment convivial. Le vin est une boisson à laquelle il n'est pas reconnu de vertu désaltérante. C'est une boisson alcoolisée, qui n'est usuellement pas consommée pour atteindre l'ivresse. C'est une boisson qui renvoie à un imaginaire de consommation ritualisée : verres spéciaux, tire-bouchon, carafe sont évoqués spontanément par les consommateurs que l'on fait parler du vin. Les consommateurs français évoquent aussi spontanément les régions et paysages viticoles et se gargarisent de grands noms de vins qu'ils ne boiront sans doute jamais.

Handicaps spécifiques

[6] Agence bio -Baromètre de consommation et de perception des produits biologiques en France Octobre 2009

[7] « La consommation du vin en France »Enquête Viniflhor –Université Montpellier2 – Vague 2005

Traditionnel et peu porté par des marques fortes, le vin est logiquement rejeté par la jeunesse qui trouvera ailleurs ses sources d'ivresse. **Statutaire** il est difficile à adapter à des modes de vie et de consommation qui se déstructurent. **Alcoolisé** il ne trouve plus sa place dans un rythme de vie marqué par le travail sédentaire et les déplacements automobiles qui requièrent une certaine vigilance. **Patrimonial**, il manque de souplesse d'adaptation gustative.

Un produit impliquant dans un emballage qui s'invite à table

De plus en plus consommé en situation festive et occasionnelle, le vin est un produit qui implique la « maitresse de maison » ou le « maître de maison » dans son domaine souvent réservé, dans sa façon d'honorer ses hôtes et qui se présente avec son emballage sur la table. Les signes de qualité présents sur l'emballage sont donc visibles par tous. D'où par exemple une grande discrétion des marques de distributeurs qui bien que représentant plus du tiers des volumes de vins vendus par la grande distribution, sont peu identifiables et peu identifiés par les consommateurs. Une autre spécificité du vin est d'être acheté pour satisfaire à des rites statutaires par des personnes qui n'en consomment pas elles mêmes et auront du mal à capitaliser par l'expérience le sens des messages du marketing produit.

Opinions et comportements d'achat de vin

Dans cette partie seront analysées différentes tendances du marché du vin à l'aune des axes « tradition, patrimoine », « environnement » et « santé, nutrition »

Signe de qualité

En France, plus de 80% de l'offre de vin est proposée avec une indication de provenance (AOP ou IGP). Un grand nombre de vins sont médaillés, les mentions traditionnelles comme « mise en bouteille à la propriété » sont apposées sur plus de la moitié des étiquettes aussi le consommateur ne compte-t-il que très peu sur les signes de qualité pour faire son choix. Ainsi, logiquement seuls 14% des personnes interrogées par le CREDOC ont cité le vin parmi les produit pour les quels ils seraient prêt à payer plus cher pour la présence d'un signe de qualité (contre 57% prêts à payer plus pour un signe de qualité en viande de boucherie, et 40% pour la volaille).

Vins de cépages

Le segment des vins vendus avec l'indication d'un cépage, limité jusqu'en 2009 à la catégorie des vins de pays connait depuis une dizaine d'année une forte croissance et représente 15% des volumes vendus en grande distribution. Cet identifiant des vins n'est pas spécialement situé sur les 3 axes de segmentation choisi. Il semble répondre tout simplement à un besoin de simplification de l'offre et de lisibilité des linéaires des magasins en self-service. Grâce au cépage, le goût du vin est annoncé, le patrimoine et la tradition sont simplifiés. La grande distribution ne s'y est pas trompée : plus de la moitié des vins de cépages sont vendus sous marque de distributeur.

Vins rosés et vins effervescents

Ces deux segments en développement jouent également sur d'autres registres que santé et environnement. En rupture par rapport au modèle de consommation statutaire lié au repas, ils sont plus portés sur la fête ou la décontraction. Ils permettent une approche du produit plus autonome : le vin effervescent peut se boire à la paille, debout, se mélanger dans des cocktails sans se dévaloriser, le vin rosé peut se boire en pique-nique dans un gobelet en plastique, allongé d'un glaçon même s'il se rêve parfois plus guindé.

Vin bio

Parmi les 40% de français qui déclarent acheter des produits de l'agriculture biologique, 18% déclarent acheter du « vin bio » soit une forte progression au cours des dernières années : 6% en 2006, 10% en 2007. Les consommateurs de produits bio, comme cela a été dit plus haut sont soucieux de leur santé.

Une enquête menée par CSA pour FranceAgriMer a montré que l'affirmation « Dans les vins, on trouve les résidus des engrais, fongicides et insecticides, qui ont servi à cultiver la vigne » qui n'était considérée comme vraie que par 41% des Français en 1998, l'est en 2008 par 67%. Le vin bio se positionne à la jonction environnement / santé et répond à cette préoccupation des français[8] par la garantie d'absence de résidus de produits phyto sanitaires. Sur le 3ème axe, tradition et patrimoine, les producteurs de vins bio font valoir que leurs vins expriment le terroir sans fard puisque les sols et la plante ne subissent pas de traitement systémique. Ce discours qui nécessite une expertise gustative pour être expérimenté par les consommateurs reste, à mon avis, réservé aux initiés.

Fontaine à vin (ou bag-in-box®)

Le bag-in-box® connait en France un succès grandissant. La quasi-totalité des points de vente le proposent et en vendent de plus en plus. Les études sur les acheteurs et consommateurs de vin en bag-in-box montrent que si ce conditionnement a trouvé sa place sur les tables festive, informelle, estivale lors de moment de consommation collectif il est également choisi pour une consommation individuelle, régulière voire quotidienne. Les arguments mis en avant par ses adeptes sont la praticité, la possibilité de fractionner et de maitriser sa consommation, l'économie d'emballages … Il se positionne à la fois sur l'axe santé et sur l'axe environnement. Sur l'axe tradition il est handicapé par son manque de prestige. Les amateurs français ont situé une limite hiérarchique au-delà de laquelle le bib ne pouvait pas conditionner les vins. S'ils considèrent que cet emballage ne doit pas se cantonner aux vins de cépages ou aux appellations régionales, il n'est pas légitime pour conditionner les « grands crus » dont il pourrait altérer l'image si ce n'est la qualité.

Les tendances à venir ?

Les vins de qualité à faible teneur en alcool que les techniques permettent d'obtenir en utilisant les levures OGM ou des procédés physiques trouveront-ils des consommateurs soucieux de limiter leur consommation d'alcool sans se restreindre sur la quantité de vin bue ?

[8] Consommateurs qui ne se préoccupent pas des solutions alternatives choisies et notamment de l'impact environnemental de l'usage du cuivre et du soufre.

La valeur patrimoniale et traditionnelle liée à l'embouteillage à la propriété garantissant la traçabilité du vin résistera-t-elle à la pression d'économie de CO_2 et de déchets qui penche en faveur du transport en vrac et du conditionnement au plus prêt du consommateur ? La bouteille 75cl sera-t-elle marginalisée par le développement du bag-in-box ?

Les promesses de santé cardiaque garantiront elle le succès des vins enrichis ou complémentés en resvératrol ?

Une production de petits producteurs de pays du sud labélisée « commerce équitable » s'inscrira-t-elle dans la dynamique de développement durable et solidaire et séduira-t-elle les consommateurs ?

Répondre à des demandes ou des sensibilités des consommateurs sans s'enthousiasmer et trop investir pour des modes sans lendemain, s'arcbouter sur les traditions au risque de laisser passer une génération de consommateurs, perdre son âme en surfant sur la tendance du moment, autant de cas de conscience pour les décideurs de la filière vins !

Françoise BRUGIERE

DEVELOPMENT OF ANALYTICAL METHODS TO DETECT EGG ALLERGENS IN WINES ACCORDING TO THE EU DIRECTIVE ON LABELLING

P. Restani(1), F. Uberti(1), C. Ballabio(1), C. Di Lorenzo(1), A. Persico(1), C.Tarantino(2)

[1] Department of Pharmacological Sciences, Università degli Studi di Milano, Italy
patrizia.restani@unimi.it
uberti_francesca@libero.it
cinzia.ballabio@unimi.it
chiara.dilorenzo@unimi.it
allergia@unimi.it
[2] Euroclone S.p.A, via Figino 20/22, 20016 Pero, Milano, Italy
c.tarantino@euroclone.it

ABSTRACT

The wine fining is the process of removing undesirable substances such as polymerized tannins, phenols and proteins. Caseinates adsorb and mechanically remove suspended materials responsible for wine turbidity. Albumen/egg albumin is a common fining agent for red wines, with colloidal nature and a positively charged surface that attracts negatively charged tannins responsible for the undesired astringency. Both proteins have very useful enological properties, but their residues could represent a risk for subjects suffering from food allergy. The EC Directive 89/2003 established that also in the case of alcoholic beverages, it should be mandatory to include in the labelling all ingredients with allergenic potential. The application was posponed for alcoholic beverages to January 2011. At present, it is not clear if a definitive exemption can be obtained for labelling of caseinates and egg white proteins, but the develoment of suitable methods to detect the allergenic residues is critical for every possible international action. In this paper, the development/validation of suitable analytical methods is described.

RIASSUNTO

La chiarifica del vino è il processo che consente l'allontanamento di sostanze indesiderate, quali i tannini polimerizzati, i fenoli e alcune proteine. I caseinati sono in grado di assorbire e di rimuovere le sostanze che impartiscono turbidità al vino bianco. L'albume/ovalbumina è un chiarificante comunemente usato nei vini rossi, in cui produce l'eliminazione delle sostanze astringenti. Entrambe queste proteine hanno ottimali proprietà chiarificanti, ma possono rappresentare un rischio per i soggetti allergici qualora rimangano nel vino al commercio.

La Direttiva Europea 89/2003 ha stabilito che anche le bevande alcoliche devono riportare in etichetta l'eventuale utilizzo di additivi/coadiuvanti allergenici; tale norma è stata però posticipata per questo settore al Gennaio 2011. Attualmente non è chiaro se sarà possibile ottenere un esonero definitivo all'etichettatura dei caseinati e dell'albume, ma per qualunque richiesta a livello internazionale risulta fondamentale la disponibilità di uno o più idonei metodi analitici. In questo lavoro si descrive la messa a punto/convalida di test analitici applicati alla ricerca delle proteine dell'albume nei vini rossi.

INTRODUCTION

The clarification (i.e. fining) of wine during winemaking is the process of removing undesirable substances such as proteins, phenols and polymerized tannins that would cloud the wine and cause bitterness and astringency.

Fining agents have organic or inorganic nature and are added to must or wine to interact with the elements responsible for turbidity and sensory imbalances.

Caseinates are positively charged proteins that flocculate in acidic media such as wine. When added to wine, caseinates adsorb and mechanically remove suspended materials responsible for turbidity. They are usually applied in association with mineral products (mainly bentonite) as secondary fining agents.

Egg white/albumin is a common fining agent for red wines; it is colloidal in nature and has a positively charged surface that attracts negatively charged tannins, responsible for the undesired astringency in red wines.

Both proteins have very useful enological properties, but their residues could represent a risk for subjects suffering for food allergy. Several national and international committees responsible for food safety underlined the necessity to improve the protection of consumers suffering for food allergies and intolerances.

The EU Directive on labeling (2003/89/EU) states that all allergenic ingredients included in Annex III must be declared in the labeling. Labeling for alcoholic beverages was delayed to June 2009 and again to January 2011 to allow new studies and evaluate the possible risk associated with allergenic residues in commercial products. After 1st January 2011, allergenic fining agents listed in Annex IIIa of the Directive 2000/13/EC (and following amendments) must be indicated on the labelling, if still present in commercial wines. At present, it is not clear if a definitive exemption can be obtained for l caseinates and egg white proteins, but the develoment of suitable methods to detect the allergenic residues is critical for every possible international action.

On these bases, the aim of this research is the development of a new ELISA method for the detection of egg white/ovalbumin in clarified red wines. Results are confirmed in parallel by immunoblotting.

MATERIALS AND METHODS

Egg proteins

Highly purified egg proteins (Sigma Chemical, St. Louis, Mo) were used in SDS-PAGE.

ELISA plates

Sandwich ELISA kit, specific for the research of egg white proteins in wine, was developed for this research

SDS-PAGE

Gradient running gel. 9-19% acrylamide; 0.08-0.17% bis-acrylamide; 0.36 M TRIS-HCl buffer pH 8.8; 35% glycerol; 0.1% SDS; 0.02% ammonium persulfate; and 0.15% N,N,N',N'-tetramethylenediamine (TEMED).

Stacking gel. 3.5% acrylamide; 0.09% bis-acrylamide; 0.125 M TRIS-HCl buffer pH 6.8; 0.1% SDS; 0.02% ammonium persulfate; and 0.15% (TEMED).

Running buffer. 25mM TRIS, 0.19M glycine and 0.1% SDS (w/v), pH 8,8. For further details, see Ballabio et al (2007).

Immunoblotting

After SDS-PAGE, proteins were transferred to PVDF membrane (Millipore) by western blotting in a Trans-blot Electrophoretic Transfer Cell (Bio-Rad). The membranes were blocked with 1% gelatin and washed three times with 0.25% gelatin solution (in 150 mM NaCl, 5mM EDTA, 50 mM Tris, 0.05% Triton-X) to prevent non-specific adsorption of the immunological reagents. The membrane was then immersed in 10 mL of 0.25% gelatin solution containing 10 μL of several rabbit anti-egg protein IgG polyclonal antibodies. Antigen-IgG complexes were detected using 10 μL of goat anti-rabbit IgG antibodies labeled with alkaline phosphatase (Sigma, Milan, Italy). The developing solution contained 15% bromochloroindolyl phosphate (BCIP) and 30% nitro blue tetrazolium (NBT) (Sigma, Milan, Italy) in alkaline phosphatase buffer (100 mM Tris-HCl, pH 9.5, 100 mM NaCl, 5 mM $MgCl_2$).

RESULTS AND DISCUSSION

Anti-egg white protein antibody characterization

The quality of antibody used in ELISA test is crucial for its validation; on these bases, we verified the specificity of three anti-egg white protein antibodies by immunoblotting. Purified egg proteins were separated in SDS-PAGE (Figure 1) and then, after transfer on PVDF membranes, were incubated with each developed antibody (Figure 2).

In Figure 1, egg white proteins are easily identified in oenological fining agent thanks to the purified standard proteins run in parallel.

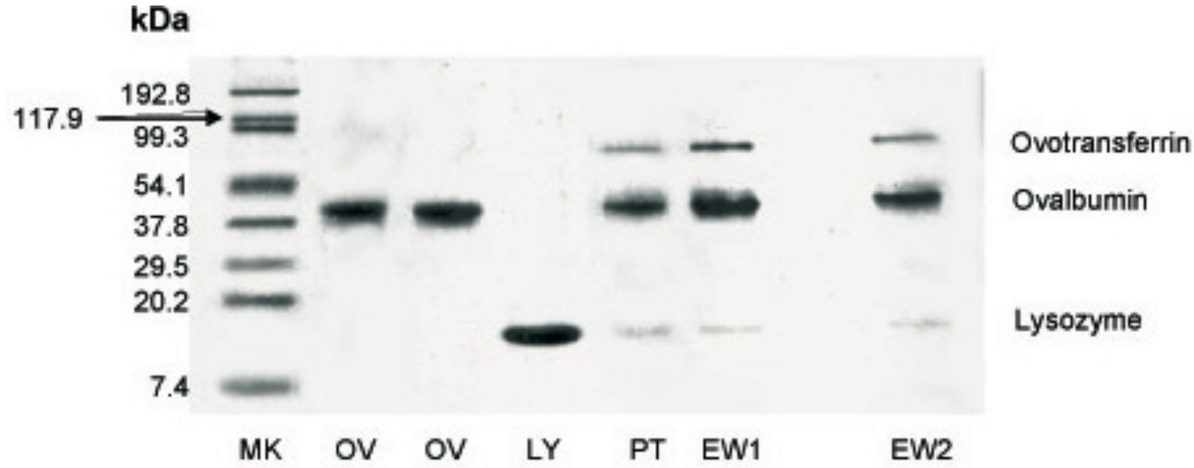

Figure 1 - SDS-PAGE of fining agent containing egg white and purified proteins

MK= molecular weight standard solution; OV=ovalbumin; LY= lysozyme; PT= Egg white total proteins; EW1= Fresh Egg White; EW2= Egg White – fining agent

Figure 2 shows the immunoblottings used to characterize the four antibodies developed for this research. All antibodies show high specificity and among them, the Antibody n. 1 was selected for ELISA plate coating.

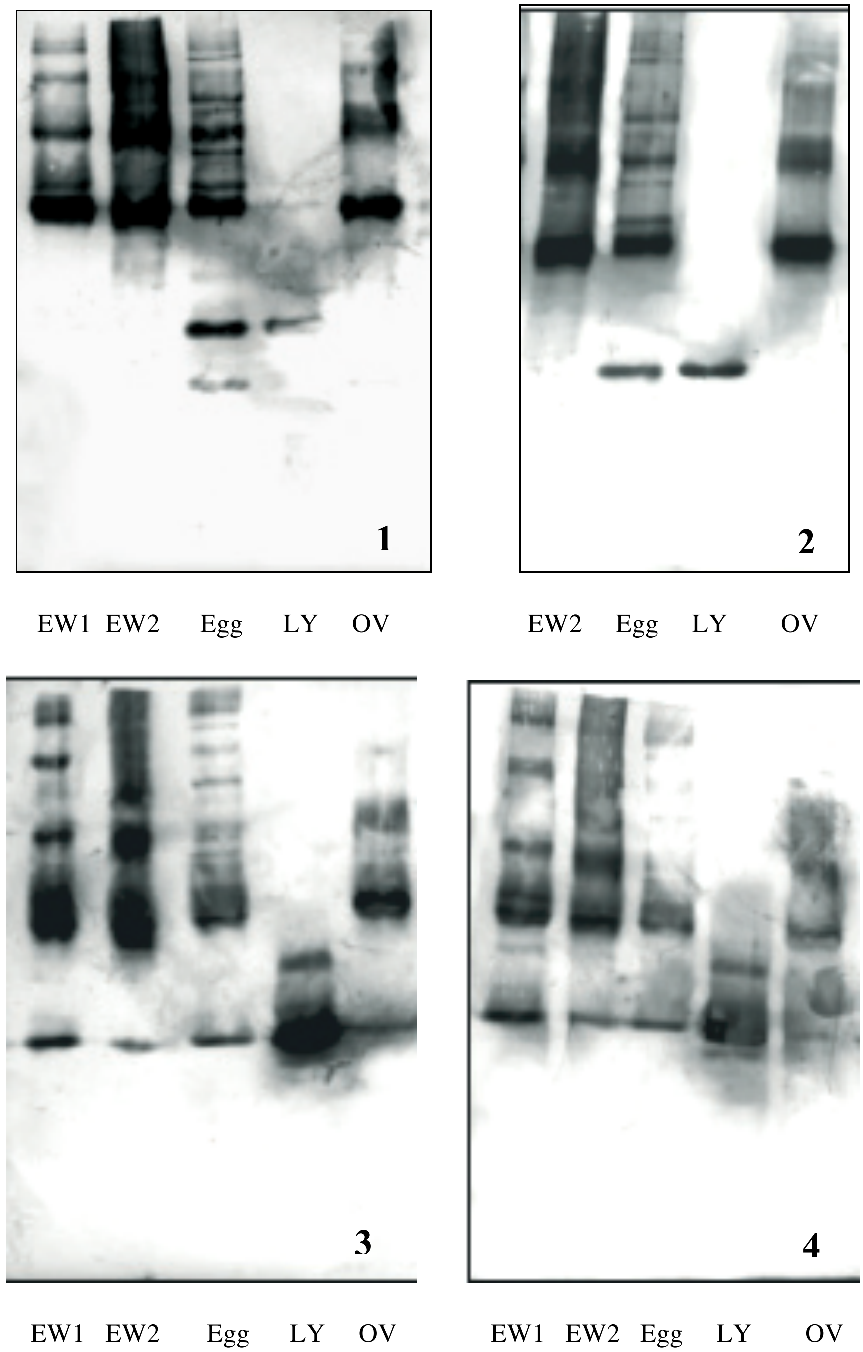

Figure 2 - Immunoblotting of commercial egg white (fining agent) and corresponding purified proteins. Four (1-4) different antibodies were tested to assess their specificity. OV=ovalbumin; LY= lysozyme; EW1= Fresh Egg White; EW2= Egg White – fining agent

ELISA method

In this research, we developed a new ELISA method specifically applied to the detection of egg white traces in wine. All procedure is internally monitored using negative and positive “wine standard solutions”.

Internal standards and wine sample preparation

The preparation of wine samples is extremely simple and requires only dilution with specific buffer. To improve the quality of test, we prepared a positive “wine matrix” standard solution.

Assay protocol

The procedure is very similar to that normally applied in any sandwich ELISA test; the loading of 100 µl of every Egg White Standard Solution, positive internal control and wine samples is followed by washing steps, and the use of chromogen reagent responsible for the colour development.

A spectrophotometric evaluation of the results is done by reading the colour intensity of the controls in comparison with the colour intensity of internal standards and the wine samples.

The performance of the developed ELISA test requires one hour and the detection is done with spectrophotometric detection at 450 nm. The absorbance value obtained must be multiplied by the dilution factor, as a consequence of the sample preparation.

An example of standard curve is reported in Figure 3. The linearity in the range of 0.02 ppm to 1 ppm is good, as shown by the R^2 value (0.9387).

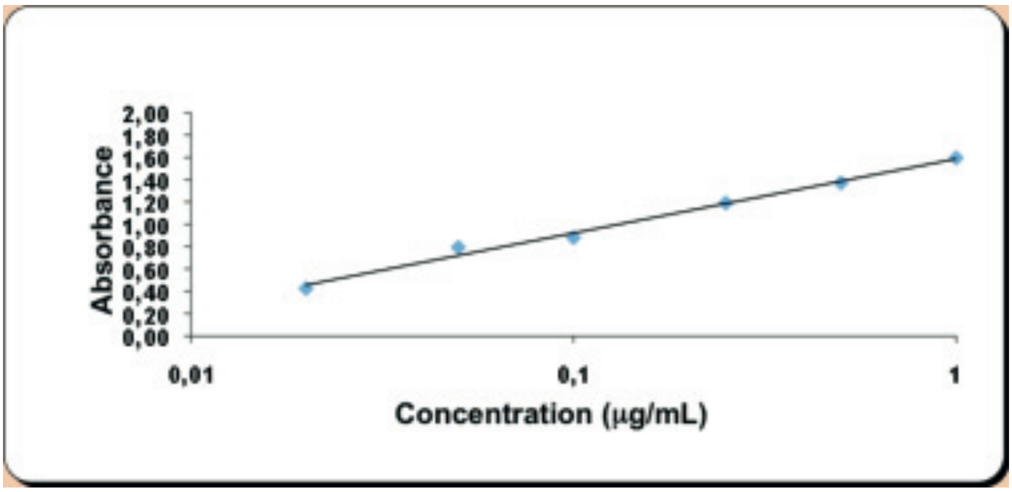

Figure 3 – An example of regression curve obtained in ELISA test

Same experimental samples were prepared by adding oenological egg white in untreated red wines and included in the ELISA test; the internal "wine sample" control was analysed in parallel. The results are reported in Table 1.

Table 1 – Egg white concentration in Internal "wine standard control" and in commercial wine samples fined with oenological egg white

Wine samples	1st measure	2nd measure	Mean	ppm
Red wine 1 as such	- 0-016	- 0.003	- 0.009	ND
Red wine 1 dil 1:2 (v/v)	- 0.023	- 0.023	- 0.023	ND
Red wine 2 as such	- 0.005	- 0.020	- 0.013	ND
Red wine 2 dil 1:2 (v/v)	- 0.009	- 0.009	- 0.009	ND
Positive standard (0.5 ppm)	0.117	0.110	0.114	0.49

ND= undetectable

All wine samples were free from egg white residues, while positive internal standard contained, as expected, egg white proteins.

SDS-PAGE and immunoblotting of red wine samples

The absence of allergenic residues in wine samples was verified by immunoblotting; Figure 4 shows the electrophoretic pattern of some red wines and the immunoblotting obtained by incubating the corresponding membrane with anti-egg white antibody n. 1.

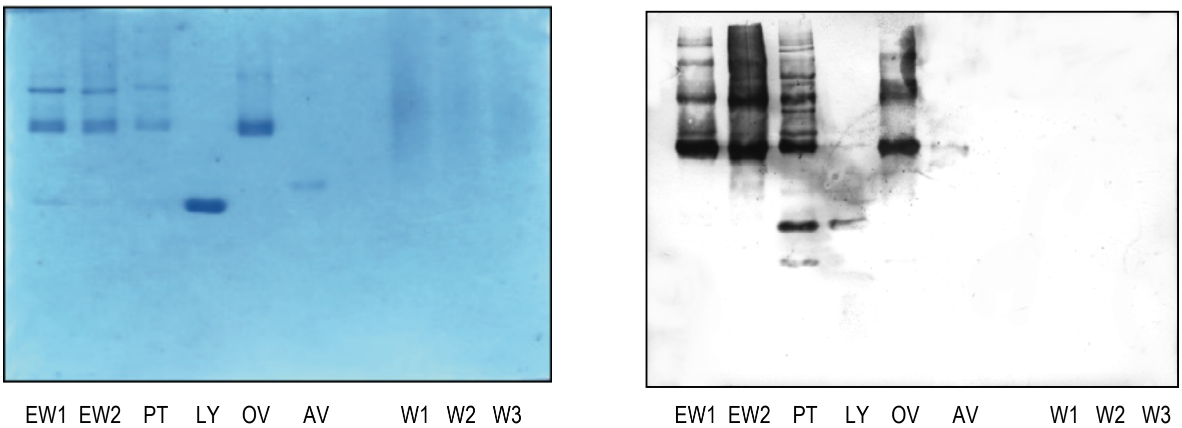

Figure 4 - SDS-PAGE (left) and Immunoblotting (right) of egg white derivatives and red wines treated with egg white fining agent.
OV=ovalbumin; LY= lysozyme; AV= avidin; PT= Total egg proteins; EW1= Fresh Egg White; EW2= Egg White – fining agent; W1-W3= red wines treated with EW2

The antibody recognizes egg white samples with high affinity, but no immunoreactivity was detected in treated red wines, confirming data found by ELISA test.

CONCLUSIONS

The ELISA test is one of the most frequently used analytical methods to detect allergens present in foods. The specificity and sensitivity of immunological procedures is well known; moreover, for the limited equipments required, ELISA test can be applied in most laboratories involved in quality control. The critical point in ELISA methods is the effect of any food composition on allergen determination. This is the reason why a specific protocol must be developed for any food/beverage. In this paper, we describe the preliminary data obtained applying an ELISA test to the detection of allergenic residues in red wine fined with egg white.

A similar method was succefully applied to the control of white wines fined with caseinates to discriminate between wines that need a specific labelling and those that could be considered safe for subjects suffering for food allergies (Restani et al, 2009).

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