1st International Symposium

Alcohol level reduction in wine

OENOVITI INTERNATIONAL network

6 September 2013 - Bordeaux

Coordinator
Pierre-Louis Teissedre

Institut des Sciences de la Vigne et du Vin
Faculté d'œnologie - Université Bordeaux Segalen
210 Chemin de Leysotte - 33882 Villenave d'Ornon cedex - France

ISVV
INSTITUT DES SCIENCES DE LA VIGNE ET DU VIN
BORDEAUX AQUITAINE

OENOVITI INTERNATIONAL Network

Alcohol level reduction in wine

Forward

The OENOVITI INTERNATIONAL Network is the first and unique international network for higher education and research in oenology & viticulture. It aims to promote the exchange of know-how and expertise between the stakeholders of the viticulture and academic communities and the industry. The network enables its members to be influent at the international level by developing the opportunities in terms of joint research and training projects and by encouraging dialogue between the involved groups. The OENOVITI INTERNATIONAL Network comprises more than 30 partners worldwide which form an international consortium of institutions with recognised excellence in the field. It is divided in nine working groups (multidisciplinary and thematic). At the root of the network, the OENODOC Joint Doctoral Programme was created in order to set up an international doctorate specific to the oenology-viticulture sector. The OENODOC consortium gathers especially the members of VINTAGE and EMaVE which both provide the VINTAGE and Vinifera Erasmus Mundus Master's degree programmes.

The different working groups are as follows : climate change, oenology, engineering and processes, biotechnology, genetic material, grape selection and production, vine diseases and pests, wine management and oenotourism, table grape, industrial transfer, development – strategic watch and international relations. The innovative approach of the OENOVITI INTERNATIONAL Network is based on staff and student mobility, the sharing of experience and good practices between the disciplines and a common core of education and training policies. The programmes also bring together numerous industrial and socio-economic partners. Beside financial support, they apply their expertise to conduct high-level R&D and provide employment opportunities for young graduates.

This network, which is coordinated by Bordeaux Segalen University, enables the academic community and the industry to meet and discuss the numerous research challenges in oenology and viticulture. The alcohol level reduction in wine is one of the issues the viticulture and oenology sector has to deal with in order to : preserve the quality of wine, bring solutions to viticulturists and ensure consumers maximum enjoyment when tasting wine. This first international symposium of the network intends to gather knowledge and suggest research work on this topic. The following subjects will be addressed so as to come with different solutions aiming to reduce alcohol level in wine : viticulture and oenology, strategies, rules, technological practices and processes, sensory impact and consumer's preferences.

The network relies especially on the support of the Château Pichon-Longueville (AXA Millésimes Group), the University of Bordeaux Foundation (original interface between the academic and socio-economic communities), IdEx Bordeaux (investment programme to foster the dynamic of transformation and development of the University of Bordeaux) and of all the academic and industrial partners.

We would like to thank Mr Christian Seely (CEO of the Château Pichon-Longueville), the Professor Manuel Tunon de Lara (President of the University of Bordeaux Segalen), the Professor Alain Boudou (President of the University of Bordeaux), Mr Rodolphe Gouin (Director of the University of Bordeaux Foundation) and all the partners which participate in the support, the activity and the progress of the network which will benefit to the whole vine and wine sector.

Pierre-Louis Teissedre
Professeur
Coordinateur Réseau Œnoviti International

OENOVITI INTERNATIONAL Network

Alcohol level reduction in wine

Préface

Le réseau OENOVITI INTERNATIONAL est le premier et unique réseau international pour la formation et la recherche en œnologie et viticulture. Il vise à favoriser les échanges de savoir-faire et d'expertise entre les acteurs du monde viticole, académiques et industriels. Le réseau offre à ses membres une forte visibilité sur la scène internationale en leur permettant de multiplier les opportunités en termes de projets conjoints de recherche, de formation et de temps d'échanges. Le réseau OENOVITI INTERNATIONAL compte plus de 30 partenaires dans le monde qui forment un consortium international d'institutions reconnues pour leur excellence dans le domaine. Il est organisé autour de ses membres en 9 groupes de travail (transverses et thématiques). À l'origine du réseau, le programme de doctorat conjoint OENODOC a été créé dans le but de développer un doctorat international, spécifique à la filière œnologie-viticulture. Le consortium OENODOC réunit notamment les membres de VINTAGE et EMaVE qui offrent respectivement les masters labellisés Erasmus Mundus VINTAGE et VINIFERA.

Parmi les groupes de travail on trouve : changement climatique, œnologie, ingénierie et procédés, biotechnologie, matériel génétique, sélection et production de raisin, maladies et parasites de la vigne, management du vin et oenotourisme, raisin de table, transfert industriel, développement - veille stratégique et relations internationales. L'approche innovante du réseau OENOVITI INTERNATIONAL est basée sur la mobilité des personnels et des étudiants, l'échange d'expériences et de bonnes pratiques entre les disciplines et l'établissement d'un socle commun en éducation et formation. Les programmes associent également de nombreux partenaires de l'industrie et du monde socio-économique. Au-delà d'un soutien financier, ils apportent leur expertise pour mener une R&D d'excellence et offrir des débouchés professionnels aux jeunes diplômés.

Ce réseau coordonné par l'Université Bordeaux Segalen permet aux mondes académique et industriel de se rassembler sur les nombreux challenges de recherche de l'œnologie et de la viticulture. La réduction d'alcool des vins est un des défis auquel le monde viticole et de l'œnologie doit répondre pour : préserver la qualité des vins, apporter des solutions aux vignerons, et garantir aux consommateurs un plaisir optimum lors de la dégustation des vins. Ce premier colloque international du réseau vise à rassembler les connaissances et de proposer des actions de recherches sur ce thème. Afin de présenter différentes possibilités pour essayer de réduire les teneurs en alcool des vins, les thématiques suivantes seront présentées : viticulture et œnologie, stratégies, réglementations, pratiques technologiques et procédés, impact sensoriel et préférences des consommateurs.

Le réseau bénéficie notamment du soutien de Château Pichon-Longueville (groupe AXA Millésimes), de la fondation Bordeaux Université (interface originale entre les mondes universitaire et socio-économique), de l'IdEx Bordeaux (programme d'investissements pour accompagner la dynamique de transformation et de développement de l'Université de Bordeaux), et de l'ensemble des partenaires académiques et industriels.

Nous tenons à remercier M. Christian Seely (Directeur général de Château Pichon-Longueville), le Professeur Manuel Tunon de Lara (Président de l'Université Bordeaux Segalen), le Professeur Alain Boudou (Président de l'Université de Bordeaux), M. Rodolphe Gouin (Directeur de la fondation Bordeaux Université) et tous les partenaires qui participent au soutien, à la vie et aux avancées du réseau qui bénéficieront à l'ensemble de la filière vigne et vin.

Pierre-Louis Teissedre
Professeur
Coordinateur Réseau Œnoviti International

OENOVITI INTERNATIONAL Network

Alcohol level reduction in wine

Welcome letter

Coordinated by the University of Bordeaux Segalen, the OENOVITI INTERNATIONAL Network is the first and unique international network in oenology and viticulture. Composed of more than 30 partners from all over the world, this network aims to promote the exchange of expertise and know-how between the stakeholders of the oenology and academic communities and the industry. It gives the opportunity to develop international collaborations in Vine and Wine Sciences research and training.

In the frame of this network, the first OENOVITI INTERNATIONAL symposium will be held in September 2013 in Bordeaux, France, in the Vine and Wine Sciences Institute. Researchers will be able to discuss the current topic of the increase of alcohol level in wine and the issue of a potential reduction of this level.

In a globalised world, viticulture and oenology will have to face major challenges. The increase of the alcohol level in wine related to climate change is one of them. This phenomenon observed all over the planet shows that grape ripens more and more early, and would mainly result from global warming.

In line with this observation, the alcohol level in wine has increased. It is now common to see quality wines with an alcohol by volume (ABV) of 13, 14 or even 15 %. Since the eighties, each ten years, alcohol level gained almost 1 % with an average increase of 2 to 3 %, if not more. This historical surge of ABV was measured in many countries : in the South-West of France, 15 years ago, the average alcohol level amounted to 11 % ; it now ranges between 13 and 14 %. In Australia, the average was 12.4 % in 1984 ; in 2004 it reached a striking 14 %. In California, the average ABV was 12.5 % in 1978 and soared to 14.8 % in 2001.

This issue is particularly important for viticulture since the reduction of alcohol level in wine without altering its quality and while preserving its specificity is now essential for the whole sector worldwide.

What are the possible actions to cope with it directly at the vine level, during the development of the wine, what are the most appropriated techniques and processes to do so ? What are the regulatory limits which exist in this field ? Will it be necessary to irrigate ? Will we have to adapt the current varietals or develop new ones producing grapes with a reduced sugar level and therefore, with less alcohol in the end, contrary to what was intended thirty or forty years ago ? Eventually, what will be the influence on sensorial perception and consumers' acceptability of the reduction of alcohol level in wine ?

All these questions will be addressed during this first OENOVITI INTERNATIONAL symposium and justify our support to this programme. Indeed, this original and unique network focuses energy on current challenges in oenology and viticulture. OENOVITI INTERNATIONAL is an ambitious network, with international and multidisciplinary influence. The topics discussed deal, among others, with research in oenology and viticulture, health or environment, subjects that we are all interested in. We are proud in AXA Millésimes to contribute to the development – of the biggest international research network in oenology and viticulture ; - of research in oenology and viticulture resulting in collective answers to the new challenges in this field ; - of courses of excellence at the international level.

Christian Seely
Directeur Général Château Pichon-Longueville
AXA Millésimes

OENOVITI INTERNATIONAL Network

Alcohol level reduction in wine

Lettre de bienvenue

Coordonné par l'Université Bordeaux Segalen, le réseau OENOVITI International est le premier et unique réseau international en œnologie et viticulture.

Composé de plus de 30 partenaires dans le monde, ce réseau vise à favoriser les échanges de savoir-faire et d'expertise entre les acteurs du monde viticole, académiques et industriels et donne l'opportunité de développer des collaborations internationales en recherche et formation des sciences de la vigne et du vin.

Dans le cadre de ce réseau, le premier Symposium OENOVITI International est accueilli en septembre 2013 à Bordeaux en France au sein de l'Institut des Sciences de la Vigne et du Vin. Il rassemble des scientifiques sur le thème actuel de l'augmentation de la teneur en alcool des vins et l'enjeu d'une possible réduction.

Dans un monde globalisé, la viticulture et l'œnologie se retrouvent aujourd'hui en face de défis majeurs. L'augmentation de la teneur en alcool des vins liée au changement climatique en est un. Ce phénomène observé un peu partout dans le monde montre que le raisin arrive de plus en plus tôt à maturité, et que ce phénomène serait lié au rôle prépondérant du réchauffement climatique.

De ce constat découle une élévation de la teneur en alcool du vin. Il est désormais courant de voir des vins de qualité titrer 13, 14, voire 15 % vol. Depuis les années 1980, le vin a gagné près de 1 % vol. par tranche de dix années, avec des gains moyens de 2 à 3 % vol. voire plus. L'ascension historique des titres alcoométriques est remarquable un peu partout dans le monde : pour le Sud-Ouest de la France, il y a 15 ans, le taux moyen d'alcool était de l'ordre de 11 % ; aujourd'hui, il se situe entre 13 % et 14 %, en Australie : la moyenne était de 12,4 % en 1984 ; en 2004, c'est un fulgurant 14 %., en Californie : le titre alcoométrique moyen était de 12,5 % en 1978 pour atteindre un sommet de 14,8 % en 2001.

L'enjeu pour la viticulture est tel que la réduction de la teneur en alcool des vins sans en modifier la qualité et en gardant sa typicité est aujourd'hui essentielle pour l'ensemble de la filière au niveau mondial.

Quels sont les moyens possibles d'actions aujourd'hui pour y remédier à la vigne, lors de l'élaboration du vin, quelles techniques et procédés sont les plus appropriés pour y parvenir ? Quelles sont les limites réglementaires qui existent dans ce domaine ? Faudra-t-il irriguer ? Faudra-t-il adapter les cépages actuels, ou mettre au point de nouveaux cépages produisant des raisins avec moins de teneur en sucre et donc, à terme, moins d'alcool ; à l'inverse de ce qui pouvait être recherché il y a trente ou quarante ans ? Enfin quelle est l'influence sur la perception sensorielle et l'acceptabilité par les consommateurs de la réduction de la teneur en alcool des vins ?

Autant de questions qui seront abordées lors de ce premier colloque d'OENOVITI International qui justifie notre soutien à ce programme ; car ce réseau unique et original concentre son énergie vers les défis actuels de l'œnologie et viticulture. OENOVITI International est un réseau ambitieux, au rayonnement international et multidisciplinaire. Les axes abordés portent entre autres sur la recherche œnologique et viticole, la santé ou l'environnement, des sujets qui nous concernent tous. Nous sommes heureux au sein d'AXA Millésimes de contribuer :

- au développement du plus grand réseau international de recherche et de formation en œnologie et viticulture,
- d'accompagner le développement de la recherche en œnologie et viticulture pour la résolution collective des nouveaux défis dans ce domaine, - de contribuer au développement de formations d'excellence au niveau international.

Que l'ensemble des scientifiques qui oeuvrent pour apporter des solutions aux défis de la viticulture et de l'œnologie soit remercié. C'est grâce à leurs travaux de recherches et d'innovations que nous espèrerons que la viticulture et l'œnologie traverseront ce siècle en apportant des améliorations qualitatives pour nos vins.

Christian Seely
Directeur Général Château Pichon- Longueville
AXA Millésimes

ŒNOVITI INTERNATIONAL Network

Alcohol level reduction in wine

Table des matières / Contents

Alcohol level reduction in wine

OENOVITI INTERNATIONAL network

organisation and contact
Pierre-Louis Teissedre - œnodoc@u-bordeaux2.fr
Tél. +33 (0)5 57 57 58 53
http://www.oenoviti.univ-bordeauxsegalen.fr
Institut des Sciences de la Vigne et du Vin
Faculté d'œnologie - Université Bordeaux Segalen
210 Chemin de Leysotte - 33882 Villenave d'Ornon cedex - France

Secretariat
Charlotte Bondu
œnodoc@u-bordeaux2.fr

ISBN
2-915883-11-4
EAN
9782915883114

Conception graphique/Graphc Design
Vigne et Vin Publications Internationales - Marylène Perreaud
edition@vigne-vin.com

Imprimé par/*Printed by*
Copy-Media (Mérignac - 33 - France)

Crédit photos/*Photo credits*
Emmanuel Baugier© - Fotolia©

Dépôt légal/*Legal deposit*
3e trimestre/*3rd trimestre 2013*

Alcohol level reduction in wine

OENOVITI INTERNATIONAL Network

Session I - Potential reduction in alcohol levels and viticulture

Viticultural strategy to reduce alcohol levels in wine

Vittorino Novello[1,*] and Laura de Palma[2]

[1]Dipartimento di Scienze Agrarie, Forestali e Alimentari, Università di Torino, Via L. da Vinci 44, 10095 Grugliasco TO, Italy
[2]Dipartimento di Scienze Agrarie, degli Alimenti e dell'Ambiente, Università di Foggia, Via Napoli 25, 71121 Foggia, Italy

Abstract: Full-bodied and deeply colored red wines are presently the most appreciated and prized. Well-ripe grapes normally have a high glucidic content that gives highly alcoholic wines. Moreover, global temperature is increasing leading to advance in berry maturation and increase of sugar accumulation; this fact enhances even more the average wine alcohol content. Due to the consumer concern about the effects of high alcohol wine drinking, several producers have started to offer low alcohol wines. This type of wines can be produced by reducing alcohol in winery or by reducing sugar accumulation in berries. Within the last approach, different strategies have been suggested: selecting specific varieties or clones, increasing crop load, shading bunches, choosing proper irrigation techniques, modulating source-sink relationships by removing leaves or topping shoots, applying anti-transpirant to leaves or plant growth regulators to grapes. The results obtained from many studies are not univocal and are likely affected by the interaction between genotype and environment and by the extent to which each technique is applied.

Keywords: sugar accumulation**,** genotype, source-sink relationship, photosynthetic limitation, plant growth regulators

Introduction

Full-bodied and deeply colored red wines are presently the most appreciated and prized. The obtaining of this wine style involves a high phenol extraction from full-ripe grapes. Less ripe grapes are richer in herbaceous aromas, show a lower anthocyanin and proanthocyanidin extractability from berry skins and a higher proanthocyanidin extractability from seeds; on the whole, they tend to give excess of astringency and of herbaceous aromas in wines (Ó-Marques *et al*., 2005; Fournand *et al*., 2006).

Well-ripe grapes normally have a high glucidic content and give highly alcoholic wines. It is provable that, on average, wines have gradually increased alcohol content in the last decade.

A high wine alcohol content has negative effects on human health and, presently, is not appreciated by a wide part of consumers that prefer drinking light and responsibly (Salamon, 2006), thus it discourages wine consumption.

Moreover, ethanol excess may exert detrimental effects on must fermentation and sensory property perception (Bisson, 1999; Fischer and Noble, 1994).

In addition, it is thought that the global warming could alter grape composition (Schultz and Jones, 2010) increasing berry sugar content and final alcoholic level in wines. It is well known that elevate temperatures during berry ripening induce faster pulp maturation and enhance must total soluble solids and pH.

Another problem related to a high temperature regime during berry ripening is the greater difficulty to harvest grapes at the most suitable aromatic and phenolic maturity; this fact leads to obtain not well-balanced wines. All the effects of global warming may impact even more in agro-environmental conditions where water depletion is more effective, such as in the case of non-irrigated hilly vineyards, vines on sandy and shallow soils, high plant density (Williams, 2012).

These considerations stimulate a greater attention for strategies directed to reduce alcohol level in wine.

This type of wines can be produced by reducing alcohol in winery or by reducing sugar accumulation in vineyard. This latter approach concerns the use of some viticultural techniques.

Contact author : vittorino.novello@unito.it

1. Revisiting basic viticulture techniques

Within vineyard management, the most obvious strategy to reduce wine alcohol level is to lower grape sugar content by increasing yield. Grape yield may be increased by enhancing the bud load, lowering the cluster thinning and choosing a vigorous rootstock. However, the yield increment should be carefully assessed and modulated in order to limit possible detrimental effects on wine quality. Part of consumers presently asks wines not only less alcoholic than in the past, but also less structured, thus, possibly, a new equilibrium between fruit quality and quantity should be individuated. Basing on studies carried out by Kliewer and Dokoozlian (2005) it is easy to predict a reduction of berry sugar accumulation in vineyards having a high unitary grape yield that is often joint to a low leaf area/grape weight ratio (less than 0.8 and 0.5 m^2/kg, respectively, for VSP trained vines and for vines trained to horizontal or divided canopy).

On the other hand, vineyard strategies looking at reducing shoot vigour and obtaining small berries and clusters as well as grapes rich in phenols are also thought to be useful to produce low alcohol wines, since they are capable to provide a good quality grape at a lower maturity level in terms of sugar concentration. These strategies include the choice of a proper irrigation management, pruning intensity and new genotypes (Clingeleffer, 2007).

As concerns irrigation, increase of water supply may cause dilution of sugars, but also that of phenols. On the other hand, deficit irrigation from berry-set to veraison does not seem able to induce a vine stress apt to limit berry sugar accumulation (Cooley *et al.*, 2005). Nonetheless, among several irrigation treatments experimented in a warm-dry climate, the applying of water supply only from veraison to harvest, calculated by ET_C using 0.6 K_C, proved to reduce sugar accumulation without modifying phenol composition and wine quality in Cabernet Sauvignon (Fernandez *et al.*, 2013). Generally speaking, late irrigation water supply could be a strategy useful to resume shoot growth in the central phase of berry sugar accumulation in order to drive more photosynthates to the new vegetation and, thus, to reduce photosynthates available for the bunches.

As for pruning systems, minimal pruning, that greatly increases bud load and shoot number per vine, is known to stimulate some compensatory behaviors resulting in numerous little and loose bunches spread over a large canopy and well lighted, having small berries richer in skin phenols than usual and thus harvestable at a lower sugar concentration (Clingeleffer, 2007). Never-theless, European viticulture is still quite reluctant to abandon the traditional pruning systems.

Among rootstocks, low-to-moderate vigour genotypes have been proposed in Australia to produce grapes for low alcohol wines. An example of their potential effect is given by the genotypes of the Merbein series: compared to traditional rootstocks, they are able to stimulate the improving of colour and phenolics by about 20 % in Shiraz grapes harvested at maturity level lower by 1.5 °Brix than usual (Clingeleffer, 2007).

The searching of new grape varieties characterized by low berry sugar accumulation and, at same time, by optimal grape flavour and colour requires long and elaborated studies. Australian breeding work obtained new varieties, adapt to warm climate, that compared to Cabernet Sauvignon harvested at a given maturity level reached higher yield, better wine chemical composition and sensorial attributes; this is the case of Cienna, released in 2000, that has been suggested for producing low alcohol wine (Clingeleffer, 2007). Nevertheless, the searching of natural and already existing genotypes apt to produce low alcohol wines could be also considered, looking for those capable to limit sugar concentration without penalizing too much the phenol content. For example, it has been observed that some selected clones may differ in sugar accumulation dynamics (Zecca *et al.*, 2013).

Apart from these techniques, the “double harvest” has been also proposed for decreasing wine alcohol level. The first harvest (“green”) coincides with the normal bunch thinning performed at veraison. The must of these grapes is conserved and blended with that of grapes harvested at normal maturity. The final wines show a significant lower alcohol and pH and a higher titratable acidity, but do not differ in

quality and sensory properties in comparison with those obtained by traditional wine-making (Kontoudakis *et al.*, 2011; Balda and Martinez de Toda, 2013). This technique however, although starts from a viticultural practice, involves the technological aspects more than the vineyard management.

Finally, among simple growing techniques, those that allow do not expose bunches to full sunlight, such as a moderate use of leaf removal in the fruit zone and/or the choice of training systems apt to protect grape from direct solar radiation (such as pergola, free cordon, GDC), could be also reconsidered providing the assessing of proper conditions apt to modulate the grape yield/quality balance.

2. Modulating source-sink relationship and reducing photosynthetic activity

The source-sink relationships, based on the ratio between the photosynthesizing leaf surface and the fruit mass that attracts a great portion of photoassimilates, are considered of fundamental importance in modulating grape ripening and quality. For example, it is well known that by limiting leaf area at berry-set it is possible to reduce the final berry size and to improve the berry composition (Ollat and Gaudillère, 1998), and that the fruit thinning reduces the level of competition for metabolites improving the final fruit quality.

However, it is to consider that either shoot or cluster thinning may reduce the grape yield and, thus, may increase sugar accumulation in maturing bunches (Dokoozlian and Hirschfelt, 1995; Sun *et al.*, 2011). Moreover, cluster and shoot thinning may result in more “fruity” wines, but they may also penalize tannin extractability in some varieties (Sun *et al.*, 2012).

On the other hand, changes of leaf-to-fruit ratio may reduce the velocity of berry maturation and the final sugar content. Stoll and collaborators (2010) proved that, by topping severely vine shoots at berry-set (leaving 6 leaves per shoot) it is possible to slow down Riesling grape maturation by 20 days and to cut final sugar accumulation by about 4 °Brix. Severe shoot topping (just over clusters) after berry-set, that induced a relevant reduction of leaf area/grape weight ratio, slow down maturation and significantly reduced sugar content, bunch and berry weight in cvs Grenache and Tempranillo; however, total polyphenol and anthocyanin concentration in the must were penalized (Balda and Martinez de Toda, 2011). Filippetti and collaborators (2011), by late-topping Sangiovese shoots one week after veraison, obtained a good lowering of must sugar concentration without modifying pH, organic acid and anthocyanins concentration, skin and seed tannins content.

The leaf removal technique is effective in modulating source-sink relationship. Leaf removal above cluster zone (-36 %) one month after veraison reduced leaf-to-fruit ratio by 41 % and proved to slow down maturation of Sangiovese grape and to lower sugar concentration and wine alcohol level (-0.6 %) (Palliotti *et al.*, 2013). Post-veraison leaf removal at distal canopy portion of Sangiovese and Montepulciano vines (removing respectively 60 % and 29 % of vine leaf area) reduced leaf/fruit ratio at harvest by 38 % (Sangiovese) and 16 % (Montepulciano) without affecting bunch and berry weight, must total acidity and pH, but lowering sugar concentration (-0.7 °Brix) in both varieties; however, also final Montepulciano anthocyanin and polyphenol concentration were reduced (Lanari *et al.*, 2013). Leaf removal of eight basal leaves, at fruit-set, lowered significantly total soluble solids in the white grapes of cv Loureira (Freire *et al.*, 2013). In Negroamaro vines, the removal (at berry pea-size) of main leaves and lateral shoots from the basal node to the second node over the clusters (eliminating about 63 % of vine leaf area), reduced grape yield (-14 %), improved wine total polyphenols (+17 %), anthocyanins (+15 %) and colour intensity (+16 %) compared with the regular thinning of 50 % main leaves and laterals along the entire canopy. Moreover, the first treatment lowered total flavonoids (-22 %) and proanthocyanidins (-16 %), and reduced berry total soluble solid (-1 °Brix) and wine alcohol but only by 4 %. Differences in the type of removed leaf, canopy microclimate, leaf ecophysiological functioning and leaf-to-fruit ratio accounted for these results (de Palma *et al.*, 2010). In Nebbiolo grape fruit zone leaf removal and bunch shading improved berry phenol composition (North-West Italy), but did not influenced berry sugar accumulation

in comparison to the control (Guidoni *et al.*, 2008; Chorti *et al*., 2010).

As concerns the influence of the training system, Sylvoz and Lyre have been found use- ful to reduce potential alcohol in Sousón grapes, likely due to the increase of shoot vigour, vine productivity and bunch shading normally associated to these training systems; Lyre induced similar results also in Godello and Loureira grapes (Diaz-Losada *et al.*, 2013).

3. Photosynthetic limitation

The limitations of photosynthetic rate per leaf area unit act as a reduction of "active" leaf area, although leaves are not removed.

The application of shade nets over the vine canopy reduces the photosynthetic photon flux at the leaf surface available for photosynthetic process. According to the net colour and density different shading levels may be obtained. Measuring diurnal rates of leaf net CO_2 uptake per leaf area unit in Sangiovese vines exposed at 100 %, 60 % and 30 % of full sunlight, it was observed, at flowering, a relevant difference between the two extreme treatments. At harvest, vine productivity decreased only by 14 %, but sugar content decreased by 2 3% (from 21.9 °Brix to 16.8 °Brix) (Palliotti *et al.*, 2012).

Anti-transpirant canopy sprays obtained by distillation of conifer resins (such as those containing 'pinolene', a product having 1-*p*-menthene as active compound) are able to reduce the leaf CO_2 influx. After spraying, the product evaporates in few hours leaving the leaves covered by a thin transparent layer that limits the rate leaf gas exchange. Rates ranging from 30 % to 70 % of those of control vines have been observed for a period of 40-50 days. After the product is degraded, leaves are able to recover their functionality. Investigations performed since 2008 proved that post-veraison anti-transpirant treatments may induce a significant reduction of must sugar concentration and, hence, of wine alcoholic level, regardless of the cultivar and the vine productivity. In Italy, trials were carried out on Sangiovese, Tocai rosso, Trebbiano toscano and Grechetto producing from 7 to 32 t/ha of grapes. However, anti-transpirant treatments may induce some detrimental effects on phenolic content, mostly in black-berry varieties and especially for anthocyanins, while total polyphenol content seems less affected. This effects are not desirable for aged red wines, but they could be acceptable for rosé and Beaujolais type of wines or for base wines to blend with others richer in color and phenolic compounds (Palliotti *et al.*, 2008; 2010; 2013). Similar results were obtained by Tittmann and coauthors (2013) in Riesling and Müller Thurgau grapes grown either in greenhouse or in open field.

4. Plant growth regulator treatments

Several hormones, such as abscisic acid and ethylene, are known to have a direct influence on the maturation processes, including colour development, while the auxin level is normally reduced when the fruit ripening begins. Hence, among techniques aiming at slow down grape maturation, the use of plant growth regulators may be proposed.

According to research of Davies and collaborators (1997), dipping Shiraz bunches for 30 seconds in benzotriazole-2-oxaloacetic acid (BTOA) 6 and 8 weeks after blooming is able to delay the evolution of physical-chemical changes related to maturation: increase of berry weight, anthocyanin and hexose accumulation and abscisic acid concentration, and degradation of chlorophyll and organic acids. The expression of genes typical of the pre-veraison stage continued for an extensive period, while that of genes typical of the ripening period was delayed.

Some cytokinins, such as CPPU (forchlorfenuron), applied at pre-veraison stage, are able to reduce total soluble solid concentration and berry skin colour; berry weight and juice total acidity are increased (Han and Lee, 2004).

Pre-veraison auxin treatments (1-naphthaleneacetic acid) proved to delay Shiraz berry ripening in terms of juice sugar accumulation and skin anthocyanin content. Wine sensory characteristics did not change in comparison to the control. Hence, auxin applications may represent a technique useful to control fruit composition (Böttcher *et al.*, 2011).

5. Conclusions

Reducing alcohol levels in wine is a challenge for the next future. Viticultural

strategies useful to face this problem are all those apt to reduce grape sugar content at harvest, since this is the starting point to obtain less alcohol in wine. Except for the searching of specific grapevine genotypes, these strategies are mostly based on the proper use of some growing techniques having a direct or indirect impact on the berry sugar accumulation, such as, for example, irrigation, pruning and canopy management; however, they have to be revisited in order to achieve the desired result. The use of anti-transpirants to spray on leaves and of plant growth regulators to apply on grapes may be considered as a new tools.

All the techniques limiting photosynthesis, or modifying the source-sink ratio or delaying fruit maturation may reach the goal. Nevertheless, this is not always easy to reach since grapevine has a high physiological plasticity that provides several compensatory responses acting as a buffer respect to the imposed treatments. Moreover, together with a low alcohol level, a high color intensity and a rich aromatic and phenol profile is any case required for quality wines; grapes harvested at a total soluble solid concentration suitable to produce low alcohol wines may not have enough phenols, especially in terms of anthocyans, also when they have been obtained by applying proper viticultural methods. It is easy to suppose that the best results could derive from optimizing the application of several growing techniques at the same time.

The experimental results available at the moment show often not univocal indications. This is a consequence of the fact that each viticultural thecnique may be applied at a different extent, and that the interaction between grape variety, rootsock and environment is very complex. Moreover, it is to consider that most of the presented experimental results derive from research on vineyard management not specifically devoted to the obtaining of grapes for low alcohol wines. To clarify many of the actual discrepancies in the results more specific studies on this argument should be done.

6. References

Balda P., Martinez De Toda F., 2011. Delaying berry ripening process through leaf are to fruit ratio *decrease. Proceedings of "17th International Symposium of the Giesco"*, August 29th-2nd September 2011, Asti-Alba, Italy, 579-582.

Balda P., Martínez de Toda F., 2013. Decreasing the alcohol level and pH in wines by the "double harvest" technique. Ciência e Técnica Vitivinícola, Volume 28, *Proceedings 18th International Symposium GiESCO*, Porto, 7-11 July 2013, 899-903.

Böttcher C., Harvey K., Forde C.G., Boss P.K., Davies C., 2011. Auxin treatment of pre-veraison grape (*Vitis vinifera* L.) berries both delays ripening and increases the synchronicity of sugar accumulation. *Australian Journal of Grape and Wine Research*, **17**, 1–8.

Chorti E., Guidoni S., Ferrandino A., Novello V., 2010. Effect of Different Cluster Sunlight Exposure Levels on Ripening and Anthocyanin Accumulation in Nebbiolo Grapes. *Am. J. Enol. Vitic.*, **61**(1), 23-30.

Clingeleffer P.R., 2007. Viticultural practices to moderate wine alcohol content. *Proceedings ASVO Seminar: Towards best practice through innovation in winery processing*. Tanunda (SA), Australia, 17 October.

Cooley N.M., Clingeleffer P.R., Walker, R.R., 2005. The balance of berry sugar accumulation, colour and phenolic concentration under deficit irrigation strategies. *Proceedings of the Twelfth Australian Wine Industry Technical Conference*, Melbourne (Vic.) Australia, 2004, pp. 94–96.

Davies C., Boss P.K., Robinson S.P., 1997. Treatment of grape berries, a nonclimacteric fruit with a synthetic auxin, retards ripening and alters the expression of developmentally regulated genes. *Plant Physiology*, **115**, 155–1161.

de Palma L., Tarricone L., Muci G., Limosani P., Savino M., Novello V., 2011. Leaf removal, vine physiology and wine quality in cv. Negroamaro (*Vitis vinifera* L.). *Progrès Agricole et Viticole, 17th GiESCO Meeting*, Asti - Alba (CN), Italy, 29th August - 2nd September 2011, 231-234.

Díaz-Losada E., Trigo-Córdoba E., Soto-Vázquez E., Bouzas-Cid Y., Mirás-Avalos J.M., Rego-Martínez F., 2013. Effects of training system on the agronomical and enological performance of Galician grapevine cultivars. Ciência e Técnica Vitivinícola, Volume 28, *Proceedings 18th International Symposium GiESCO*, Porto, 7-11 July 2013, 578-582.

Dokoozlian N. K., Hirschfelt D. J., 1995. The influence of cluster thinning at various stages of fruit development on flame seedless table grapes. *Am. J. Enol. Vitic.*, **46**, 429-436.

Filippetti I., Allegro G., Movahed N., Pastore C., Valentini G., Intrieri C., 2011. Effects of late-season source limitation induced by trimming and antitraspirants canopy spray on grape composition during ripening in *Vitis vinifera* cv Sangiovese. *Proceedings 17th International Symposium GiESCO*, Asti - Alba (CN), Italy, 29th August - 2nd September 2011, 259-262.

Fernández O., Sánchez S., Rodríguez L., Lissarrague J.R., 2013. Effects of different irrigation strategies on berry and wine composition on Cabernet sauvignon grapevines grown in Madrid (Spain). Ciência e Técnica Vitivinícola, Volume 28, *Proceedings 18th International Symposium GiESCO*, Porto, 7-11 July 2013, 112-117.

Fischer U., Noble A.C., 1994. The effect of ethanol, catechin concentration, and pH on sourness and

bitterness of wine. *American Journal of Enology and Viticulture*, **45**, 6–10.

Fournand D., Vicens A., Sidhoum L., Souquet J.M., Moutounet M., Cheynier V., 2006. Accumulation and extractability of grape skin tannins and anthocyanins at different advanced physiological stages. *J. Agric. Food Chem.*, **54**, 7331-7338.

Freire L., Canosa P., Rodríguez-Vega I., Vilanova M., 2013. Effect of early leaf removal on yield components, must composition and wine volatiles of *Loureira* cv from NW Spain. Ciência e Técnica Vitivinícola, Vol. 28, *Proceedings 18th International Symposium GiESCO*, Porto, 7-11 July 2013, 422-425.

Guidoni S., Ferrandino A., Novello V., 2008. Effects of Seasonal and Agronomical Practices on Skin Anthocyanin Profile of Nebbiolo Grapes. *Am. J. Enol. Vitic*., **59** (1), 22-29.

Han D.H., Lee C.H., 2004. The Effects of GA3, CPPU and ABA Applications on the Quality of Kyoho (*Vitis vinifera* L. x *V. labrusca* L.). *Grape. Acta Hort*., 653, 193-197.

Kliewer W.M., Dokoozlian N.K., 2005. Leaf area/crop weight ratios of grapevines: influence on fruit composition and wine quality. *Am. J. Enol. Vitic*., 56, 170-181.

Kontoudakis N., Esteruelas M., Fort F., Canals J.M., Zamora F., 2011. Use of unripe grapes harvested during cluster thinning as a method for reducing alcohol content and pH of wine. *Aust. J. Grape Wine Res.*, **17**, 230–238.

Lanari V., Lattanti T., Borghesi L., Silvestroni O., Paliotti A., 2013. Post-Veraison Mechanical Leaf Removal Delays Berry Ripening on 'Sangiovese' and 'Montepulciano' Grapevines. *Acta Hort.*, **978**, 327-333.

Martinez de Toda F., Balda P., 2011. Decreasing the alcohol level in quality red wines by the "double harvest" technique. *Progrès Agricole et Viticole, Proceedings 17th International Symposium GiESCO*, Asti - Alba (CN), Italy, 29th August - 2nd September 2011, 463-466.

Ó-Marques J., Reguinga R., Laureano O., Ricardo da Silva J.N., 2005. Changes in grape seed, skins and pulp condensed tannins during berry ripening: effect of fruit pruning. *Ciência Tècnica Vitivinicola*, **20**, 35-52.

Ollat N., Gaudillère J.P., 1998. The Effect of limiting leaf area during stage I of berry growth on development and composition of berries of *Vitis vinifera* L. cv. Cabernet Sauvignon. *Am. J. Enol. Vitic.*, **49**, 251-258.

Palliotti A., Poni S., Petoumenou D., Vignaroli S., 2008. Limitazione modulata della capacità fotoassimilativa delle foglie mediante antitraspiranti ed effetti su qualità e composizione dell'uva. *Atti: "II Convegno Nazionale di Viticoltura"*, Marsala 14-19 Luglio 2008. Italus Hortus 17 (suppl. n. 3), 21-26, 2010.

Palliotti A., Poni S., Berrios J. G., Bernizzoni F., 2010. Vine performance and grape composition as affected by early-season source limitation induced with anti-transpirants in two red *Vitis Vinifera* L. cultivars. *Austr. J. Grape Wine Res*., **16**, 426-433.

Palliotti A., Silvestroni O., Leoni F., Poni S., 2012. Maturazione dell'uva e gestione della chioma in *Vitis vinifera*: processi e tecniche da riconsiderare in funzione del cambiamento del clima e delle nuove esigenze di mercato. *Italus Hortus*, **19** (2), 1-15.

Palliotti A., Silvestroni O., Leoni F., Cini R., Poni S., 2013. Effect of late mechanized leaf removal to delay grape ripening on Sangiovese vines. *Acta Hort.*, 978, 301-307.

Salamon A., 2006. Techniques to achieve moderate alcohol levels in South African wine. Assignment submitted in partial requirement for the Cape Wine Master Diploma. capewineacademy.co.za.

Schultz H.R., Jones G.V., 2010. Climate Induced Historic and Future Changes in Viticulture. *Journal of Wine Research*, **21** (2–3), 137-145.

Stoll M., Lafontaine M., Schultz H.R., 2010. Possibilities to reduce the velocity of berry maturation through various leaf area to fruit ratio modifications in *Vitis vinifera* L. Riesling. *Progrès Agricole et Viticole*, **127**(3), 68-71.

Sun Q., Sacks G.L., Lerch, S.D., Vanden Heuvel J.E., 2011. Impact of shoot thinning and harvest date on yield components, fruit composition, and wine quality of Marechal Foch. *Am. J. Enol. Vitic.*, **62** (1), 32-41.

Sun Q., Sacks G.L., Lerch S.D., Vanden Heuvel J.E., 2012. Impact of shoot and cluster thinning on yield, fruit composition, and wine quality of Corot noir. *Am. J. Enol. Vitic.*, **63** (1), 49-66.

Tittmann S., Stöber V., Bischoff-Schaefer M., Stoll M., 2013. Application of anti-transpirant under greenhouse conditions of grapevines (*Vitis vinifera* cv. Riesling and cv. Müller-Thurgau) reduce photosynthesis. Ciência e Técnica Vitivinícola, Volume 28, *Proceedings 18th International Symposium GiESCO*, Porto, 7-11 July 2013, 276-282.

Williams L., 2012. Potential vineyard evapotranspiration (ET) due to global warming: comparison of Vineyard et at three locations in California differing in mean seasonal temperatures. *Acta Hort*., **931**, 221-236.

Zecca O., Valentini S., Domeneghetti D., 2013. Comparison of six Gamay noir clones in a high altitude environment. Ciência e Técnica Vitivinícola, Volume 28, *Proceedings 18th International Symposium GiESCO*, Porto, 7-11 July 2013, 800-804.

Vineyards adaptation and varieties: The effect of varieties, clones and rootstocks on must sugar content

Gabor Jakab[1,2,*], Anna Csikasz-Krizsics[2], Beatrix Hartman[2], Janos Werner[2] and Pal Kozma[2]

[1]Deparment of Plant Physiology, Faculty of Sciences, University of Pecs, Ifjusag u. 6, H-7624-Pecs, Hungary
[2]Research Institute for Viticulture and Oenology, University of Pecs, Pazmany P. u. 4, H-7634-Pecs, Hungary

Abstract: Production site can primarily determine the choice of a grapevine variety since variety needs to be adapted to the climate of the geographic region. During centuries, grape cultivars were selected by the growers in order to adapt them to their wants. This way a huge genetic variation of grape cultivars were selected and maintained in the wine-growing regions. This variation can serve as a basis of further selection exploiting the genetic capacity of the different autochthonous cultivars under rapidly changing environmental conditions to fulfill producer expectations. Unfortunately, climate change can further encumber the prediction of the productivity of the different grape varieties in a certain region. In this context, reevaluation of local varieties, selection of new clones may help to determine genetic backgrounds ensuring low sugar content but high flavor quality of the must. Rootstock combinations had further effect on these parameters, so investigation of rootstock-scion interactions may also help us to react to the demands of customers for lowering wine alcohol content.

Keywords: autochthonous cultivar, clonal selection, must sugar content, genetic variation, affinity selection of rootstock

1. Introduction

In an attempt to meet the demands of customers wine companies around the world are looking for strategies lowering wine alcohol content. The alcohol in the wine is the product of the fermentation process of the sugars in the must during wine making. Therefore the alcohol content of the wine in general is proportional with the starting sugar content of the must. The obvious option to lowering the must sugar content by harvesting grape earlier when its sugars are not excessive is often not an option since unripe grape may have unenhanced flavors.

There is a close relationship between the balance of Photosynthetic Active Radiation (PAR) and total dry matter as well as qualitative characteristics such as sugar concentration, must and wine quality (Carbonneau, 1980; Schultz, 1995). Basic relationship between PAR and leaf photosynthesis is well known in grapevine (Schultz *et al*., 1996; Medrano *et al*., 2002; Flexas *et al*., 2008), and intensively studied in case of different varieties indicating the importance of genetic background. Environmental factors such as temperature, altitude, soil type, water, nutritional status, pathogenesis further modulate vineyard production. Climate change can further encumber the prediction of the productivity of the different grape varieties in a certain region. In grapevine as a C3 plant photosynthesis is CO_2 limited and therefore any increase in atmospheric CO_2 level may increase assimilation, unless plants experience other limitations such as water scarcity. Due to global climate change, high solar irradiation, temperature, and drought are becoming more widespread. The capacity of a grapevine plant to express adaptive responses depends on multiple signal transduction pathways selected during previous breeding demands. The emerging picture today is that the exploitation of the genetic capacity of the different autochthonous cultivars is important in both economical and agronomical aspect under rapidly changing environmental conditions. The autochthonous cultivars of grapevine represent a historical and national heritage and their genetic diversity ensuring good adaptation to local environmental conditions could contribute to a unique adaptation response and must composition. This way global warming and an increasing demand of low alcohol wine seem to be in conflict. There-

Corresponding author : jakab@gamma.ttk.pte.hu

fore, there is need of more data regarding the characterization of the local varieties under the different growing and stress conditions. In the frame of our analysis we could study the effect of temperature and water stress on different cultivars in the same research field in three consecutive years (2010, 2011, 2012).

2. Field conditions and meteorological characterization of the experimental years

A valuable collection of grapevine cultivars (a matter of 1500) is available at the Research Institute of Viticulture and Oenology (RIVO, University of Pecs). The vines grow on the south-facing slopes and terraces of the Mecsek Hills (latitude: 46º07' N, longitude: 18º17' E, 200-240 m above sea level) under non-irrigated field-conditions.

Vintage data, must and wine analysis results were collected each year according to varieties, clones and rootstocks, as well as field conditions and exposition.

2010 was an extremely wet year with 50% more precipitation, while in 2011 the precipitation was with 30% less and in 2012 with 40% less than the average of the last 50 years. The average temperature during the growing season showed also big variations among the years: in 2010 it was 17.4°C, in 2011 it was 19.1°C and in 2012 it was as high as 20.3°C. So in summary we can state that 2010 was a cool, wet year, 2011 a good average year, while 2012 was a warm and dry year. As a consequence the harvest in 2010 was delayed and the must had lower sugar content, the vintage of 2011 gave a high quality must while 2012 resulted increased sugar content and reduced quantity of the must.

This situation allowed us to compare the behavior of grapevine under extreme weather conditions presumptive during the next years due to climate change.

3. Results and discussion

3.1. Comparison of varieties side by side

The choice of a variety of vine is influenced by primarily by the site since variety needs to be adapted to the climate of the region. During centuries, growers have empirically selected grapes in order to adapt the cultivars to local conditions and needs. This way a lot of grape cultivars were selected and maintained in the traditional wine-growing regions of the old world. Unfortunately, during the last fifty years the number of varieties in the world wine production was significantly reduced preferring the so-called noble cultivars, e.g. Cabernet-Sauvignon, Merlot, Pinot noir, Chardonnay, Sauvignon blanc. The autochthonous cultivars became effaced in several regions.

During the years of its existence our Institute created a collection of different grape species, varieties, cultivars, hybrids and clones with more than 1500 items. These plants grow side-by-side on our experimental field under similar geographical and climatic conditions. The yearly collected vintage data allow us to compare their biological potential determined by divergent genotypes.

As an example the sugar contain of the musts of red grape varieties from the three consecutive years of 2010-2012 is shown in Figure 1.

The effect of the vintage is obvious showing an elevated sugar content in the warmer years. However, the impact of the rising temperature on the sugar content was much lower in the case of local cultivars (Kadarka, Portugieser, Blaufränkisch) compared to the world varieties Cabernet Franc, Cabernet Sauvignon and Merlot.

3.2. Clonal selection as a tool to reduce must sugar content

Clonal selection is one of the most ancient plant breeding methods (Sartorius, 1928). Mainly due to bud mutations, genetic variability

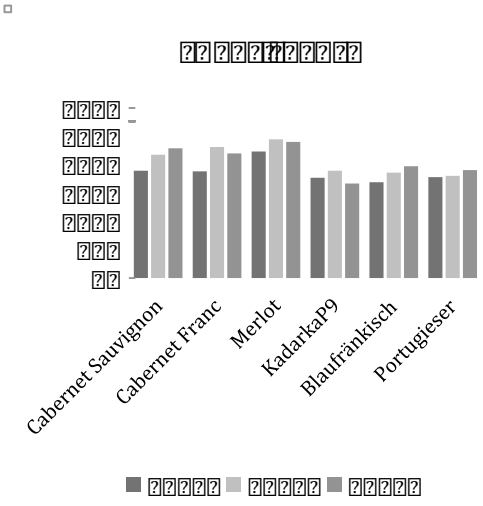


Figure 1. Sugar content of the musts of different varieties at harvest in vintage 2010, 2011 and 2012.

can be accumulated in the vegetatively propagated plants determining favorable and unfavorable attributes (Mullins *et al*., 1992). Although the ancient grapevine varieties (for example 'Pinot noir') are rather heterogeneous, the genetic analogy of their propagated clones stands between 95-99 % (Bessis, 2007; Wegscheider *et al*., 2009). The maintenance of the divergent varieties and valuable mutations of the grapevine varieties may be assured by the selection and preservation of clones (Boursiquot, 1999).

The new market and environmental challenges in the field of wine production necessitate the preservation and improvement of the biological basis. As an example here we present the case of clonal selection of Kadarka, a popular red grape variety in Hungary originated from the Balkans. Kadarka is a grapevine variety being fertile, resistant to drought, having frost sensitivity and susceptibility to rot and shriveling. The sugar degree of the must and the color depth of Kadarka vary in accordance with its vintage. White, rosé, "siller", red and "aszu" wine can be produced from its yield, but it can be utilized as a table grape. Its wine is characteristic, mildly aromatic, elegant with a fresh acid content (Kozma, 1963; Németh, 1967), representing an internal part in one of the most famous wine brand of Hungary, Bikavér (Eperjesi *et al*., 1998).

In 2001 the Institute of Viticulture and Enology Pecs analyzed an old stock of Kadarka having high variability in phenotype. This old Kadarka stock (planted in 1898) was suitable for finding entities appropriate for the selection. Clones of great biological value were selected from it, with which the quality and yield security of Kadarka can be increased. Regarding to the 56 elite stocks, a considerable difference could be observed in the morphological characteristic of the yield and even in the performance of the vine-stocks. On the basis of the accomplished statistic analysis, including the comparison of each clones and vintages with each other, it is shown that the values of the measured parameters were influenced by the variables collectively and significantly, which fact even justifies the diversity of the clones and of the vintages. The analysis of the 16 most valuable elite stocks was continued in the second step of the selection in the medium-parceled experiment.

The sugar content of the must of the selected clones of 2010 is compared to the P. 9 control

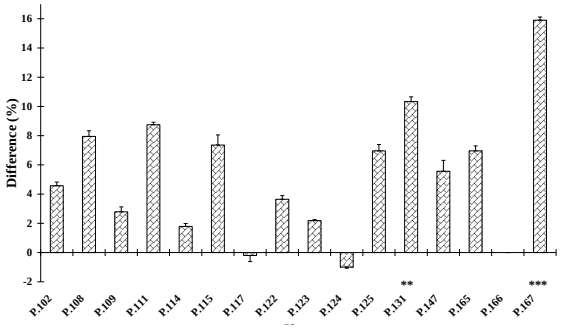


Figure 2. The divergences in sugar content of the must of selected 'Kadarka' clones from the P. 9 cloin in vintage 2010. significance level: **p≤0,01; ***p≤0,001

clone, and it is given in percentile divergence (Figure 2.). It can be observed that most of the clones selected ripe with higher sugar content of the must in an unfavorable vintage.

Harvest data of 2011 compared to the P. 9 showed a significant divergence in the quantity of yield, in the average bunch weight and in the sugar content of the must. The P. 114 clone had a lower value and P. 108, P. 117, P. 125 and P. 166 clones had a higher value in the average bunch weight. The divergences in the sugar content of the musts of the selected clones were statistically lower in case of three selected clones (P. 109, P. 166, P. 167), the remaining clones, similarly to the P. 9 clone, showed a higher value (Figure 3).

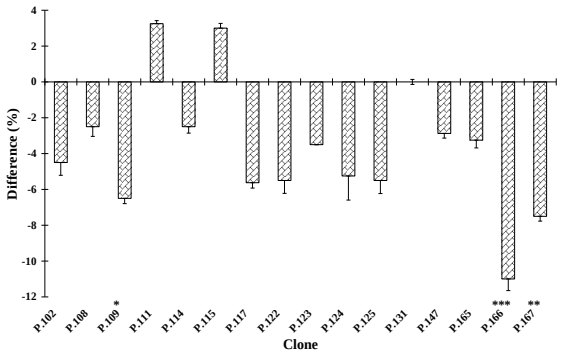


Figure 3. The divergences in sugar content of the must of selected 'Kadarka' clones from the P. 9 cloin in vintage 2011. significance level: **p≤0,01; ***p≤0,001

3.3. Interaction between rootstocks and scions influencing must sugar content

It is well accepted that a rootstock can affect foliar water and nutrient level, but the complex interactions of rootstock-scion combinations

and climate are less investigated (Csikasz-Krizsics and Diofasi, 2007). For studying root-stock-scion affinity interactions, a plantation was established in 1999 on our experimental field with several varieties on different root-stocks.

During the years 2010-1012 cluster crops were measured by repetition and calculated per plant. After the processing of harvested grapes the quality parameters (cluster weight, titratable acid, sugar, pH) of each must sample were immediately determined.

Here we present the results of two varieties (Chardonnay and Sauvignon blanc) on six different rootstocks (125AA, Fercal, Richter 110, Ruggeri 140, Teleki 5BB and 5C). We could see a strong effect of the year on the sugar content of the musts having the highest sugar level in vintage 2012 in both varieties (Figure 4).

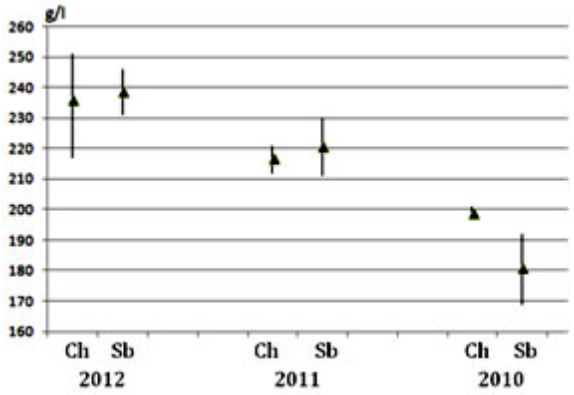


Figure 4. Average sugar content (g/L) of the musts of Chardonnay (Ch) and Sauvignon blanc (Sb) on different rootstocks at harvest in vintage 2010, 2011 and 2012.

In the case of Chardonnay the effect of the rootstocks on must sugar content was clean-cut in the year 2012, while in the case of Sauvignon blanc there was less variation (Figure 5). In both 2011 and 2012 the lowest sugar content was observed on Teleki 5C, while on the Richter 110 the productivity was the highest.

4. Conclusion

Wine production faces with new market demands and rapidly changing environmental conditions. Therefore it requires the improvement of the biological basis of grapevine. According to the presented examples exploration of the genetic capacity of different autochthonous cultivars, clonal selection, as well as the exploitation of rootstock-scion interactions may help answering to the new challenges.

Acknowledgement : This work was supported by the NKFP 4/036 project of the Szechenyi Plans, the NKFP4-00017/2005 project of Jedlik Anyos, and the Hungarian Scientific Research Foundation (OTKA K101430). We would like to thank the work of the technical staff of the RIVO, who provided an essential assistance in the recording, systematisation of the data and in the production of wine samples.

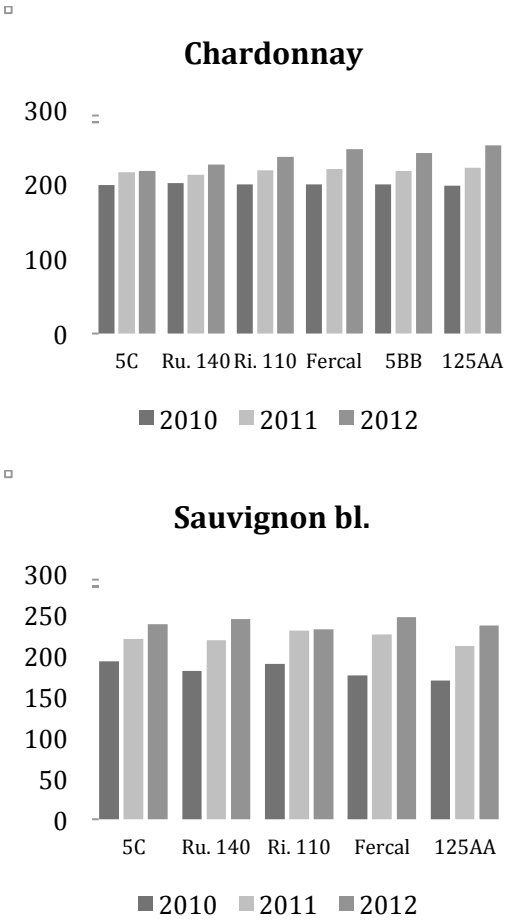


Figure 5. Sugar content (g/L) of the musts of Chardonnay and Savignon blanc on different rootstocks (125AA, Fercal, Richter 110 (Ri.110), Ruggeri 140 (Ru140), Teleki 5BB and 5C) at harvest in vintage 2010, 2011 and 2012.

5. References

Bessis R., 2007. Evolution of the grapevine (*Vitis vinifera* L.) imprinted by natural and human factors. *Canadian Journal of Botany*, **85** (8), *679-690.*

Boursiquot J-M. and This P. (1999): Essai de définition du cépage. *Le Progrès Agricole et Viticole. 116 (17): 359-361.*

Carbonneau A. (1980). Recherche sur les systèmes de conduite de la vigne : essai de maîtrise du microclimat et de la plante entière pour produire économiquement du raisin de qualité. Thèse Université Bordeaux 2, Lavoisier, Payot Ed., 240 p.

Csikasz-Krizscs A and Diofasi L (2007): Investigations into the correlation between boron concentration and yield characteristics with the grape cultivar Cabernet-

Sauvignon on different rootstocks. Mitteilungen Klosterneuburg, **57**, 213-223.

Eperjesi, I., Kállay, M. & Magyar, I. (1998): Borászat. *Mezőgazda Kiadó. Budapest.*

Flexas, J., Ribas-Carbo, M., Diaz-Espejo, A., Galmes, J., Medrano, H. (2008). Mesophyll conductance to CO_2: current knowledge and future prospects. *Plant Cell Environ*. 31: 602-621.

Kozma P. (1963). A szőlő termékenységének és szelektálásának virágbiológiai alapjai. *Akadémiai Kiadó. Budapest.*

Medrano H., Escalona J.M., Bota J., Gulías J., Flexas J. (2002). Regulation of photosynthesis of C_3 plants in response to progressive drought: Stomatal conductance as a reference parameter. *Ann. Bot.,* **89**, 895-905.

Németh M. (1967). A klónszelekciós szőlőnemesítés módszere Pécsett és az elért eredmények. *Szőlő és gyümölcstermesztés III. Budapest. 41-86.*

Schultz HR (1995). Leaf absorptance of visible radiation in *Vitis vinifera* L. estimates of age and shade effects with a simple field method. *Scientia Horticulturae*, **66**, 93–102.

Schultz, H.R., Kiefer, W., Gruppe, W. (1996): Photosynthetic duration, carboxylation efficiency and stomatal limitation of sun and shade leaves of different ages in field-grown grapevine (*Vitis vinifera* L.). *Vitis*, 35: 169-176.

Wegscheider, E., Benjak, A. & Forneck, A. (2009): Clonal variation in Pinot noir revealed by S-SAP involving universal retrotransposon-based sequences. American *Journal of Enology and Viticulture*, **60**, 1, 104-109.

Vines accumulating less sugars

Louis Bordenave[1*], ZhanWu Dai[2], Alexandra Lusson[3], Nathalie Ollat[2] and Serge Delrot[3*]

[1]ISVV, EGFV, UMR 1287, INRA, 33140 Villenave d'Ornon, France (bordenave@bordeaux.inra.fr)
[2]ISVV, EGFV, UMR 1287, INRA, 33140 Villenave d'Ornon, France (zhanwu.dai@bordeaux.inra.fr)
[3] Chambre d'Agriculture de Gironde, 39 rue Michel Montaigne, 33294 Blanquefort cedex, France (a.lusson@gironde.chambagri.fr)
[4] ISVV, EGFV, UMR 1287, University of Bordeaux, 33140 Villenave d'Ornon, France (serge.delrot@u-bordeaux2.fr)

Abstract: During the past decades, viticultural practices and climate change have resulted in an increase of sugar berry content at harvest**.** This results in wines which may have a too high alcohol potential. Although dealcoholization techniques exist and selected yeast strains may produce a lower alcohol content in the wine, it is less time-consuming and expensive to try to reduce the sugar content directly at the berry level. The best strategy is therefore to combine adequate vineyard management with the varietal and clonal genetic diversity of grapevine in order to decrease berry sugar content while maintaining the other properties required for making premium wines. This requires a good knowledge of the physiological and biochemical processes participating in the control of berry sugar content, and the design of modelling strategies and molecular markers. This short paper illustrates some of these aspects.

Keywords: Berry, sugar content, climate change, genetic diversity

1. Introduction

Grape quality is a complex trait that mainly refers to berry chemical composition, including sugars, acids, phenolics and other aroma compounds (Conde *et al.*, 2007). Among others, berry sugars play a crucial role in shaping wine quality, because they largely determine the alcohol content of the wine and provide precursors for other compounds (e.g. anthocyanins) and regulate their synthesis (Vitrac *et al.*, 2000; Agasse *et al.*, 2009). The ongoing climate change is affecting the physiology of grapevine and ultimately wine quality and typicity (Schultz, 2000). For example, a survey on Alsace wine showed that the wine potential alcohol level increased linearly from 9% to 12% during the period from 1970 to 2005 (Duchêne and Schneider, 2005). A too high sugar content leads to an excess level of alcohol, which may be detrimental for the health of consumers and raises the concern of policy makers. Partial dealcoholization after vinification is possible, but its cost is significant, and it may alter the organoleptic properties of the wine. Selected yeasts producing lower amounts of alcohol from a given amount of sugar may also be used. However, the simplest way to avoid excess alcohol production during vinification is to start from berries containing the right amount of sugars. This can be reached by combining adequate vineyard management with grape genotypes that naturally accumulate less sugar. The present contribution focuses on the last point. It summarizes our present understanding of the physiological mechanisms controlling sugar accumulation by grape berries, and some attempts to exploit the varietal and clonal diversity related to this trait.

2. Physiology of sugar accumulation

The ripening grape berry is a strong sink for dry matter transported from current photosynthesis and wood reserves (Coombe, 1989). Sugar composition and concentration change along grape development and can be affected by many factors, including genotype, environment and viticulture management (e.g. defoliation or fruit thinning which is extensively used in practice to modulate source-sink relationship) (Kliewer, 1966; Coombe, 1992; Kliewer and Dokoozlian, 2005). From veraison on, berry starts to accumulate equal amounts of glucose and fructose in mesocarp cell vacuoles, with very low level of sucrose in *Vitis vinifera* cultivars, to which most wine grape cultivars belong (Robinson and Davies, 2000; Shiraishi *et al*., 2010). The sugars

Corresponding author : serge.delrot@u-bordeaux2.fr

accumulated in the vacuoles of mesocarp cells, account for 65 to 91 % of the fresh weight in a mature berry.

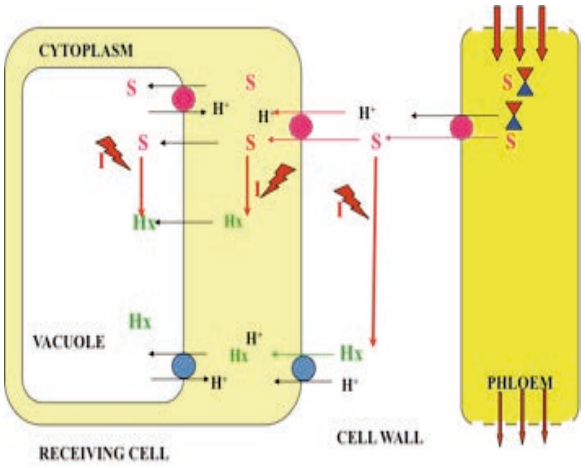


Figure 1. Mechanism of sugar accumulation by grape mesocarp vacuoles. After veraison, sucrose imported by the phloem in the berry is unloaded in the apoplast and retrieved by a series of sucrose transporters (red circles), and hexose transporters (blue circles) localized either on the plasma membrane or vacuolar membrane of the flesh cells. At some stage, the disaccharide sucrose is split into its constitutive monosaccharides (glucose and fructose) by invertases (broken arrows) that may be localized in the cell wall, cytoplasm or vacuole.

Sucrose is imported from phloem into berries through a symplasmic unloading pathway before veraison and through an apoplasmic pathway after veraison (Figure 1) (Zhang *et al*., 2006). Since the sugars accumulated in the vacuoles of flesh cells are glucose and fructose, the accumulation of these hexoses in the berries involves the activity of sucrose metabolizing enzymes, sucrose transporters, and monosaccharide transporters (Agasse *et al*., 2009). Thus, genotypic difference in sugar composition and concentration may, in theory, result from variations in biochemical properties of metabolism enzymes and/or transporters that regulate sugar storage and depletion.

In the berry, imported sucrose is hydrolyzed into glucose and fructose, which are then accumulated in the vacuoles or depleted by respiration or synthesis of other metabolites. Invertases hydrolyze sucrose into glucose and fructose and has an important role in regulating sugar composition and concentration in fruits (Takayanagi and Yokotsuka, 1997). According to their subcellular localizations, invertases are categorized into cell-wall bounded- (cwINV), cytoplasmic- (cINV), and vacuolar invertase (vINV). In grape, the protein level and gene expression of a cell wall invertase is induced just prior the onset of veraison (Zhang *et al.*, 2006; Hayes *et al.*, 2007) and the activity and transcripts levels of two vacuolar invertases *VvGIN1* and *VvGIN2* are detected in developing berries with a peak at veraison in Shiraz berries and gradually decrease thereafter (Davies and Robinson, 1996). The natural reduction of vINV activity in the “Steuben” grapevine hybrid berries leads to a decrease in vacuolar hexose accumulation and to an increase in sucrose storage (Takayanagi and Yokotsuka, 1997). However, cwINV enzyme activity in berry represents only 6% of total INV activity (Ruffner et al., 1995) and microarray results showed a constant expression level of both *cwINV* and cINV throughout berry development (Deluc et al., 2007). These results suggest that invertase alone cannot be responsible for all the increase of hexose concentration in the ripening berry. Instead, sugar metabolism during fruit development is most likely coordinately regulated together with other enzymes. In grapevine, at least 6 SuSy (sucrose synthase) genes have been reported, with relative stable activities and expression during development, suggesting limited role in regulating hexose accumulation by sucrose cleavage in mesocarp cells (Zhang *et al.*, 2006). Moreover, sucrose in cytoplasm can be synthesized by sucrose phosphate synthase (SPS), and one SPS gene is preferentially expressed in the grape pericarp, indicating a probable participation in the re-synthesis of sucrose (Grimplet *et al.*, 2007). The simultaneous synthesis and degradation of sucrose are generally termed “futile cycle”. It implicates the re-synthesis of sucrose from the hexoses present in the cytoplasm, and consequently may modify the unloading and storage of sugars into the ripening fruits (Nguyen-Quoc and Foyer, 2001; Agasse *et al.*, 2009). However, this process is rarely studied in grape because the turnover rates of metabolites in the futile cycle cannot be quantified from steady-state metabolite levels but need carbon-labeling approach (Alonso *et al.*, 2005). Interestingly, a recent comparison between Cabernet-Sauvignon and a *V. quinquangularis* Rehd wild grape (Shang-24) showed that Shang-24 had the same level of SuSy and SPS activities, higher vINV activity but lower hexose concentration. This led to the hypothesis that the accumulated hexose may be more shifted towards other metabolite pathways (eg. flavonoid pathway) (Pan *et al.*, 2009).

In the grape genome, sucrose transporter genes constitute a small multigenic family of 4 members in this species (Agasse *et al.*, 2009; Afoufa-Bastien *et al.*, 2010). Three sucrose transporters

cDNAs have been cloned from Shiraz and Cabernet-Sauvignon berries (*VvSUC11*; AF021808, also identified as *VvSUT1* AF182445; *VvSUC12* AF021809; *VvSUC27* AF021810) and characterised as proton-dependent sucrose transporters by heterologous expression in *Saccharomyces cerevisiae*. VvSUC11 and VvSUC12 are intermediate affinity sucrose transporters (K_m, 0.9 mM and 1.4 mM, respectively) (Ageorges *et al.*, 2000; Manning *et al.*, 2001), and VvSUC27 is a low affinity sucrose transporter (K_m of 8-10 mM; Zhang *et al.*, 2008). VvSUT2, whose sequence is close to VvSUC27 is weakly expressed in the berries (Afoufa-Bastien *et al*., 2010).

The sucrose transporters expressed in the berries must fulfill two opposite functions: maintain sucrose into the conducting bundles until it reaches the site of unloading, and at this site, they must allow its leakage or mediate efflux. Heterologous expression in yeast indicates that these three sucrose transporters act as proton-sucrose symporters, and are thus expected to mediate sucrose uptake (in the phloem, or in the mesocarp cells). *VvSUC11* and *VvSUC12* transcripts concomitantly increase with post-véraison sugar accumulation, contrarily to *VvSUC27* transcripts whose amounts significantly decrease at this stage (Davies *et al*., 1999). VvSUC11 is the closest homologue to AtSUC4 (68 %), a vacuolar sucrose transporter characterized in *A. thaliana*, but it has a higher affinity for sucrose (K_m = 0.88 mM) than AtSUC4. Its subcellular localization is unknown. VvSUC12 is homologous to AtSUC3, a low affinity sucrose transporter that has been mistakenly considered as a sucrose sensor in *A. thaliana*.

Fifty-nine putative hexose transporter homologues have been identified in the grape genome based on protein motif recognition (Samson *et al.* 2004 ; Jaillon *et al.*, 2007 ; Agasse *et al*., 2009 ; Afoufa-Bastien *et al*., 2010). Six full length cDNAs encoding for MST and named *VvHT1* to *6* (*V. vinifera* Hexose transporter, *VvHT1* AJ001061, *VvHT2* AY663846, *VvHT3* AY538259 and AY854146, *VvHT4* AY538260, *VvHT5* AY538261, *VvHT6* AY861386, DQ017393) have been cloned from Pinot noir, Ugni blanc, Chardonnay, Cabernet sauvignon and Syrah (Fillion *et al.*, 1999; Vignault *et al*., 2005; Hayes *et al*., 2007). In spite of the high number of putative monosaccharide genes in the grape genome, no other VvHT was identified in grape berries, suggesting that the most important transporters for sugar accumulation in this organ have already been cloned.

VvHT1, *VvHT2* and particularly *VvHT3* are highly expressed compared to the other *VvHT*s, at all stages of berry development. Except VvHT6, all VvHTs isolated so far from grape berries present high homologies with AtSTPs, *i.e.* functional hexose transporters. VvHT1, the first transporter of this family identified in grape (Fillion *et al*., 1999), and functionally characterized (Vignault *et al*., 2005) is homologous to AtSTP1.

The plasma membrane localization of VvHT1, VvHT4 and VvHT5 has been demonstrated by immunofluorescence, immunolabelling and GFP fusion proteins (Vignault *et al.*, 2005; Hayes *et al.*, 2007).

Functional studies showed that *VvHT1*, *VvHT4* and *VvHT5* code high affinity, H^+-dependent glucose transporters. VvHT1 has a higher affinity for glucose (K_m of 70 µM) than VvHT4 and VvHT5 (K_m about 150 µM and 100 µM respectively) and displays broad substrate specificity. Both *VvHT1* transcripts and protein levels (Conde *et al.*, 2006) are much higher at pre-véraison stages, indicating that it is not directly responsible for the post-véraison sugar accumulation (Vignault *et al.*, 2005; Conde *et al.*, 2006). *VvHT1* transcripts are abundant in the phloem region of the conducting bundles of the leaf, petiole and berry (Vignault *et al.*, 2005). This localization, and its high affinity for hexoses suggest that it could be involved in the retrieval of minor amounts of hexoses leaking from the conducting complex.

While VvHT4 transport activity may be restricted to glucose, VvHT5 is able to transport both glucose and fructose (Hayes *et al.*, 2007). *VvHT5* transcripts are relatively more abundant at late ripening, but their amount remains weak. *VvHT3*, whose transcripts levels are reduced at véraison but high at both green and ripening stages (Hayes *et al.*, 2007), is homologous to *AtSTP7*, which has not been functionnally characterized. VvHT3 is not able to transport any of the tested radiolabelled sugars in the deficient yeast model (Vignault *et al.*, 2005; Hayes *et al*., 2007), and its transport function is also unknown. A gene (re)named *VvHT8*, which has a high similarity to *VvHT1* (99.4 %) was identified as a target of grape selection that has led to high sugar contents in domesticated grapes (Xin *et al*., 2013).

VvHT2 and VvHT6 seem localized in the tonoplast (Vignault *et al.*, unpublished), and VvHT6 has high sequence similarity with the previously described tonoplast transporter of *Arabidopsis thaliana* AtTMT2 (Afoufa-Bastien *et al*., 2010). The TMT (Tonoplast Monosaccharide Transporter)

subfamily of MFS transporters contains tonoplast hexose-proton antiporters. This family is represented by three members in *V. vinifera* (Afoufa-Bastien *et al*., 2010). VvTMT1 has been cloned, localized and characterized as a functional monosaccharide transporter localized in the tonoplast of grape cells (Zeng *et al*., 2011). Its expression decreases strongly along berry development. Unlike *VvTMT1*, *VvHT6/TMT2* transcripts are highly accumulated at véraison (Terrier *et al.,* 2005; Deluc *et al.,* 2007), suggesting that this transporter may be responsible for vacuolar accumulation of hexose at the inception of ripening. *VvHT2*, whose expression is mainly associated with véraison is homologous to AtSTP5, but its transport activity has not yet been characterized.

The expression and activity of sugar transporters in the grape berry is controlled by sugars and abscisic acid (ABA). The promoter region (2500 pb upstream of the site for initiation of transcription) of *VvHT1* contains several sugar responsive elements (Fillion *et al*., 1999) which drive the expression of this transporter when either glucose, sucrose are present at relatively low concentrations (60 mM), whereas fructose is inactive in this regard (Atanassova *et al*., 2003). The proximal 160 pb region of *VvHT1* promoter upstream to the "TATA box" was used to develop a one hybrid approach and allowed to identify a grape *ASR*, named *VvMSA*, interacting with these DNA elements (Cakir *et al*., 2003). *VvMSA* and *VvHT1* share similar patterns of expression during the ripening of grape. Both genes are inducible by sucrose in grape cells suspension. Sugar induction of *VvMSA* is strongly enhanced by ABA, suggesting that VvMSA is involved in a common transduction pathway of sugar and ABA signaling (Cakir *et al*., 2003). Several *ASR* orthologous and paralogous genes are transcriptionally regulated by ABA and sugars (Dominguez *et al*., 2013 and references therein). Saumonneau *et al*. (2008) described some polymorphism for grape ASRs and characterized them as chromosomal non-histone proteins. By a yeast two-hybrid approach and functional analysis, they identified a protein partner of VvMSA which was characterized as an APETALA2 domain transcription factor named VvDREB. Later, three ASR genes were isolated from two grape varieties, Cabernet-Sauvignon and Ugni Blanc (Saumonneau *et al*., 2012). One was specific for Cabernet-Sauvignon, one for Ugni Blanc, and one common to both varieties.

Conde *et al*. (2006) used grape heterotrophic suspension-cultured cells as a model system to study glucose (Glc) transport and its regulation. In this experimental system, glucose regulated its own uptake through hexokinase-dependent repression of transcription, and through hexokinase-independent posttranscriptional regulation. High glucose down-regulated *VvHT1* transcription and glucose uptake, whereas low glucose increased these parameters.

A transcriptomic approach allowed to identify and characterize VvSK1, a protein kinase which control the expression of several hexose transporters in grape cell suspensions (Lecourieux *et al*., 2010).

3. Genotypic variations in sugar concentration

Large natural genotypic variation exist in sugar concentration among different *V. vinifera* cultivars (Kliewer, 1966). Among 78 *V. vinifera* varieties including table grape, red and white wine varieties, Kliewer (1966) recorded a variation of total soluble solids (TSS, °Brix) at maturity of 18.7 to 27. Large surveys including wild species (Decroocq, unpublished) and interspecific hybrids (Shiraishi *et al.,* 2010) display a higher range of variability for the same traits. Among 26 species of Vitis including species from North America and Middle East regions, Kliewer (1967) reported that TSS ranged from 13.7 to 31.5 °Brix. In a more recent study with 60 varieties from 20 species characterized in the south-west of France, a range of TSS of 11.5 to 26 was recorded 30 days after veraison, with *V. candicans* varieties always characterized by the lowest TSS (Decroocq, unpublished).

In Canada, several varieties have been selected on the basis of their low sugar content. The sugar content of the Sabrevois variety rarely exceeds 21 °Brix. This variety exhibits medium vigour, low internode development, an average yield of 8t/ha and medium acidity. The Radisson variety (formerly called ES 5-17) does not exceed 20° Brix and has a low acidity. Like Sabrevois, it exhibits a weak sensitivity to fungal diseases. In contrast to Sabrevois, its bud fertility is high.

Clonal selection is one of the strategies that may be used to adapt the plant material to climate change. This may concern the rootstock, which control the vigour of the plant, and the scion. In the Bordeaux area, this is especially important for Merlot and Sauvignon blanc, which exhibit an early phenology and are thus more exposed to hot summer periods.

Merlot, which is issued from the cross between Magdeleine noire and Cabernet Franc, is one of the most famous and widespread varieties in the world (249 000 ha). It occupies about 50 % of the Bordeaux vineyard. Sauvignon blanc, which was formerly used only for the sweet white wines is the most planted white variety in the Bordeaux vineyards.

Twelve clones of Merlot are presently agreed, among which 10 are issued from a repository planted by INRA in Gironde in 1964. At this time, they were selected on the basis of their medium productivity and high alcohol potential. Improvement of viticultural practices now allows to obtain ripe berries more regularly, and low yields are desired to reach better quality. The clonal diversity of Merlot is presently revisited to select 20-30 genotypes out of a population of 256 genotypes present in the Bordeaux INRA repository. The screening is first based on the amount of berries and the shape of the cluster, and on early phenology. In the Bordeaux area, a lag phase of about 110 days and 45 days are observed between mid-flowering and maturity, and between mid-veraison and maturity respectively. Other measurements concern sugar content, total acidity, malic acid, pH, polyphenols, berry weight and berry tasting.

Twenty clones of Sauvignon blanc are presently agreed, and there are 3 French repositories gathering 400 accessions, including one at INRA Bordeaux (Château Couhins). Five clones (108, 242, 316, 317, 905 et 906) have been particularly studied by the Chambre d'Agriculture of Gironde and exhibit an interesting variability in terms of yield, sugar content, acidity, phenology, resistance to *Botrytis* and wine aromas.

4. Environmental cues and viticultural practices influencing sugar accumulation.

Assessment of the plasticity of a given genotype should be considered with similar source-sink ratio. Generally, sugar concentration gradually increases with leaf area to fruit ratio and reaches a plateau (Figure 2) (Zufferey, 2000; Kliewer and Dokoozlian, 2005). The plateau can be considered as the potential sugar concentration that a genotype can accumulate under non-source limitation condition, giving a reliable measure of the genotypic effect. Despite the well-known natural variation in sugar concentration and its response to source-sink ratio, limited information is available for the underlying mechanisms

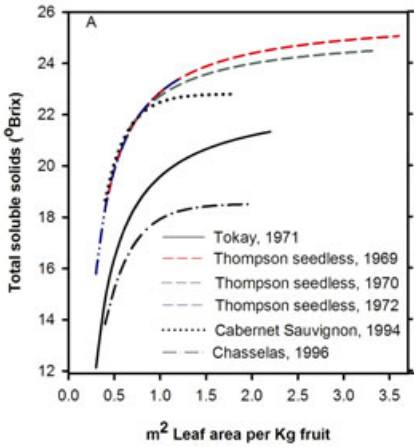


Figure 2. Sugar concentration in function of leaf area to fruit ratio, which is an indicator of source-sink relationship (Redrawn from Zufferey, 2000; Kliewer and Dokoozlian, 2005). Generally, total soluble solids (an indicator of sugar concentration) gradually increase with leaf area to fruit ratio and then reach a plateau. The plateau can be considered as a non-source limitation condition, under which the difference in sugar concentration should be due mainly to the difference in sink activities that is regulated by enzyme activities.

5. Mathematic model simulating sugar accumulation

Mathematic models can integrate and quantitatively compare the relative response of sugar import, sugar metabolism, and water budget, offering new insights into the regulation of sugar concentration. Keeping the model complexity at a relative low level and meanwhile reflecting the major mechanic control of sugar metabolism, the SUGAR model (Génard and Souty, 1996) developed for peach provides a valuable framework to simulate sugar accumulation in fruits. This model simulates sugar content in relation to fruit carbon balance and partitioning and also integrates water accumulation, to model the dynamics of sugar concentration. After refinement with considerations of specific features of sugar metabolism in grape berry, the SUGAR model has been adapted to simulate sugar accumulation in grape berry (Dai *et al.*, 2009). This SUGAR-vitis model correctly simulated the negative effect of lowered source-to-sink ratio and the positive effect of water shortage on sugar concentration. Recently, this SUGAR-vitis model has been used to analyze the genetic determinism of sugar content over three growing seasons in the vineyard in progeny from a Riesling x Gewurztraminer cross (Duchêne *et al.*, 2012; Duchene *et al.*, 2013). Model analysis showed that a coefficient (k) related to the non-sugar use of carbon imported in berries differs between the individuals of the

progeny, explaining part of the variability in sugar content (Duchene *et al.*, 2013). The QTLs (genome regions) linked with this model parameter will be determined to identify underlying gene candidates that control the utilization of imported carbon for the non-sugar compounds in grape berry as did in tomato fruits (Prudent *et al.*, 2011). The combination of physiological observations with model analysis provides an alternative way to identify gene candidates that are involved in berry quality regulation. It will be interesting to determine whether some of these QTLS include sugar transporters and enzymes of sugar metabolism, which are suggested to have been under specific selection pressure during frape domestication (Xin *et al*., 2013).

6. References

Afoufa-Bastien D, Medici A, Jeauffre J, Coutos-Thévenot P, Lemoine R, Atanassova R, Laloi M (2010) The *Vitis vinifera* sugar transporter gene family: phylogenetic overview and macroarray expression profiling. *BMC Plant Biology*, **10**, 245.

Agasse A, Vignault C, Kappel C, Conde C, Geros H, Delrot S (2009). Sugar transport & sugar sensing in grape. I*n KA Roubelakis-Angelakis, ed, Grapevine Molecular Physiology & Biotechnology*, Ed Second. Springer, New York, pp 105-128.

Ageorges A, Issaly N, Picaud S, Delrot S (2000). Characterization of an active sucrose transporter gene expressed during the ripening of grape berry (*Vitis vinifera* L.). *Plant Physiology and Biochemistry*, **38**, 177-185.

Alonso AP, Vigeolas H, Raymond P, Rolin D, Dieuaide-Noubhani M (2005). A new substrate cycle in plants. evidence for a high glucose-phosphate-to-glucose turnover from *in vivo* steady-state and pulse-labeling experiments with [^{13}C]Glucose and [^{14}C]Glucose. *Plant Physiology*, **138**, 2220-2232.

Atanassova R, Leterrier M, Gaillard C, Agasse A, Sagot E, Coutos-Thévenot P, Delrot S (2003). Sugar-regulated expression of a putative hexose transport gene in grape. *Plant Physiology*, **131**, 326-334.

Çakir B, Agasse A, Gaillard C, Saumonneau A, Delrot S, Atanassova R (2003). A grape ASR protein involved in sugar and abscisic acid signaling. *Plant Cell*, **15**, 2165-2180.

Conde C, Agasse A, Glissant D, Tavares R, Gerós H, Delrot S (2006). Pathways of glucose regulation of monosaccharide transport in grape cells. *Plant Physiology*, **141**, 1563–1577.

Conde BC, Silva P, Fontes N, Dias ACP, Tavares RM, Sousa MJ, Agasse A, Delrot S, Geros H (2007). Biochemical changes throughout grape berry development and fruit and wine quality. *Food*, **1**, 1-22.

Coombe BG (1989). The grape berry as a sink. *Acta Horticulturae*, **29**, 149-158.

Coombe BG (1992). Research on development and ripening of the grape berry. *American Journal of Enology and Viticulture*, **43**, 101-110.

Dai ZW, Vivin P, Robert T, Milin S, Li SH, Génard M (2009). Model-based analysis of sugar accumulation in response to source-sink ratio and water supply in grape (*Vitis vinifera*) berries. *Functional Plant Biology*, 36, 527-540.

Davies C, Robinson SP (1996). Sugar accumulation in grape berries. *Plant Physiology*, **111**, 275-283.

Davies C, Wolf T, Robinson SP (1999). Three putative sucrose transporters are differentially expressed in grapevine tissues. *Plant Science*, **147**, 93-100.

Deluc LG, Grimplet J, Wheatley MD, Tillett RL, Quilici DR, Osborne C, Schooley DA, Schlauch KA, Cushman JC, Cramer GR (2007). Transcriptomic and metabolite analyses of Cabernet-Sauvignon grape berry development. BMC Genomics, **8**, 429.

Dominguez PG, Frankel N, Mazuch J, Balbo I, Iusem N, Fernie AR, Carrari F (2013). ASR1 mediates glucose-hormone cross talk by affecting sugar trafficking in tobacco plants. *Plant Physiology*, **161**, 1486-1500.

Duchêne E, Dumas V, Jaegli N, Merdinoglu D (2012). Deciphering the ability of different grapevine genotypes to accumulate sugar in berries. *Australian Journal of Grape and Wine Research*, **18**, 319-328.

Duchene E, Dumas V, Jaegli N, Merdinoglu D, Dai Z (2013). Deciphering the genetic variability of berry sugar content in grapevine varieties. *In IX International Symposium on Grapevine Physiology and Biotechnology* La Serena, Chile, *In press*.

Duchêne E, Schneider C (2005). Grapevine and climatic changes : a glance at the situation in Alsace. *Agronomy for Sustainable Development*, **25**, 93-99.

Fillion L, Ageorges A, Picaud S, Coutos-Thevenot P, Lemoine R, Romieu C, Delrot S (1999). Cloning and expression of a hexose transporter gene expressed during the ripening of grape berry. *Plant Physiology*, **120**, 1083-1094.

Génard M, Souty M (1996). Modeling the peach sugar contents in relation to fruit growth. *J. Am. Soc. Hortic. Sci.*, **121**, 1122-1131.

Grimplet J, Deluc L, Tillett R, Wheatley M, Schlauch K, Cramer G, Cushman J (2007). Tissue-specific mRNA expression profiling in grape berry tissues. BMC *Genomics*, **8**, 187.

Hayes MA, Davies C, Dry IB (2007). Isolation, functional characterization, and expression analysis of grapevine (*Vitis vinifera* L.) hexose transporters: differential roles in sink and source tissues. *J. Exp. Botany*, **58**, 1985-1997.

Jaillon O, Aury JM, Noel B, Policriti A, Clepet C, Casagrande A, Choisne N, Aubourg S, Vitulo N, Jubin C, Vezzi A, Legeai F, Hugueney P, Dasilva C, Horner D, Mica E, Jublot D, Poulain J, Bruyere C, Billault A, Segurens B, Gouyvenoux M, Ugarte E, Cattonaro F, Anthouard V, Vico V, Del Fabbro C, Alaux M, Di Gaspero G, Dumas V, Felice N, Paillard S, Juman I, Moroldo M, Scalabrin S, Canaguier A, Le Clainche I, Malacrida G, Durand E, Pesole G, Laucou V, Chatelet P, Merdinoglu D, Delledonne M, Pezzotti M, Lecharny A, Scarpelli C, Artiguenave F, Pe ME, Valle G, Morgante M, Caboche M, Adam-Blondon AF, Weissenbach J, Quetier F, Wincker P (2007) The grapevine genome sequence suggests ancestral hexaploidization in major angiosperm phyla. *Nature*, **449**, 463-467.

Jin Y, Ni DA, Ruan YL (2009). Posttranslational elevation of cell wall invertase activity by silencing its Inhibitor in tomato delays leaf senescence and increases seed weight and fruit hexose level. *Plant Cell*, **21**, 2072-2089.

Kliewer WM (1966). Sugars and organic acids of *Vitis vinifera*. *Plant Physiology*, **41**, 923-931.

Kliewer WM (1967). The glucose-fructose ratio of *Vitis Vinifera* grapes. *American Journal of Enology and Viticulture*, **18**, 33-41.

Kliewer WM, Dokoozlian NK (2005). Leaf area/crop weight ratios of grapevines: influence on fruit composition and wine quality. *Am. J. Enol. Vitic.*, 56, 170-180.

Lecourieux F, Lecourieux D, Vignault C, Delrot S. 2010. A sugar inducible protein kinase, VvSK1, regulates sugar transport and accumulation in grapevine cells. *Plant Physiology*, **152**, 1096-1106.

Manning K, Davies C, Bowen HC, White PJ (2001) Functional characterization of two ripening-related sucrose transporters from grape berries. *Annals of Botany*, **87**, 125-129.

Nguyen-Quoc B, Foyer CH (2001) A role for 'futile cycles' involving invertase and sucrose synthase in sucrose metabolism of tomato fruit. *J. Exp. Botany*, **52**, 881-889.

Ohyama A, Ito H, Sato T, Nishimura S, Imai T, Hirai M (1995) Suppression of acid invertase activity by antisense RNA modifies the sugar composition of tomato fruit. *Plant and Cell Physiology*, **36**, 369-376.

Pan Q-H, Yu X-C, Zhang N, Zou X, Peng C-C, Wang X-L, Zou K-Q, Zhang D-P (2006) Activity, but not expression, of soluble and cell wall-bound acid invertases is induced by abscisic acid in developing apple fruit. *Journal of Integrative Plant Biology*, **48**, 536-549.

Pan QH, Cao P, Duan CQ (2009). Comparison of enzymes involved in sugar metabolism from Shang-24 (Vinifera quinguangularis) and Cabernet Sauvignon (Vinifera vinifera) at veraison. *Australian Journal of Grape and Wine Research*, **15**, 9-17.

Prudent M, Lecomte A, Bouchet J-P, Bertin N, Causse M, Génard M (2011). Combining ecophysiological modelling and quantitative trait locus analysis to identify key elementary processes underlying tomato fruit sugar concentration. *J. Exp. Botany*, **62**, 907-919.

Robinson SP, Davies C (2000). Molecular biology of grape berry ripening. *Aust. J. Grape Wine Res.*, **6**, 175-188.

Ruffner HP, Hurlimann M, Skrivan R (1995) Soluble invertase from grape berries: purification, deglycosyla-tion and antibody specificity. *Plant Physiology and Biochemistry*, **33**, 25-31.

Samson F, Brunaud V, Duchêne S, De Oliveira Y, Caboche M, Lecharny A, Aubourg S (2004) FLAGdb++: a database for the functional analysis of the Arabidopsis genome. *Nucleic Acids Research*, 32, D347–D350.

Saumonneau A, Agasse A, Bidoyen MT, Lallemand M, Cantereau A, Medici A, Laloi M, Atanassova R (2008) Interaction of grape ASR proteins with a DREB transcription factor in the nucleus. *FEBS Letters*, **582**, 3281–3287.

Saumonneau A, Laloi M, Lallemand M, Rabot A, Atanassova R (2012) Dissection of the transcriptional regulation of grape ASR and response to glucose and abscisic acid. *J. Exp. Botany*, **63**, 1495-1510.

Schultz HR (2000) Climate change and viticulture: A European perspective on climatology, carbon dioxide and UV-B effects. *Aust. J. Grape Wine Res.*, **6**, 2-12.

Shiraishi M, Fujishima H, Chijiwa H (2010) Evaluation of table grape genetic resources for sugar, organic acid, and amino acid composition of berries. *Euphytica*, **174**, 1-13.

Takayanagi T, Yokotsuka K (1997) Relationship between sucrose accumulation and sucrose-metabolizing enzymes in developing grapes. *Am. J. Enol. Vitic.*, **48**, 403-407.

Terrier N, Glissant D, Grimplet J, Barrieu F, Abbal P, Couture C, Ageorges A, Atanassova R, Léon C, Renaudin JP, Dédaldéchamp F, Romieu C, Delrot S, Hamdi S (2005) Isogene specific oligo arrays reveal multifaceted changes in gene expression during grape berry (*Vitis vinifera* L.) development. *Planta*, 222, 832-847.

Vignault C, Vachaud M, Çakir B, Glissant D, Dédaldechamp F, Büttner M, Atanassova R, Fleurat-Lessard P, Lemoine R, Delrot S (2005) *VvHT1* encodes a monosaccharide transporter expressed in the conducting complex of the grape berry phloem. *Journal of Experimental Botany*, **56**, 1409-1418.

Vitrac X, Larronde F, Krisa S, Decendit A, Deffieux G, Mérillon JM (2000) Sugar sensing and Ca^{2+}-calmodulin requirement in *Vitis vinifera* cells producing anthocyanins. *Phytochemistry*, **53**, 659-665.

Xin H, Zhang J, Zhu W, Wang N, Fang P, Han Y, Ming R, Li S (2013) The effects of artificial selection on sugar metabolism and transporter genes in grape. *Tree Genetics & Genomes*. doi 10.1007/s11295-013-0643-7.

Zeng L, Wang Z, Vainstein A, Chen A, Ma H. (2011) Cloning, localization and expression analysis of a new tonoplast monosaccharide transporter from *Vitis vinifera* L. *Journal of Plant Growth Regulators*, **30**, 199-212.

Zhang XY, Wang XL, Wang XF, Xia GH, Pan QH, Fan RC, Wu FQ, Wu XC, Zhang DP (2006). A shift of phloem unloading from symplasmic to apoplasmic pathway is involved in developmental onset of ripening in grape berry. *Plant Physiology*, **142**, 220-232.

Zhang YL, Meng QY, Zhu HL, Guo Y, Gao HY, Luo YB, Lu J (2008). Functional characterization of a LAHC sucrose transporter isolated from grape berries in yeast. *Plant Growth Regulators*, **54**, 71-79.

Zheng Y, Tian L, Liu H, Pan Q, Zhan J, Huang W (2009). Sugars induce anthocyanin accumulation and flavanone 3-hydroxylase expression in grape berries. *Plant Growth Regulation*, **58**, 251-260.

Zufferey V (2000). Échanges gazeux des feuilles chez *Vitis vinifera* L. (cv. Chasselas) en fonction des parametres climatiques et physiologiques et des modes de conduite de la vigne. Ecole Polytechnique Federale de Zurich, Zurich, Switzerland.

Viticultural practices to match future challenges

M. Stoll[1,*], V. Stoeber[1] and S. Tittmann[1]

[1]Deparment of General and Organic Viticulture, University of Geisenheim, Von-Lade-Str. 1, 65366 Geisenheim, Germany

Abstract: In high quality viticultural regions around the world, average temperatures during the growing season have increased by more than one degree Celsius, impacting on fruit quality and wine style. To adapt to these conditions several seasonal management and leaf removal practices were investigated. Whether maturation would be altered by a change in the leaf area to fruit weight ratio was tested in a field trial (*Vitis vinifera* L. cv. Riesling) during several growing seasons. Leaf area was reduced using various manual as well as mechanical defoliation treatments post-flowering, differing in their position within the canopy and their intensity. Both the application of an anti-transpirant agent and the application of shading nets were trialed for their impact on the transpiration from the vine's canopy. Large reductions in the ratio of leaf area to fruit weight significantly reduced the sugar content of Riesling berries at harvest. The specific impacts were dependent on the timing of carbohydrate limitation, the position of defoliation, and its severity.

Keywords: leaf area manipulation; leaf area to fruit weight ratio, defoliation, summer pruning; ripening

1. Background

In many crops, including grapevines, a trend of recent warming has advanced the phenological development affecting the onset of bud break, flowering or veraison. Even though a higher risk of spring frost events is not predicted in the near future, the loss due to late frost events of further advanced canopies may hit the viticultural industry severely. Furthermore, warmer weather conditions may cause a risk of higher disease pressure and almost certainly will impact on the harvest date. Such warmer temperature will need to be taken into consideration when processing the fruit. Overall fruit quality in terms of total soluble solids or harvest maturity may alter and hence affect the wine style.

With a view to these constraints, long or short term adaptation strategies will need to be investigated. Apart from making use of the genetic resources of scions and rootstock combination and their adaptation to different environmental conditions, the selection of vineyard sites as well as the direction of row orientation will need to be taken into consideration or investigated more deeply in future. However, either the customers' demands for a specific wine style, varietal characters or limitations in choosing a site may limit the freedom of choice. Furthermore, once such decisions are taken the vineyard will stay in production for decades.

On the other hand, seasonal vineyard management practices do also have high potential and play a complementary role affecting microclimate and photosynthesis.

Mechanically defoliated canopy (MDC) and severe summer pruning (SSP) with approximately half of the height of the control plants were compared. The treatments were applied after flowering and above the fruit zone. The leaf area to crop weight ranged from 8 cm^2 g^{-1}, 14 cm^2 g^{-1} to 19.5 cm^2 g^{-1} for SSP, MDC and control respectively. At different developmental stages over the growing season both topping and leaf removal showed no detrimental effects on leaf photosynthesis. However, further removal of the youngest leaves of the SSP dramatically affected leaf ageing. Therefore berry ripening was heavily affected by leaf area, and the preliminary results indicated that

Corresponding author : Manfred.Stoll@hs-gm.de

altered leaf area to crop weight has an effect on the velocity of harvest maturity and berry composition.
Initial sensory evaluation showed that wines from a reduced ratio of leaf area to crop weight (MDC) were considered well-balanced and capable of two weeks' delay in ripening and high production.

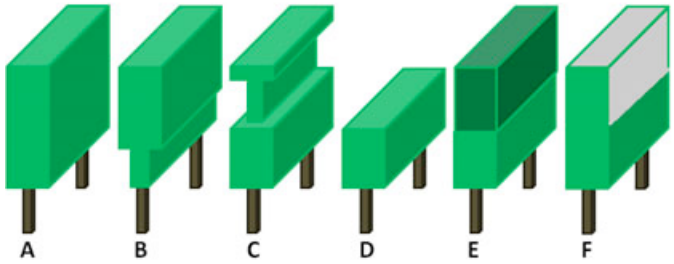


Figure 1 - Several seasonal options to impact on Leaf Area to Fruit Weight:
A) control; B) defoliation of the bunch zone; C) defoliation above bunch zone; D) severe summer pruning; E) application of anti-transpirant oil above bunch zone; F) shading above bunch zone.

2. Conclusions

This experiment addressed the opportunities for reducing the 'velocity' of berry maturation through different canopy management practices. The successful application of treatments which restricted carbohydrates to the developing berry may have been able to offset maturity by at least one or two weeks. Mechanised removal of leaves shows promise as an alternative to traditional hand removal, as does anti-transpirant applications to temporarily reduce photosynthesis efficiency.

Acknowledgements : This work was mainly funded by the Federal Ministry of Food, Agriculture and Consumer Protection through the Federal Office for Agriculture and Food (BLE) [grant number: 2810HS018, ´viticultural practices to reduce sugar content in the field´]. We would also like to thank the *Deutscher Wetterdienst Beratungsstelle Geisenheim* for providing meteorological data related to the experiment.

Alcohol level reduction in wine

OENOVITI INTERNATIONAL Network

Session II - Potential reduction in alcohol levels and oenology (microorganisms & chemistry)

Non microbiological strategies to reduce alcohol in wines

Mariona Gil, Nickolaos Kontoudakis, Sandra Estévez, Elena González-Royo, Mireia Esteruelas, Francesca Fort, Joan M. Canals and Fernando Zamora*

Departament de Bioquímica i Biotecnologia, Grup de Recerca en Tecnologia Enològica, Facultat d'Enologia de Tarragona, Universitat Rovira i Virgili. Campus de Sescelades, C/ Marcel.li Domingo, s/n. 43007-Tarragona, Spain. Tel:+ 34 977 55 86 79; Fax:+ 34 977 55 86 86

Abstract: The general aim of this article is related with the problem generated by climatic change on grape ripening, how it affects red wine composition and quality and how we can mitigate these problems applying some technical procedures in the winery. Specifically, global warming provokes an increasing unbalance between primary and secondary metabolism of vines. This phenomenon causes that grapes attain quickly very high sugar content, very low acidity and very high pH. This new scenario represents a new challenge for wine industry which needs to develop new strategies for better winemaking. Faced with this situation oenologists have only two possibilities. First, to harvest when alcoholic degree and/or pH are at the correct level and then, adapt winemaking to conditions of unripe grapes. Or second, to wait for the complete maturity and harvest when grapes are really well ripe and then, apply techniques for decreasing alcoholic degree and pH. Within this context, this article discusses the possible non microbiological strategies to reduce alcohol in wines.

Keywords: Partial dealcoholization, wine composition, reverse osmosis, unripe grapes

1. Introduction

The concept of climate change is not new. In fact, many years ago it was described by some scientists who were then dismissed as alarmists. Today, everybody know that the consumption of fossil fuels is causing an increase in the concentration of carbon dioxide and other gases, which, by reflecting the radiation off, are causing a greenhouse effect (Crowley, 2000, Zamora, 2005a) which is responsible of the actual global warming of the planet. Data are truly frightening. In 1958, the concentration of CO2 was 315 ppm. Today has reached more than 400 ppm, and in the best case scenario, it will be higher than 500 ppm by the end of the twenty-first century (IPCC, 2012).

But what impact climate change will result in viticulture? Basically, the global warming provokes that grapes stored sugars and degrade acids faster than in normal conditions (Jones *et al.*, 2005; Mira de Orduña, 2010). Therefore, grapes arrive at very high potential alcoholic degree and pH sooner than usual. This phenomenon provokes the advance of the date of the harvest. However, skins and specially seeds remain already unripe. Therefore and increasing imbalance between industrial and phenolic maturity takes place as a consequence of climatic change. In those conditions, when grapes are not well-ripe, winemaker has a very difficult decision. If he realizes a short maceration, the wines would have not color enough. At the contrary, if he realizes a large maceration, the risk of extracting astringent, herbaceous and bitter tannins is really very high (Llaudy *et al.*, 2008).

For these reasons, winemakers usually wait until the skins and seeds of grapes are suitably ripe. In fact, the term phenolic maturity is usually used to describe this phenomenon. Nevertheless, grapes with complete phenolic maturity frequently have higher sugar concentrations and lower acidity, which can cause some problems. First, high ethanol content can lead to stuck and sluggish fermentations. Secondly, ethanol clearly participates in wine sensory perception and, consequently, an excess can lead to unbalanced wines that are unpleasant for consumers. Thirdly, the current wine market is very concerned with health prevention policies and, therefore, is interested in wines with a moderate alcohol level. Finally, some countries penalize high alcohol wines with higher taxes, which considerably increase their final price.

In recent years, wines have (on average) gradually increased in alcohol content probably because winemakers are looking for grapes with a high phenolic maturity. Moreover, it is

Corresponding author : Fernando.zamora@urv.cat

generally considered that climate change increases this tendency (Jones *et al*., 2005). If the temperature during ripening is higher than the optimum, the grape pulp matures faster, and the pH and sugar concentration become too high. Therefore, the period between veraison and industrial maturity decreases, making it more difficult to pinpoint proper aromatic and phenolic maturity, and leading to unbalanced wines.

In this situation, there are only two possibilities. Grapes can be harvested when the potential alcohol value is appropriate or when complete phenolic maturity has been reached. In the first case, it must be assumed that complete phenolic maturity has not been reached. In the second case, the ethanol content of the grapes would probably be excessive. Neither of these possibilities is conducive to obtaining high quality wines and winemakers are obviously concerned.

Several ways of mitigating the impact of global warming have been proposed. One is to change certain viticultural practices by introducing new cultivars, modifying culture techniques and even relocating vineyards to other production areas to delay pulp sugar accumulation (Schultz and Jones, 2010). Another is to use unripe grapes harvested during cluster thinning to reduce alcohol content (Kontoudakis *et al*., 2011). And yet a third is to use yeast with a lower ethanol yield even though natural Saccharomyces cerevisiae strains seem to provide similar ethanol yields (Malherbe *et al*., 2003). Despite these proposals, nowadays, the most commonly used methods for reducing alcohol content in wines (Pickering, 2000) are physical ones (Schmidtke *et al*., 2012): for example, the spinning cone column (Belisario-Sánchez *et al*., 2009) and reverse osmosis (Catarino and Mendes, 2011). Little is known about how these methods affect wine composition and quality. There are very few references in the scientific literature and most of them are aimed to obtain alcohol-free wines (Takács *et al*., 2007; Gómez-Plaza *et al.*, 1999) or very low-alcohol wines (Diban *et al*., 2008; Varavuth *et al*., 2009, Bogianchini *et al*., 2011). However, most wineries are only interested in reducing alcohol content by one or two degrees in order to obtain more balanced wines (Meillon *et al*., 2010a, 2010b, Gambutti *et al*., 2011). Within this context, this article discusses two non microbiological strategies, reverse osmosis and the employment of unripe grapes harvested during cluster thinning, to reduce alcohol content**.**

2. Reverse osmosis

Reverse osmosis is nowadays probably the most employed procedure. This technique is now a reality and even some enterprises offer the possibility of renting this equipment to wineries. Table 1 shows an experimental assay of partial dealcoholization by reverse osmosis with two red wines of AOC Priorat and Penedès (Gil *et al*., 2013).

The results showed that only significant differences were found in alcohol content while the other laboratory parameters remained unchanged. These wine were also tasted by a trained sensory panel using the discrimination triangular test. In general tasters were able to distinguish between the control and the partially dealcoholized wines but all the tasters

Table 1. Partial dealcoholization by reverse osmosis

Parameter	AOC Penedès			AOC Priorat		
	Control	-1%	-2%	Control	-1%	-2%
Ethanol content (%)	14.8 ± 0.2 A	13.8 ± 0.2 B	12.8 ± 0.2 C	16.2 ± 0.2 A	15.1 ± 0.2 B	14.1 ± 0.1 C
Titratable acidity (g/l)	4.8 ± 0.1 A	4.8 ± 0.1 A	4.9 ± 0.1 A	5.2 ± 0.1 A	5.2 ± 0.1 A	5.6 ± 0.1 B
Color intensity	15.3 ± 1.5 A	15.6 ± 0.9 A	15.4 ± 0.7 A	15.4 ± 0.2 A	15.2 ± 0.4 A	14.5 ± 0.5 A
Hue	67.7 ± 1.1 A	67.9 ± 0.4 A	68.3 ± 1.5 A	59.3 ± 1.2 A	60.0 ± 0.4 A	59.2 ± 0.5 A
Anthocyanins (mg/l)	567 ± 41 A	546 ± 19 A	574 ± 14 A	200 ± 13 A	206 ± 23 A	226 ± 11 A
IPT	72.9 ± 2.5 A	73.9 ± 2.3 A	75.8 ± 20.6 A	62.4 ± 0.5 A	62.2 ± 0.2 A	62.1 ± 0.8 A
Proanthocyanidins (g/l)	1.8 ± 0.3 A	1.6 ± 0.2 A	1.7 ± 0.2 A	1.6 ± 0.2 A	1.7 ± 0.3 A	1.5 ± 0.2 A
mDP	6.8 ± 1.2 A	7.5 ± 1.8 A	7.2 ± 0.6 A	6.8 ± 1.8 A	5.8 ± 0.3 A	6.5 ± 0.7 A

All data are expressed as the average of the three replicates standard deviation (n = 3). Statistical analysis: one-factor ANOVA and Scheffe's test (both p<0.05). Different letters indicate the existence of statistically significant differences.

confessed that it was quite much harder than they thought at the beginning of the test. Therefore it seems that reverse osmosis can be a useful procedure to compensate the excess of ethanol content in red wines since it hardly alters their composition and sensory characteristics. Moreover, the cost of the process can be considered as affordable since the equipment manufacturer provides the service at a price of 0.15 €/L for removing 1 % of ethanol. Reverse osmosis is, therefore, an interesting tool for improving the balance of the wines from regions where grapes can easily reach high alcohol content. This is particularly important nowadays because climatic change is increasingly causing a mismatch between the pulp and phenolic maturity of grapes.

3. Employment of unripe grapes

Another possible strategy is the use of unripe grapes harvested during cluster thinning as a method for reducing alcohol content and pH of wine. Kontoudakis *et al.* (2011) have proposed that grapes from cluster thinning can be used to produce a very acidic low-alcohol wine. This wine can be then treated with high doses of charcoal and bentonite. This odourless and colourless wine can be used to reduce pH and ethanol content of wine produced from very ripe grapes, which had reached complete phenolic maturity. In their study, authors have employed grapes of the cultivar *Vitis vinifera* cv. Cabernet Sauvignon and Merlot from AOC Penedes and Bobal from the AOC Utiel-Requena, which were harvested at two different ripening stages. The first harvest was carried out when the potential degree of alcohol was between 13.0 and 14.0%. The second harvest was carried out when the grapes reached optimum phenolic maturity. Three tanks from the first harvest and three tanks from the second harvest were elaborated without any addition of the low-ethanol wine. The other three tanks from the second harvest were used for the alcohol-reduction experiment. Specifically, a part of the total volume of the grape juice was removed and replaced with the same volume of low alcohol wine.

Table 2 shows the analytical parameters of the Merlot obtained wines. Similar results were obtained in wines from the other cultivars.

As expected, the wines of which part of their juice had been replaced by the low-alcohol wine had a lower ethanol content and pH than their corresponding controls. In fact, the ethanol content, the pH and the titratable acidity of these wines were closer to the control wines of the first harvest than to those of control wines of the second for the three cultivars. The results concerning phenolic compounds and color were very clear. In fact, the anthocyanin and proanthocyanidin concentrations as well as the proanthocyanidin mean degree of polymerization (mDP) and the percentage of (-)-epigallocatechin of wines from the second harvest were significant higher than those of the first harvest. This data confirms the great influence of phenolic maturity on these parameters. On the other hand, all the treated wine has similar parameters than the control wine of the second harvest. Furthermore, since the pH of the treated wines was significant lower than those of the non treated wines, its color intensity was considera-

Table 2. Influence of partial dealcoholization by using grapes from cluster thinning

Parameter	First harvest	Second Harvest	
		Control	Dealcoholized
Ethanol (%)	13.4 ± 0.1 ***A***	15.9 ± 0.1 ***B***	14.2 ± 0.1 ***C***
TA (g/L)	7.0 ± 0.2 ***A***	6.3 ± 0.2 ***B***	7.1 ± 0.1 ***A***
pH	3.45 ± 0.01 ***A***	3.76 ± 0.03 ***B***	3.55 ± 0.07 ***C***
Anthocyanidins (mg/L)	191 ± 20 ***A***	252 ± 25 ***B***	271 ± 5 ***B***
Color Intensity	8.7 ± 0.9 ***A***	12.6 ± 1.5 ***B***	17.0 ± 1.9 ***C***
Proanthocyanidins (mg/L)	427 ± 115 ***A***	1070 ± 17 ***B***	969 ± 41 ***C***
mDP	2.72 ± 0.13 ***A***	4.80 ± 1.84 ***B***	4.43 ± 0.36 ***B***
(+)-Catechin (%)	26.2 ± 2.2 ***A***	21,8 ± 1.4 ***B***	19 ± 2.1 ***B***
(-)-Epicatechin (%)	57.8 ± 0.7 ***A***	57.6 ± 0.1 ***A***	57.6 ± 1.9 ***A***
(-)-Epicatechin-3-O-Gallate (%)	4.4 ± 0.4 ***A***	4.9 ± 0.2 ***B***	5.7 ± 0.3 ***C***
(-)-Epigallocatechin (%)	11.6 ± 1.3 ***A***	16.5 ± 0.6 ***B***	17.7 ± 0.7 ***C***

All data are expressed as the average of the three replicates standard deviation (n = 3). Statistical analysis: one-factor ANOVA and Scheffe's test (both p<0.05). Different letters indicate the existence of statistically significant differences.

Corresponding author : Fernando.zamora@urv.cat

bly higher than their controls.

It can be concluded that the proposed procedure may be useful for partial reduction of alcohol content and simultaneous decrease of pH of wines. The colour of the reduced-alcohol wines was better than their corresponding controls and their phenolic composition was similar. Moreover, this procedure does not require additional equipment and is easy to apply in standard wineries. Further experimentation is needed to better adapt the process to obtain more balanced wines without the problems of excess alcohol and high pH.

The climate change is inevitable. We only can adapt to it and try to mitigate their effects. These techniques are now available and their use can be very useful to compensate the effects of global warming in our wineries. However, Global warming is a major problem and, evidently, the real solution is other one.

Acknowledgements : We thank CICYT (AGL2011-29708-C02-01) and CDTI (Project CENIT Demeter) for financial support. We also thank the Société Michael Paetzold for providing the reverse osmosis equipment and to Juvé y Camps SA and Vinícola del Priorat SCCL wineries for providing the wines.

4. References

Belisario-Sánchez, Y., Taboada-Rodríguez, A., Marín-Iniesta, F., Loópez-Gómez, A., 2009. Dealcoholized wines by spinning cone column distillation: phenolic compounds and antioxidant activity measured by the 1,1-diphenyl-2-picrylhydrazyl method. *J. Agric. Food Chem*., **57**, 6770-6778.

Bogianchini, M., Cerezo, A.B., Gomis, A., López, F., García-Parrilla, M.C., 2011. Stability, antioxidant activity and phenolic composition of commercial and reverse osmosis obtained dealcoholised wines. LWT - *Food Sci. Technol*., **44**, 1369-1375.

Catarino, M., Mendes, A., 2011. Dealcoholizing wine by membrane separation processes. *Innovative Food Sci. Emerg. Technol.*, **12**, 330-337.

Crowley, T.J., 2000. Causes of climate change over the past 1000 years. *Science*, **289**, 270-277.

Diban, N., Athes, V., Bes, M., Souchon, I., 2008. Ethanol and aroma compounds transfer study for partial dealcoholization of wine using membrane contactor. *J. Membr. Sci*., **311**, 136-146.

Gambuti, A., Rinaldi, A., Lisanti, M.T., Pessina, R., Moio, L., 2011. Partial dealcoholisation of red wines by membrane contactor technique: influence on colour, phenolic compounds and saliva precipitation index. *Eur. Food Res. Technol.*, **233**, 647-655.

Gil, M., Estévez, S., Kontoudakis, N., Fort, F., Canals, J.M., Zamora, F. 2013. Influence of partial dealcoholization by reverse osmosis on red wine composition and sensory characteristics. *Eur Food Res Technol. In press.* DOI 10.1007/s00217-013-2018-6.

Gómez-Plaza, E., López-Nicolás, J.M., López-Roca, J.M., Martínez-Cutillas, A., 1999. Dealcoholization of wine. Behaviour of the aroma components during the process. LWT - *Food Sci. Technol*., **32**, 384-386

Intergovernamental Panel on Climate Change. http://www.ipcc.ch/index.html

Jones, G. V., White, M. A. and Cooper, O. R., 2005. Climate change and global wine quality. *Clim. Change*, **73**, 319-343.

Kontoudakis, N., Esteruelas, M., Fort, F, Canals, J.M., Zamora, F., 2011. Use of unripe grapes harvested during cluster thinning as a method for reducing alcohol content and pH of wine. *Austr. J. Grape Wine Res*., **17**, 230-238.

Llaudy, M.C., Canals, R., Canals, J.M. and Zamora, F., 2008. Influence of ripening stage and maceration length on the contribution of grape skins, seeds and stems to phenolic composition and astringency in a wine simulated macerations. *Eur. Food Res. Technol.,* **226**, 337-344.

Malherbe, D.F., Du Toit, M., Cordero Otero, R.R., Van Rensburg, P., Pretorius, I.S., 2003. Expression of the Aspergillus niger glucose oxidase gene in Saccharomyces cerevisiae and its potential applications in wine production. *Appl. Microbiol. Biotechnol.,* **61**, 502-511.

Meillon. S., Urbano, C., Guillot, G., Schlich, P. 2010a., Impact of partial alcohol reduction in wine on perceived complexity and temporality of sensations and link with preference. *Food Qual. Pref.,* **21**, 763-773.

Meillon, S., Dugas, V., Urbano, C., Schlich, P. 2010b., Preference and Acceptability of Partially Dealcoholized White and Red Wines by Consumers and Professionals. *Am. J. Enol. Vitic*., **61**, 42-52.

Mira de Orduña, R., 2010. Climate change associated effects on grape and wine quality and production. *Food Res. Int., 43*, 1844–1855.

Pickering, G,J,, 2000. Low- and Reduced-alcohol Wine: A Review. *J. Wine Res.*, **11**, 129-144.

Schmidtke, L.M., Blackman, J.W., Agboola, S.O., 2012. Production Technologies for Reduced Alcoholic Wines. J. Food Sci., 77, 25-41.

Schultz, H.R., Jones, G.V., 2010. Climate induced historic and future changes in viticulture. J. Wine Res., 21, 137-145.

Takács, L., Vatai, G., Korány, K., 2007. Production of alcohol free wine by pervaporation. J. Food Eng., 78, 118-125.

Varavuth, S., Jiraratananon, R., Atchariyawut, S., 2009. Experimental study on dealcoholization of wine by osmotic distillation process. Sep. Purif. Technol., 66, 313-321.

Zamora, F., 2005a. El cambio climático: una amenaza para nuestra viticultura. *Enólogos*, **39**, 28-31.

Microbiological strategies to reduce alcohol levels in wines

Valentin Tilloy[1,2], Axelle Cadière[1,2], Maryam Ehsani[1,2] and Sylvie Dequin[1*]

[1]INRA, UMR1083, 2 Place Viala, 34060 Montpellier, France
[2]Lallemand SAS, 19 rue des Briquetiers 31700 Blagnac, France

Abstract: Microbial strategies to develop low alcohol *Saccharomyces cerevisiae* wine yeasts have been explored for nearly 20 years. Reducing the ethanol yield in yeast represents a considerable challenge, as a large proportion of sugar entering the cell must be diverted to other by-products than ethanol, while preserving yeast strain performances during wine fermentation and avoiding the accumulation of by-products detrimental to wine quality. Various approaches, mainly based on engineering central carbon and redox metabolism have been developed. Using multi step metabolic engineering, low alcohol wine yeasts with carbons rerouted towards glycerol and 2,3-butanediol have been generated, having an ethanol yield reduced by up to 19%. Due to the low public acceptance of GMOs (genetically modified organisms) in the food industry, non-GM approaches, such as evolutionary engineering have been recently favored. Using adaptive evolution, we compared the effect of various selective pressures to reroute carbons towards alternative pathways to glycolysis, including the pentose phosphate and the glycerol pathways. Evolved wine yeast strains producing higher glycerol, 2,3-butanediol and succinate and substantially lower ethanol production were generated. This is the first example of a non GMO, low alcohol *S. cerevisiae* wine yeast strain.

Keywords: low alcohol, glycerol, wine yeast, redox, adaptive evolution

1. Introduction

The reduction of the alcohol content in wine has become a major objective in most winemaking areas worldwide. Since two decades, various strategies have been considered including the improvement of viticulture or winemaking practices, the development of physical approaches and the generation of low alcohol yeasts. As microbial strategy is an easy to implement and costless option, metabolic engineering of *Saccharomyces cerevisiae* for reduced ethanol production has been a very active field for the past two decades.

Developing low alcohol yeast raises a number of scientific issues. First, a major challenge is to redirect a huge amount of sugars of grape musts (16.8 g/L sugar per degree alcohol) towards other(s) by-product(s) that ethanol, while maintaining redox and energy homeostasis. Second, as the metabolic network is strongly interconnected, modification of central carbon metabolism often leads to large, sometimes unpredictable effects on metabolite production. A critical issue is to maintain yeast performance and to avoid accumulation of metabolites that may have a detrimental effect on wine quality.

Various approaches, mostly based on the use of genetic engineering strategies have been developed to redirect metabolism away from ethanol (reviewed by Kutyna *et al.*, 2010; Schmidtke *et al.*, 2012). Most of them rely on modification or redox balance or of central carbon metabolism. Although low alcohol wine yeasts with carbons rerouted towards glycerol and 2,3-butanediol have been generated by these approaches (Ehsani *et al.*, 2009), the use of such strains is limited by public attitude regarding GMO-based food products. Alternative strategies such as adaptive evolution have been recently used to manipulate yeast metabolism for reducing ethanol yield. Various approaches have been assessed, and for the first time, a non GM, high glycerol wine yeast strain with reduced ethanol yield has been generated (Tilloy *et al.*, submitted).

We will present the main strategies based on genetic engineering and evolutionary engineering and we will discuss the potential of the low alcohol yeasts generated.

2. Metabolic engineering strategies

Two main strategies have been developed: (i) the conversion of sugars into metabolites that cannot be fermented, thereby rendering it unavailable for ethanol production (e.g. expression of a glucose oxidase) (ii) the

Corresponding author : dequin@supagro inra.fr

modification of the central carbon pathway to divert carbons away from ethanol production, thereby resulting in the accumulation of other end-products. This has been achieved through targeted modification of specific metabolic pathways involved in redox metabolism (e.g. by increasing the production of lactate or glycerol) or by engineering directly NADH metabolism to decrease the availability of this cofactor for the alcohol dehydrogenase reaction (e.g. by expression of a NADH oxydase) (Figure 1).

A first approach (Figure 1A) is based on the expression of a NADH-dependent bacterial lactate dehydrogenase (LDH) in *S. cerevisiae* (Dequin & Barre, 1994). In these cells, pyruvate is rerouted towards the formation of lactic acid at the detriment of the alcohol pathway. Lactic acid is an interesting compound because of its lack of flavor and its acidifying properties. In engineered yeast, this compound plays the same role as ethanol as electron acceptor. Redirection of yeast metabolism towards the production of lactic acid resulted in a concomitant reduction of the alcohol production, without affecting the oxido-reduction balance. However, in order to reduce substantially the ethanol yield, an amount of lactate, highly above acceptable levels in wine (> 10 g/L), has to be produced.

Another strategy (Figure 1B) has been based on the expression of a bacterial NADH oxidase in yeast. The objective was to reduce the intracellular level of NADH by introducing a system for NADH utilisation which competes with the fermentative alcohol dehydrogenase. Expression of this gene allowed a 15% reduction in the ethanol yield under microaerobic conditions, accompanied by decreased fermentation rate, increased acetaldehyde production and stuck fermentation (Heux *et al.*, 2006b). Using a two-step strategy where oxygen addition was limited to the stationary phase, a reduction of 7% in the ethanol yield was achieved, but oxidized compounds were accumulated (Heux *et al.*, 2006b).

The most efficient strategy was to reroute metabolism towards increased production of glycerol (Figure 1C). In *S. cerevisiae*, this compound plays major roles in redox homeostasis and in osmotic stress resistance as it is the main compatible solute in yeast (Blomberg and Adler, 1992). This polyol, the most abundant by product of fermentation (5 to 8 g/L) after CO_2 and ethanol, is thought to contribute to the smoothness and overall body of wine (Noble and Bursick, 1984). Rerouting carbons towards glycerol led to a substantial decrease in ethanol (Michnick *et al.*, 1997; Remize *et al.*, 1999; de Barros Lopes *et al.*, 2000) and accumulation of various compounds of which acetate and acetoin, undesirable for wine sensorial quality (Remize *et al.*, 1999; Cambon *et al.*, 2006).

Rational engineering of key reactions at the acetaldehyde branch point allowed to limit the accumulation of these compounds, resulting in the development of low-alcohol strains with carbon flux redirected towards glycerol and 2,3-butanediol, a polyol with no sensorial impact in wines (Cambon *et al.*, 2006; Ehsani *et al.*, 2009). In this strain, the ethanol yield was reduced by 19%.

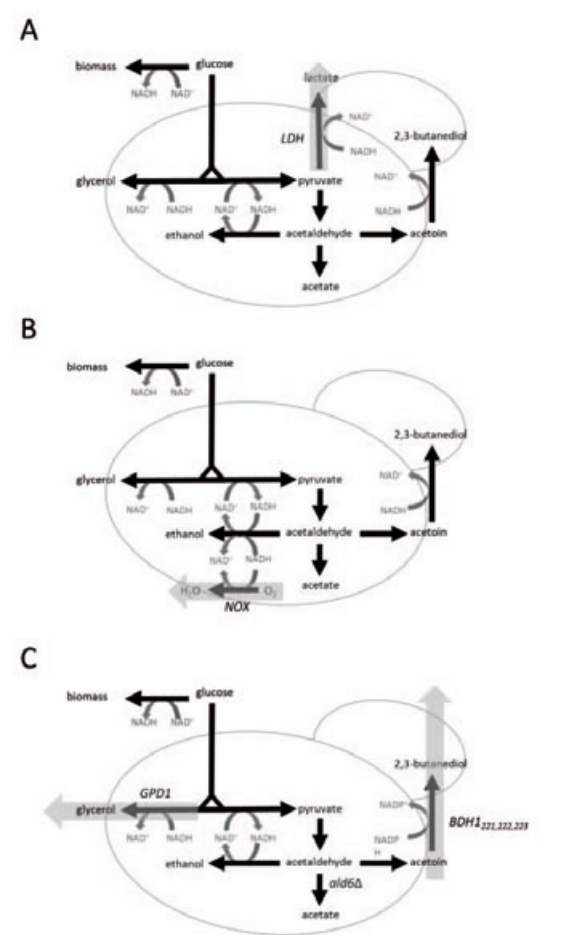


Figure 1 - Metabolic engineering strategies to divert carbons away from ethanol production.
A: Expression of a bacterial lactate dehydrogenase in *S. cerevisiae*, resulting in a mixed alcoholic and lactic fermentation. B: Expression of a bacterial NADH oxidase acting in competition with the yeast alcohol dehydrogenase for NADH, C: Deviation of sugars towards glycerol and 2,3-butanediol by (i) overexpression of *GPD1* encoding a glycerol 3-phosphate dehydrogenase, (ii) deletion of *ALD6* encoding an acetaldehyde dehydrogenase and (iii) overexpression of a NADPH-dependent mutated form of the butanediol dehydrogenase Bdh1p.

3.

An alternative approach to rational engineering is the selection of yeast variants using adaptive evolution. Adaptive evolution involves culturing yeast populations over a long period of time in selective conditions. Variants with increased fitness to the environment will be selected. These approaches were successfully developed in recent years to improve several features of interest for various yeast industries. Such approach can in principle be used to alter yeast metabolism in different ways, but finding the selective conditions to guide metabolism towards a particular desired product remains challenging.

The potential of these approaches to remodel the metabolism of wine yeasts was recently assessed (Figure 2). First, we targeted the pentose phosphate pathway, an alternative route to glycolysis for sugar catabolism (Figure 2A). The diversion of carbons towards the PP pathway was expected to have various consequences on yeast metabolism including (i) a lower availability of carbons for ethanol production, as for each sugar molecule entering this pathway, 1 carbon on 6 is eliminated under the form of CO_2. (ii) a reduced production of acetate, which is the second NADPH producing pathway in addition to the PP pathway. Using this approach, we obtained evolved strains with 1.5-fold increase in the flux through the PP pathway. This increase had a very low impact on ethanol yield, but the evolved strains had various novel properties, including reduced acetate production and remarkably increased ester production (Cadiere *et al.*, 2011; Cadiere *et al.*, 2012).

Adaptive evolution strategies were also used with the objective to increase glycerol production. A first approach was based on the use of sulfite at alkaline pH as a selective agent (Kutyna *et al.*, 2012). A variant producing 30% more glycerol than the parental strain in anaerobic conditions and enhanced sulfite tolerance was obtained. However, this diversion was not sufficient to substantially reduce the production of ethanol.

Besides its key role as redox sink, glycerol is also the main osmolyte produced in response to hyperosmotic stress and this response is mediated by the HOG (high osmolarity glycerol) MAP kinase pathway.

This pathway is also required for the growth of *S. cerevisiae* in the presence of methylglyoxal, a toxic glycolytic byproduct. We therefore tested various conditions expected to stimulate the HOG pathway for generating high glycerol producer strains. Using osmotic/salt stressors or methylglyoxal, we obtained a variety of responses ranging from no impact to various levels of glycerol overproduction (Figure 2B). Evolved strains that had 167% increase in glycerol production and 6% decrease in ethanol production were generated. This metabolic shift was accompanied with a substantial re-direction of carbon fluxes towards succinate and 2,3-butanediol, the latter compound being produced at 356% compared to the ancestral strain. On the other hand, acetate production was slightly reduced, the biomass yield was not affected and the evolved strains did not accumulate undesirable organoleptic compounds such as acetaldehyde or acetoin. This demonstrates that adaptive evolution is a potentially valuable alternative to rational design for engineering low alcohol wine yeast strains.

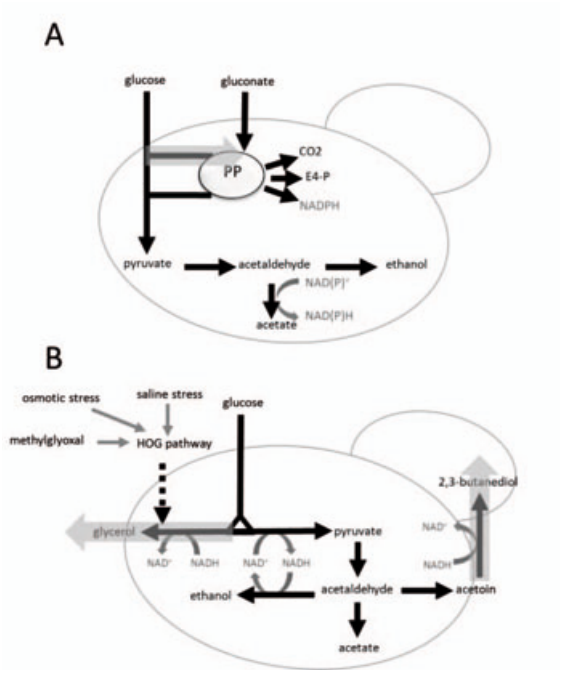


Figure 2 - Evolutionary engineering strategies to reduce alcohol yield

A Rerouting carbons towards pentose phosphate pathway by adaptive evolution on gluconate.

B Adaptive evolution under hyperosmotic or salt stress conditions or in the presence of methylglyoxal, to favour glycerol production.

4. Conclusion

Today, health concerns and consumer's expectations for wines easy to drink has made ethanol reduction a focal point of research. A multitude of research efforts in this area has been made for over 20 years, demonstrating the potential of GM technology to develop low alcohol yeasts. A current major challenge is to develop "low-alcohol yeasts" using technologies that are acceptable to the consumer and which can be used immediately by winemakers. We have recently obtained evolutionary engineered yeasts with sugars diverted towards glycerol and 2.3-butanediol. This is the first example of a low-alcohol yeast strain for the non-GMO wine market. Although such strategies cannot not generate a diversion of carbon flux as high as that achieved by genetic engineering, the availability of non-GM strains, with good overall attributes, able to reduce the alcohol content of wine by 0.5 to 1 % vol/vol offers exciting perspectives. These strains could also represent an essential tool in an integrated strategy involving a combination of different approaches (vine-plant variety, physical and biological methods) to fine tune the alcohol content of wines.

5. References

Cadière A, Ortiz-Julien A, Camarasa C, Dequin S., 2011. Evolutionary engineered *Saccharomyces cerevisiae* wine yeast strains with increased in vivo flux through the pentose phosphate pathway. *Metab. Eng*. **13**, 263–271.

Blomberg A and Adler L., 1992. Physiology of osmotolerance in fungi. *Adv Microb Physiol*. **33**, 145-212.

Cambon B, Monteil V, Remize F, Camarasa C, Dequin S., 2006. Effects of GPD1 overexpression in *Saccharomyces cerevisiae* commercial wine yeast strains lacking ALD6 genes. *Appl. Environ. Microbiol*., **72**, 4688–4694.

de Barros Lopes M, Rehman A, Gockowiak H, Heinrich AJ, Langridge P, Henschke PA. 2000. Fermentation properties of a wine yeast over-expressing the *Saccharomyces cerevisiae* glycerol 3-phosphate dehydrogenase gene (GPD2). *Aust. J. Grape Wine Res*., **6**, 208–215.

Dequin S. and Barre P., 1994. Mixed lactic acid-alcoholic fermentation by *Saccharomyces cerevisiae* expressing the *Lactobacillus casei* L(+)-LDH. *Bio/Technology*, **12**, 173-177.

Ehsani M, Fernández MR, Biosca JA, Julien A, Dequin S., 2009. Engineering of 2,3-butanediol dehydrogenase to reduce acetoin formation by glycerol-overproducing, low-alcohol *Saccharomyces cerevisiae*. *Appl. Environ. Microbiol*., **75**, 3196–3205.

Heux S., Cachon R. and Dequin S., 2006a. Cofactor engineering in Saccharomyces cerevisiae: expression of a H_2O-forming NADH oxidase and impact on redox metabolism. *Metab. Eng*. **8**, 303-314.

Heux S., Sablayrolles J.M., Cachon R. and Dequin S., 2006b. Engineering S. cerevisiae wine yeast that exhibit reduced ethanol production during fermentation under controlled microoxygenation conditions. *Appl. Environ. Microbiol*. **72**, 5822-5828.

Kutyna DR, Varela C, Henschke PA, Chambers PJ, Stanley GA., 2010. Microbiological approaches to lowering ethanol concentration in wine. *Trends Food Sci. Technol*., **21**, 293–302.

Kutyna DR, Varela C, Stanley GA, Borneman AR, Henschke PA, Chambers PJ., 2012. Adaptive evolution of *Saccharomyces cerevisiae* to generate strains with enhanced glycerol production. *Appl. Microbiol. Biotechnol.*, **93**, 1175–1184.

Michnick S, Roustan JL, Remize F, Barre P, Dequin S., 1997. Modulation of glycerol and ethanol yields during alcoholic fermentation in *Saccharomyces cerevisiae* strains overexpressed or disrupted for *GPD1* encoding glycerol 3-phosphate dehydrogenase. *Yeast Chichester Engl.*, **13**, 783–793.

Noble AC, Bursick GF., 1984. The contribution of glycerol to perceived viscosity and sweetness in white wine. *Am. J. Enol. Vitic.*, **35**, 110–112.

Remize F, Roustan JL, Sablayrolles JM, Barre P, Dequin S., 1999. Glycerol overproduction by engineered *Saccharomyces cerevisiae* wine yeast strains leads to substantial changes in by-product formation and to a stimulation of fermentation rate in stationary phase. Appl. Environ. Microbiol., **65**, 143–149.

Schmidtke LM, Blackman JW, Agboola SO., 2012. Production technologies for reduced alcoholic wines. *J. Food Sci*., **77**, R25-41.

Non-conventional yeasts and alcohol levels reduction

Marina Bely[1,*], Philippe Renault[1,2], Telma da Silva[3], Isabelle Masneuf-Pomarède[1,4], Warren Albertin[1], Virginie Moine[2], Joana Coulon[2], Delphine Sicard[3], Dominique de Vienne[3] and Philippe Marullo[1, 2]

[1]ISVV, EA 4577 Œnologie, Université de Bordeaux, 33140 Villenave d'Ornon, France
[2]Laffort, BP17, 33072 Bordeaux cedex, France
[3]CNRS, UMR 0320/UMR 8120 Génétique Végétale, Univ. Paris Sud, 91190 Gif-sur-Yvette, France
[4]Bordeaux Sciences Agro, 1 cours du Général de Gaulle, 33170 Gradignan, France

Abstract: The yeast *Saccharomyces cerevisiae* is one of the most widely used in wine industry. The development of low-alcohol *S.cerevisiae* is a current challenge. Up to now, despite of many efforts, the results are still too poor to answer professional demands. We report here an evaluation of the potential of non-conventional yeasts such as *S.uvarum* species, interspecies hybrids of *S.cerevisiae* and *S.uvarum* and two non-*Saccharomyces* yeasts. EU23 interspecific hybrid showed a mean 0.34%vol. ethanol decrease compared to 10 *S.cerevisiae* starter strains, at 18°C. Interestingly, glycerol increase was not associated with volatile acidity increase. This confirms the interest of interspecific hybrids for lowering alcohol production.
No significant differences were observed between *Torulaspora delbrueckii* and *S.cerevisiae* species for alcohol/sugar yield. In contrast, *Candida zemplinina* species possessed a low alcohol/sugar yield compare to *S.cerevisiae* strains, which can be partially explained by the overproduction of glycerol and volatile acidity. Mixed *C.zemplinina / S.cerevisiae* cultures at 5:1 ratio produced up to 0.90% vol. ethanol less than a pure culture of *S.cerevisiae.* These results appear promising regarding to low-ethanol production but the sensory evaluations of the mixed cultures were not satisfying.

Keywords: low-alcohol wine, hybrid wine yeast, non-*Saccharomyces*, *Candida zemplinina*

1. Introduction

The development of low-alcohol yeasts is a current challenge in wine industry since wines have more and more ethanol contents. During the last years, researchers have been investigating *Saccharomyces cerevisiae* metabolism to reduce the yield ethanol/sugar consumed. Two approaches were used: metabolic engineering strategies diverting sugar metabolism towards products other than ethanol (GMO strategy) (Cambon *et al.*, 2006; Ehsani *et al.*, 2009; Remize *et al.*, 1999) and more recently an adaptive evolution-based strategy, (Cadière *et al.*, 2012, 2011). An alternative to these approaches is to select low-ethanol producers in *S.cerevisiae* species by screening wild yeast population or to use breeding strategies (Marullo *et al.*, 2006). Up to now, despite of many efforts, the results are still too poor to answer professional demands.

In this context, in recent years we have investigated the potential of various non-conventional-yeasts such as *S.uvarum* species, interspecies hybrids of *S.cerevisiae* and *S.uvarum* and non-*Saccharomyces* yeasts. Among the phenotypic traits studied, the capacity of these yeasts to reduce the alcohol levels has also been evaluated.

2. Potential of *Saccharomyces uvarum* species and its hybrid with *S.cerevisiae*

In some specific ecological niches, *S.uvarum* contributes to alcoholic fermentation in a mixed population with *S.cerevisiae*. The physiological and technological properties of *S.uvarum* were investigated in several studies (Masneuf-Pomarède *et al.*, 2010; Naumov *et al.*, 2002; Castellari *et al.*, 1994; Rainieri *et al.*, 1999; Walsh and Martin, 1977; Kishimoto and Goto, 1995). This species is characterized by its cryotolerance and its capacity to produce high levels of 2 phenyl-ethanol and its corresponding acetate. In contrast, at 24 °C, its low ethanol tolerance was clearly revealed as discriminative trait, distinguishing this species from *S.cerevisiae*. Natural interspecies hybrids of *S.cerevisiae* and *S.uvarum* are not rare in wine environments (Le Jeune *et al.*, 2007) thus promoting adaptation through the heterosis phenomenon to new ecological niches.

Corresponding author : marina.bely@u-bordeaux2.fr

Sixty six yeast strains were used in this study: 7 *S.cerevisiae*, *4 S.uvarum* and 55 synthetic hybrids (28 interspecifics and 27 intraspecifics). The parental strains came from different beverage industries (wine, distillery and cider) as well as from nature. Fermentations were carried out in triplicate at 2 temperatures (18 °C and 26 °C) in Sauvignon Blanc grape must containing 188 g/L sugar. Several phenotypic traits were measured: fermentation kinetics, yeast population, aromatic profiles and fermentation products.

Most strains completed alcoholic fermentation with less than 1.6 g/L residual sugar. The analytical results for ethanol production during fermentation at 26 °C showed variations in the dataset but there was no significant difference in alcohol/sugar yield among the three strains groups (*S.cerevisiae*, *S.uvarum*, interspecific hybrids). In contrast, those obtained at 18 °C were interesting since as shown in figure 1, *S.uvarum* group (parental and intraspecific hybrid strains) produced 0.30 %vol. less ethanol than *S.cerevisiae* group (parental and intraspecific hybrid strains), whereas intermediate ethanol production was observed for the interspecific hybrid group with high performances for some strains, closed to *S.uvarum* group. In particular, EU23 interspecific hybrid showed 0.30 %vol. ethanol decrease compared to *S.cerevisiae* group. This interesting hybrid was further tested in synthetic medium with higher sugar content (230 g/L), and compared to 10 strains commercialized for winemaking. EU23 showed, at 18°C, a mean 0.34 %vol. ethanol decrease compared to the 10 starter strains, related to glycerol overproduction (9.0 g/L compared to 6.5 g/L for commercial strains). Interestingly, glycerol increase was not associated with volatile acidity increase. This confirms the interest of interspecific hybrids for lowering alcohol production. Future experiments should combine other selection method (like adaptive evolution) with interspecific hybrids production and selection.

3. Potential of non-*Saccharomyces* yeasts

The fermentation of grape must is a complex microbial process, involving sequential development of various yeast communities. During the few hours of fermentation, the predominant yeast belongs to the *Hanseniaspora*, *Kloeckera*, *Pichia*, *Candida* and *Metschnikowia* genera. However, other non-*Saccharomyces* yeast, such as *Torulaspora delbrueckii*, *Schizosaccharomyces pombe* *Kluyveromyces* spp., *Issatchenkia* spp., *Zygosaccharomyces bailli*, etc, may also be present. These species start the fermentation until nutrient depletion, the increasing ethanol content and the heat production fermentation (Salvadó *et al.*, 2011; Goddard, 2008) gradually eliminating the less tolerant species, thus favouring the development of *S.cerevisiae* which completes the fermentation (Heard and Fleet, 1985). Many researchers have investigated the specific metabolisms of the various non-*Saccharomyces* yeast species and their potential applications in the wine industry.

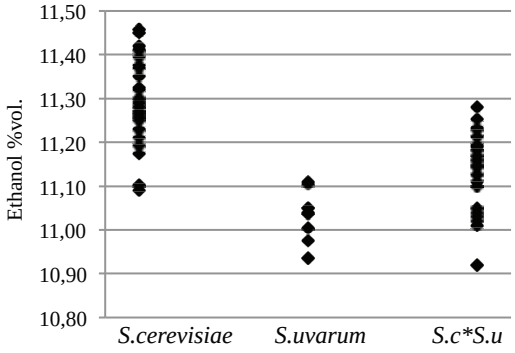


Figure 1. Ethanol production in Sauvignon wine of *S.cerevisiae* group (parental and intraspecific hybrid), *S.uvarum* group (parental and intraspecific hybrid) and interspecific hybrids (*S.c*S.u*). Fermentations were carried out at 18 °C.

Two species, *Torulaspora delbrueckii* and *Candida zemplinina* were evaluated in our laboratory and compared to *S.cerevisiae* species.

3.1 Torulaspora delbrueckii species

Thirty *T.delbrueckii* strains of various origins (wine, cider, milk) were used. A model synthetic medium was used to simulate standard grape juice (Renault *et al.*, 2009). All fermentations were performed in triplicate at 24 °C.

Our experiments confirmed the low volatile acidity and glycerol production of *T.delbrueckii*, as previously described (Herraiz *et al.*, 1990; Ciani and Maccarelli, 1997; Ciani and Picciotti, 1995; Moreno *et al.*, 1991). This species is characterized, among non-*Saccharomyces* yeasts, by a good ethanol production: the majority of strains produced 8 to 11 % and 7 to 10 % ethanol vol. at 17 °C and 24 °C, respectively, with a maximum ethanol

concentration of 12.35 %vol. at 17 °C and 10.90 %vol. at 24 °C (Renault *et al.*, 2009). Nevertheless, no significant differences were observed between *T.delbrueckii* and *S.cerevisiae* species for alcohol/sugar yield, regardless of the temperature.

3.2 Candida zemplinina species

Recent studies carried out over 5 years suggested the possible use of *C.zemplinina* species in winemaking.
Comparative evaluation of some technological properties of different wine species showed that this species is highly fructophilic, osmotolerant, and is a high glycerol producer (Tofalo *et al*., 2012; Sadoudi *et al.*, 2012; Sipiczki, 2003; Rantsiou *et al.*, 2012). Magyar and Tóth showed a lower alcohol/sugar yield in 5 *C.zemplinina* strains (Magyar and Tóth, 2011). The aim of our study was to investigate potential application of this species in mixed starters with S.*cerevisiae*. Firstly, we characterized this species by analysing fermentation traits of a wide sample of yeasts. The second step was the selection of pertinent strains to associate them to S.*cerevisiae*.

Forty-eight *C.zemplinina* isolates, mainly from Bordeaux must and also from Hungary and Italy were used. Three *S.cerevisiae* were also tested for their fermentation performance.

Fermentations were carried out in pasteurized Merlot grape must containing 240 g/L sugar at 24°C. All fermentations were performed in triplicate.

Table 1. Main oenological characters in Merlot wine of pure cultures at 24 °C, means of triplicate fermentations ± SD.
* Fermentations stopped before the end of fermentation

	C. zemplinina (n=48)			*S.cerevisiae* (n=3)	
	Minimum	*Maximum*	*Average*	*Average*	*Average*
Ethanol (%vol.)	6.6 ± 0.1	9.9 ± 0.7	7.7 ± 0.8	7.94* ± 1.49	13.9 ± 0.3
Residual sugar (g/L)	48.3 ± 13.9	94.3 ± 1.9	79.1 ± 11.2	91.1 ± 21.5	1.3 ± 0.1
Yield ethanol/sugar (g/g)	0.35 ± 0.00	0.41 ± 0.00	0.37 ± 0.01	0.42 ± 0.01	0.46 ± 0.01
Volatile acidity (g/L acetic acid)	0.48 ± 0.01	1.15 ± 0.06	0.72 ± 0.17	0.18 ± 0.05	0.33 ± 0.02
Glycerol (g/L)	11.9 ± 0.8	14.8 ± 0.8	13.6 ± 0.7	6.09 ± 0.34	7.6 ± 0.3

3.2.1 Pure cultures

Under these conditions, all *S.cerevisiae* strains completed the alcoholic fermentation while inoculating musts with *C.zemplinina* yeast resulted in stuck fermentations, confirming the low fermentation capacity of this species reported in the literature. In order to compare the two species, some additional fermentations of *S.cerevisiae* were stopped when an ethanol concentration of approximately 8%vol. was reached. As expected, this species displayed a clear fructophilic character (not shown). The results indicate relevant differences between yeast strains in terms of ethanol, volatile acidity and glycerol production (Table 1). *C.zemplinina* group possessed a poor alcohol/sugar yield, 12% less compare to *S.cerevisiae* strains, which can be partially explained by the overproduction of glycerol.

3.2.2 Co-cultures and sequential cultures

We investigated the possibility of using *C.zemplinina*, as partner of *S.cerevisiae*, in mixed fermentations in order to reduce the alcohol production. For this purpose, some strains were selected for their fermenting characteristics (high fermentation rate, low yield and low volatile production) and their olfactive properties. Indeed, *C.zemplinina* species was described to be a high producer of sulfure derived compounds (Tofalo *et al.*, 2012), by-products highly detrimental to wine quality. *C.zemplinina* yeasts were classed in 2 groups depending on the olfactive perception. Thirty four strains (group 1) were rejected due to high level of undesirable flavors in wine, 14 were perceived as acceptable or neutral (group 2). A subset of 5 *C.zemplinina* strains from the last group was chosen for the following tests.

Mixed cultures were inoculated with 10^7 viable cells/mL *C.zemplinina* and 2.10^6 viable cells/mL *S.cerevisiae*. Sequential fermentations were inoculated with *C.zemplinina* (10^7 viable cells/mL), followed by *S.cerevisiae* (2.10^6

viable cells/mL) after 24 or 48 hours' fermentation.

All mixed cultures were able to complete fermentation. In our conditions, no significant differences in ethanol production were observed between *S.cerevisiae* FX10 Zymaflore culture and co-cultures (simultaneous inoculations) (data no shown). In contrast, sequential fermentations gave reductions of ethanol production from to 0.39 to 0.90 % vol. (Table 2). Note that for the sequential culture with *C.zemplinina* 401 a higher reduction is observed when *S.cerevisiae* is added earlier during fermentation.

Table 2. Main oenological characters in Merlot wine of sequential cultures *C.zemplinina/S.cerevisiae* at 24 °C. Means of triplicate fermentations ± SD

	Sequential cultures *C. zemplinina*/*S.cerevisiae*						Pure culture
	Strain 401		Strain 629	Strain 261	Strain 153	Strain 278	*S. cerevisiae*
	24h	48 h	48h	48h	48h	48h	FX10
Ethanol (%vol.)	13.16 ± 0.07	13.52 ± 0.18	13.46 ± 0.05	13.41 ± 0.08	13.51 ± 0.04	13.01 ±0.04	13.91 ± 0.00
Residual sugar (g/L)	0.87 ± 0.14	0.90 ± 0.20	0.90 ± 0.10	1.33 ± 0.75	0.83 ± 0.06	0.95 ± 0.07	0.95 ± 0.07
Yield ethanol/sugar (g/g)	0.43 ± 0.00	0.45 ± 0.00	0.44 ± 0.00	0.44 ± 0.00	0.45 ± 0.00	0.43 ± 0.00	0.46 ± 0.00
Volatile acidity (g/L acetic acid)	0.45 ± 0.01	0.84 ± 0.15	0.83 ± 0.07	0.76 ±0.04	0.81 ± 0.07	1.01 ± 0.06	0.31 ±0.04
Glycerol (g/L)	13.03 ± 0.87	14.72 ± 0.80	15.36 ± 0.95	15.76 ± 0.62	15.21 ± 0.46	15.76 ±0.01	7.30 ± 0.48

Sequential cultures: musts were inoculated with 10^7 viable cells/mL of *C. zemplinina* , followed by *S.cerevisiae* (2.10^6 viable cells/mL) after 24 or 48 hours' fermentation

Sequential cultures produced 1.8 to 2.2-fold more glycerol than *S.cerevisiae* alone, confirming the specific characteristic of high production of these yeasts. Final volatile acidity concentrations were up to 3.3 fold more than *S.cerevisiae pure* culture. Considering the reduction in alcohol/sugar yield, glycerol and volatile acidity, the best sequential multistarter was *C.zemplinina* 401 with *S.cerevisiae* added after 24 hours' fermentation. A sensory evaluation was then performed. Mixed culture wines were judged to be significantly different from *S.cerevisiae*'s wine which was the best evaluated. Further investigations are required to clarify the molecular mechanism underlying the reduction of ethanol production in *C.zemplinina* species. Its use in sequential culture appears promising regarding low-ethanol production, but an important effort has to be done to propose *C.zemplinina* strains with neutral impact on the organoleptic perception of wine. In this respect, traditional selection/breeding programs, using non-GMO strategies, could be undertaken.

Acknowledgments : Authors thank Matthias Sipiczki (University of Debrecen) for the loan of their strains and Sarco laboratory for wine analysis. This work was supported by the ANR program (ANR-08-ALIA-009) and Laffort company.

4. References

Cadière A., E. Aguera, S. Caillé, A. Ortiz-Julien and S. Dequin. 2012. Pilot-scale evaluation the enological traits of a novel, aromatic wine yeast strain obtained by adaptive evolution. *Food Microbiology*, **32**, 332–337.

Cadière A., A. Ortiz-Julien, C. Camarasa and S. Dequin. 2011. Evolutionary engineered *Saccharomyces cerevisiae* wine yeast strains with increased *in vivo* flux through the pentose phosphate pathway. *Metabolic Engineering*, **13**, 263–271.

Cambon B., V. Monteil, F. Remize, C. Camarasa and S. Dequin. 2006. Effects of GPD1 Overexpression in *Saccharomyces cerevisiae* Commercial Wine Yeast Strains Lacking ALD6 Genes. *Applied and Environmental Microbiology*. **72**, 4688–4694.

Castellari L., M. Ferruzi, A. Magrini, G. Giudici, P. Passarelli and C. Zambonelli. 1994. Unbalanced wine fermentation by cryotolerant vs. non-cryotolerant *Saccharomyces* strains. *Vitis*, **33**, 49–52.

Ciani M., and F. Maccarelli. 1997. Oenological properties of non-*Saccharomyces* yeasts associated with wine-making. *World Journal of Microbiology and Biotechnology*, **14**, 199–203.

Ciani M., and G. Picciotti. 1995. The growth kinetics and fermentation behaviour of some non-*Saccharomyces* yeasts associated with wine-making. *Biotechnology Letters*, **17**, 1247–1250.

Ehsani M., M.R. Fernandez, J.A. Biosca, A. Julien and S. Dequin. 2009. Engineering of 2,3-Butanediol Dehydrogenase To Reduce Acetoin Formation by Glycerol-Overproducing, Low-Alcohol *Saccharomy-ces cerevisiae*. *Applied and Environmental Microbiology*, **75**, 3196–3205.

Goddard M.R. 2008. Quantifying the complexities of *Saccharomyces cerevisiae* ecosystem engineering via fermentation. *Ecology*, **89**, 2077–2082.

Heard G.M. and G.H. Fleet. 1985. Growth of Natural Yeast Flora during the Fermentation of Inoculated Wines. *Appl. Environ. Microbiol.*, **50**, 727–728.

Herraiz T., G. Reglero, M. Herraiz, P.J. Martin-Alvarez and M.D. Cabezudo. 1990. The influence of the yeast and type of culture on the volatile composition of wines fermented without sulfur dioxide. *American Journal of Enology and Viticulture*, **41**, 313–318.

Le Jeune C., M. Lollier, C. Demuyter, C. Erny, J.L. Legras, M. Aigle and I. Masneuf-Pomarède. 2007. Characte-rization of natural hybrids of *Saccharomyces cerevisiae* and *Saccharomyces bayanus var. uvarum*. *FEMS Yeast Research*. **7**, 540–549.

Kishimoto M. and S. Goto. 1995. Growth temperatures and electrophoretic karyotyping as tools for practical discrimination of *Saccharomyces bayanus* and *Saccharomy-ces cerevisiae*. *Journal of General and Applied Microbiology*. **41**, 239–247.

Magyar I., and T. Tóth. 2011. Comparative evaluation of some oenological properties in wine strains of *Candida stellata*, *Candida zemplinina*, *Saccharomyces uvarum* and *Saccha-romyces cerevisiae. Food Microbiology*, **28**, 94–100.

Marullo P., M. Bely, I. Masneuf-Pomarède, M. Pons, M. Aigle and D. Dubourdieu. 2006. Breeding strategies for combining fermentative qualities and reducing off-flavor production in a wine yeast model. *FEMS Yeast Research*. **6**, 268–279.

Masneuf-Pomarède I., M. Bely, P. Marullo, A. Lonvaud-Funel and D. Dubourdieu, 2010. Reassessment of phenotypic traits for *Saccharomyces bayanus var. uvarum* wine yeast strains. *International Journal of Food Microbiology*, **139**, 79–86.

Moreno J.J., C. Millán, J.M. Ortega and M. Medina. 1991. Analytical differentiation of wine fermentations using pure and mixed yeast cultures. *Journal of Industrial Microbiology*. **7**, 181–189.

Naumov G.I., E.S. Naumova, Z. Antunovics and M. Sipiczki. 2002. *Saccharomyces bayanus var. uvarum* in Tokaj wine-making of Slovakia and Hungary. *Applied Microbiology and Biotechnology*, **59**, 727–730.

Rainieri S., C. Zambonelli, J.E. Hallsworth, A. Pulvirenti, and P. Giudici. 1999. *Saccharomyces uvarum*, a distinct group within *Saccharomyces sensu stricto*. *FEMS Microbiology Letters*, **177**, 177–185.

Rantsiou K., P. Dolci, S. Giacosa, F. Torchio, R. Tofalo, S. Torriani, G. Suzzi, L. Rolle and L. Cocolin. 2012. *Candida zemplinina* can reduce acetic acid produced by *Saccharomy-ces cerevisiae* in Sweet Wine Fermentations. *Applied and Environmental Microbiology*, **78**, 1987–1994.

Remize, F., J.L Roustan, J.. Sablayrolles, P. Barre and S. Dequin. 1999. Glycerol overproduction by engineered *Saccharomyces cerevisiae* wine yeast strains leads to substantial changes in by-Product formation and to a stimulation of fermentation rate in stationary phase. *Applied and Environnemental Microbiology*, **65**, 143–149.

Renault P., C. Miot-Sertier, P. Marullo, P. Hernández-Orte, L. Lagarrigue, A. Lonvaud-Funel and M. Bely. 2009. Genetic characterization and phenotypic variability in *Torulaspora delbrueckii* species: Potential applications in the wine industry. *International Journal of Food Microbiology*, **134**, 201–210.

Sadoudi M., R. Tourdot-Maréchal, S. Rousseaux, D. Steyer, J.J. Gallardo-Chacón, J. Ballester, S. Vichi, R. Guérin-Schneider, J. Caixach and H. Alexandre. 2012. Yeast–yeast interactions revealed by aromatic profile analysis of Sauvignon blanc wine fermented by single or co-culture of non-*Saccharomy-ces* and *Saccharomy-ces* yeasts. *Food Microbiology*, **32**, 243–253.

Salvadó Z., F.N. Arroyo-López, E. Barrio, A. Querol and J.M. Guillamón. 2011. Quantifying the individual effects of ethanol and temperature on the fitness advantage of *Saccharomyces cerevisiae*. *Food Microbiology*, **28**, 1155–1161.

Sipiczki M. 2003. *Candida zemplinina* sp. nov., an osmotolerant and psychrotolerant yeast that ferments sweet botrytized wines. *Int. J. Systematic Evolutionary Bacteriology*, **53**, 2079–2083.

Tofalo R., M. Schirone, S. Torriani, K. Rantsiou, L. Cocolin, G. Perpetuini and G. Suzzi. 2012. Diversity of *Candida zemplinina* strains from grapes and Italian wines. *Food Microbiology*, **29**, 18–26.

Walsh R.M., and P.A. Martin. 1977. Growth of *Saccharomyces cerevisiae* and *Saccharomyces uvarum* in temperature gradiant incubator. *Journal of Institute of Brewing*. **83**, 169–172.

Finding novel carbon sinks in *S. cerevisiae*

Florian F. Bauer[1]*, Debra Rossouw[1] and Jaco Franken[1]

[1]Institute for Wine Biotechnology, Department of Viticulture and Oenology, Stellenbosch University, Matieland, 7602, South Africa

Abstract: The demand for wines with reduced alcohol levels has led to the development of technologies to reduce ethanol concentrations without compromising wine sensory characteristics. One approach to achieve such a target is to divert carbon flux in fermenting yeast away from ethanol formation. However, several attempts have had mixed successes, with reduction in alcohol frequently being accompanied by the generation of compounds that negatively affect wine quality. Here we investigate the use of new carbon sinks as metabolic endpoints for sugar-derived carbon. A first approach screened a collection of metabolism mutants for decreased ethanol formation, and identified the trehalose-phosphate synthase, TPS1, as a target for lower ethanol fermentation when deleted or over-expressed. TPS1 was placed under the control of two different growth stage specific promoters to modify expression and subsequently trehalose production. The data indicate that ethanol was successfully lowered as a consequence of these modifications without significant impacts on fermentation behavior. A second approach investigated the possibility of introducing novel pathways for the production and accumulation of sugar polymers in yeast. Production of such heterologous polymers was successfully achieved and has laid the groundwork for diverting carbon flux to foreign carbon sinks in future.

Keywords: Trehalose, levan, glycolysis, alcohol

1. Introduction

Methods to achieve wines with lower ethanol concentrations have been a major focus of wine research in the past decade, due mainly to viticultural practices which favour harvesting berries at later stages of development to ensure phenolic ripeness, optimal flavour balance and lower acidity (Hoffmann 1990; Scudamore-Smith and Moran, 1997; Pickering, 2000). The resulting higher berry sugar levels lead to higher ethanol levels at the end of alcoholic fermentation. This presents several problems on a sensory, health and financial level. High ethanol levels can be perceived as 'hotness' on the palate and can also alter the volatility and sensory perception of other aroma compounds (Guth and Sies, 2002). Concerns are also raised on a health and safety level regarding the consumption of such high alcohol wines. Furthermore, taxes are levied according to the ethanol content of wines. This is particularly troublesome for the South African wine export sector due to the high temperatures experienced in many of the traditional winemaking regions of the country (de Barros Lopes *et al*., 2003; Pickering *et al*., 1998).

Several different pre- and post-fermentation approaches to lowering ethanol levels in wine have been pursued in the past, with measured success. Physical post fermentation processes such as spinning cone columns, reverse osmosis, filtration and distillation processes are associated with a loss of flavor, while adding to the expense of wine production (Bui *et al*., 1986; Schobinger *et al*., 1986; Wright and Pyle, 1996; Gómez-Plaza *et al*., 1999; García-Martín *et al*., 2010; Belisario-Sánchez *et al*., 2011; Catarino and Mendes, 2011). Microbiological approaches focusing on yeast strains and using traditional breeding have been largely unsuccessful thus far. Genetic modification on the other hand has been successful in the generation of lower ethanol yielding strains. Several of these approaches have focused on using glycerol as a carbon sink. However, these lower ethanol strains are widely characterised by an increase in other unwanted end-products of fermentation (such as acetaldehyde and butanediol) and/or a decrease in fermentation efficacy (Remize *et al*., 1999; Remize *et al*., 2000; Varela *et al*., 2012).

Here we present two alternative strategies for the lowering of wine ethanol levels (Figure 1), both using storage carbohydrates as a carbon sink. The first is aimed at directing carbon to an alternative endogenous carbon sink as opposed to ethanol, by modification of endogenous yeast gene expression. The second approach involves the introduction of heterologous metabolic pathways to introduce an alternative carbon sink for fructose, thus decreasing flux to ethanol.

In our first strategy, 44 haploid strains from the EUROSCARF library with deletions in carbon metabolic genes were screened. And genes were identified as possible targets for ethanol reduction via either down-regulation or overexpression. One gene in particular, namely *TPS1* (trehalose-6-phosphate synthase) was identified as a suitable target for overex-

Corresponding author : fb2@sun.ac.za

pression in a commercial wine yeast strain. *TPS1* encodes the synthase subunit of the trehalose-6-phosphate synthase/phosphatase complex, which synthesizes the storage carbohydrate trehalose (Bell *et al*. 1998). The overexpression strategy that we decided to follow was unique in the sense that growth stage –specific promoters were used to target overexpression to a particular phase of fermentation. Also, the promoters selected for this purpose were of intermediate strength. This meant that increases in TPS expression were moderate and controlled, avoiding the complications and unrelated phenotypes associated with extreme and unregulated overexpression of transgenes. The controlled promoter strategy was successfully applied and resulted in moderate and stage –specific increases in TPS gene expression in a commercial wine yeast, VIN13. Trehalose accumulation was increased and ethanol levels decreased, without significantly inhibiting the growth and fermentation rate of the transformed yeast.

In the second strategy fructans, where used as a point of accumulation for fructose units. These polymers consist of multiple fructose units, which occur in a broad range of micro-organisms and a limited number of plant species as non-structural storage carbohydrates (Banguela *et al.*, 2011). Fructans have been attracting attention as potential pharmaceutical preparations or as functional foods due to pre-biotic and health enhancing properties (Lafraya *et al.*, 2011). Synthesis of fructans is catalyzed by a group of enzymes referred to as fructosyltransferases (FTFs) that synthesize fructan polymers, using sucrose as substrate. As these carbohydrates are foreign to yeast, no native degradation is expected in *S. cerevisiae*. Furthermore, this strategy should generate a limited metabolic burden as there is no theoretical impact on redox cycle. In addition, the production of unwanted metabolites would theoretically be avoided due to the metabolically non-intrusive nature of such an engineered system.

To determine the viability of producing and accumulating these fructose polymers in yeast, two separate sucrose accumulation strains where constructed in a Δ*suc2* genetic background. These yeast were transformed with the genes encoding either sucrose synthase (Susy; cloned from potato; Salanoubat *et al*. 1987) or the tobacco sucrose transporter (SUT; Reismeier *et al*., 1992). These sucrose accumulating strains were transformed with the plasmids bearing M1FT, a fuctosyltransferase from *Leuconostoc mesenteroides* (Kang *et al*., 2005) or a truncated version of the same gene, with its N-terminal secretion signal removed. The data indicates that the engineered strains are indeed able to accumulate sucrose and to functionally express M1F. Furthermore, the strains synthesized and accumulated levan inside the yeast cells in the presence of oxygen. However, no levan was produced during fermentative conditions. The findings open the possibility of engineering lower alcohol producing yeast strains that use levan as a carbon sink.

2. Materials and methods

2.1. Selection of deletion strains

Saccharomyces cerevisiae strains from the EUROSCARF library were selected from the haploid BY4742 (MATa ; his3Δ1; leu2Δ0; lys2Δ0; ura3Δ0) background. Homozygous deletion strains for essential genes were selected from the diploid BY4743 (MATa/MATa; *his3*Δ1/*his3*Δ1; *leu2*Δ0/*leu2*Δ0; *met15*Δ0/*MET15*; *LYS2*/*lys2*Δ0; *ura3*Δ0/*ura3*Δ0) background. All strains from the EUROSCARF library were confirmed as described by Rossouw *et al*. (2013). For the industrial yeast, the commercial strain VIN13 (Anchor yeast) was used.

2.2. Construction of TPS-overexpression plasmids and transformation of industrial yeast

A centromeric plasmid was constructed as described by Rossouw *et al*. (2013) to express the *TPS1* gene cloned from S. cerevisiae under the control of two low strength promoters. The first (*DUT1*) is active during the exponential growth phase of the yeast while the second (*GIP2*) is active during stationary phase.

Final constructs were transformed into the VIN13 yeast strain by electroporation (Wenzel *et al*. 1992,; Lilly *et al*. 1996).

Four strains were generated in this part of the study: Two control strains transformed with the plasmids containing only the *DUT1* or *GIP2* promoter regions (named DUT-control and GIP-control) and two *TPS1* over-expression strains transformed with plasmids containing the promoter regions as well as the *TPS1* gene (named DUT-TPS and GIP-TPS).

2.3. Fermentation conditions

All strains were maintained on YPD plates and overnight cultures grown in YPD broth. For the initial screen, small scale fermentations were conducted in 100 ml tubular bottles, sealed with a rubber bung and an S-bend airlock. These fermentations were conducted in 80 ml synthetic wine must MS300 (Bely *et al.*, 1990) with 100, 200 or 250 $g.l^{-1}$ initial hexoses as indicated in the text. Glucose and fructose were added in equimolar quantities. All fermentations were inoculated to an initial cell density of OD_{600} = 0.1. All fermentations were conducted with at least three independent replicates. The progression of fermentations was monitored by weight loss (indicative of CO_2 generation) and allowed to run to completion at room temperature.

Fermentations conducted with control and transformed industrial yeast strains were carried out in 250 ml Erlenmeyer flasks containing 200 ml MS300 and sealed with rubber bung and S-bend airlocks.

Glucose and fructose were added in equal amounts (125 $g.l^{-1}$). Pre-inoculated cultures were grown overnight at 30°C in YPD broth containing 1 $g.l^{-1}$ zeocin and used to inoculate fermentations to a cell density (OD_{600}) of 0.1. All fermentations were carried out in triplicate. Fermentations were monitored by weight loss and cell proliferation was determined by OD_{600} readings.

2.4. Real-time PCR

To verify gene expression in the transformed and control strains, RNA isolations were performed on samples taken at time points (corresponding to days) T2, T5, T 11 and T18 to cover the range of different growth phases of the yeast during fermentation. RNA was extracted using the hot phenol extraction protocol (Schmitt *et al*. 1990). Real-time PCR was carried out as described by Rossouw *et al*. (2013). Data analyses were conducted using Signal Detection Software (SDS) v. 1.3.1 (Applied Biosystems) to determine the corresponding Ct values and PCR efficiencies respectively for the samples analysed (Ramakers *et al*. 2003).

2.5. Metabolite analysis

Glucose, fructose, glycerol, ethanol and trehaolse were quantified as outlined by Rossouw *et al*. (2013).

2.6. Construction of levan producing strains

All experiments testing levan production in *S. cerevisiae* was tested in invertase negative (*suc2*Δ) yeast strains. Sucrose accumulation strains where constructed by expressing either a potato sucrose synthase (SuSy) or a sucrose transporter (SUT) from spinach in a *suc2*Δ genetic background. Both SUT and SuSy was expressed from *PGK1* based expression cassettes, that where integrated at the *SPR3* locus. The M1FT levansucrase, from *Leuconostoc mesenteroids* was cloned using known sequence information (Kang *et al*, 2005), and expressed using the constitutive *PGK1* promoter/terminator expression cassette that was cloned into the yeast shuttle vector YCpLac33. Similarly a truncated version of M1FT, without its native secretion signal, was also expressed using the same vector. The M1FT constructs where expressed in both the BY4742Δ*suc2* genetic background and also in the two sucrose accumulation strains. Cell extracts and culture supernatants were analyzed for the presence of fructose polymers using thin layer chromatography.

2.7. Levan production conditions and media

For sucrose accumulation and levan production experiments, cultures were grown in SCD media containing either 4% (w/v) glucose and 4% (w/v) fructose (SCGF) for the sucrose synthase (SuSy) expressing strains or with 5% (w/v) sucrose and 3% (w/v) glucose (SCDS) in the case of the sucrose transporter (SUT) carrying strains. Similarly, for experiments using rich media, strains were grown in 1% yeast extract, 2% peptone and either 4% (w/v) glucose and 4% (w/v) fructose (YPGF) for the sucrose synthase (SuSy) bearing strains or with 5% (w/v) sucrose and 3% (w/v) glucose (YPDS) for the SUT bearing strains. Fermentations where performed in either rich or minimal media with a 100g/L of total sugar ($YPDS^{Fermentative}$; containing 100g/L glucose with 3% sucrose for the SUT strains or $YPGF^{Fermentative}$; containing glucose and fructose in a 1:1 ratio to a total of 100g/L for the SuSy strains).

3. Results and discussion

3.1. Initial screen for altered ethanol yield

Sixty four single deletion mutants (EUROSCARF, haploid background BY4742 with the exception of essential genes in which case the diploid BY4743 background deletion was used) were systematically selected with the aim of identifying steps in central carbohydrate metabolism that could potentially cause changes in carbon flux and metabolite yields under simulated wine fermentation conditions (Table 1). Of particular interest were strains with dual abilities to consume all sugars and increase flux either towards or away from ethanol. Deletion mutants in the BY7472 genetic background showed huge variations in improved or inferior abilities to yield ethanol and utilize sugars (Table 1).

Of the haploid strains there were five with significantly lower and 30 with significantly higher ethanol yields. Knockout strains of *TPS1* and *TDH3* had the lowest ethanol yields as well as showing improved abilities to utilize sugars, making these strains attractive for further study.

For the *tdh3*Δ and *tps1*Δ strains independent knockouts were created to confirm phenotypes. Fermentations with these strains were repeated and previous results were reproduced using the newly-generated knockout strains. Overexpressions were also carried out with the *TDH3* and *TPS1* genes in a wild-type BY4742 genetic background. Both strains demonstrated a reduced fermentation capacity as defined by slower rates of CO_2 production. The *TPS1* overexpression strain was the only one to render a significantly lower ethanol yield. The significantly reduced ethanol yields of the *TPS1* overexpressing lab yeast suggested the possibility of using *TPS1* overexpression (albeit at less extreme levels) to decrease flux to ethanol without significantly impacting fermentative performance.

3.2. Over-expression of the TPS1 gene in industrial yeast

Based on the results of the mutant screen in the haploid laboratory yeast several genes would be potential knockout targets for future modification in wine yeast. *TPS1* was initially selected for overexpression

in an industrial yeast genetic background (VIN13 strain) as 'proof-of-concept' to extend the results from experiments conducted with the laboratory strains to diploid wine yeast strains used in industry. For this purpose, two different low-strength promoters were selected to drive expression of the transgene. The promoters (*DUT1* and *GIP1*) drive gene expression during early exponential growth of the yeast and early stationary phase respectively (Figure 1). The aim was to slightly increase *TPS1* gene expression and enzyme activity without burdening the yeast cell and challenging redox balance. Relative gene expression was determined at four time points during fermentation (data not shown). The first time point (day 2 of fermentation) is representative of the exponential growth phase. A significant (> 2-fold) over-expression of the TPS1 gene under the control of the DUT1 promoter was observed during the exponential growth phase compared to the wild type (VIN13) and DUT control (plasmid containing the promoter only). The second time point (day 5) is representative of early stationary phase and a significant over-expression (up to 60% increase) of the *TPS1* gene under the control of the *GIP2* promoter is observed (data not shown).

3.3. Trehalose production by TPS1 over-expressing strains

Trehalose levels show typical trends, increasing as fermentation progresses through to the stationary growth phase of the yeast, subsequently declining towards the end of fermentation. Sampling for trehalose was also carried out at time points T2, T5, T11 and T18 (corresponding to days). At T2 the DUT-TPS strain shows significantly higher levels of trehalose production compared to all other strains and the wild-type control (Figure 2, frame A). This increase in trehalose in the DUT-TPS strain persists throughout fermentation, though the difference is less obvious and not statistically significant for the T18 timepoint. Trehalose levels in the GIP-TPS strain increase beyond wild-type levels by T5, aligning with the increase in TPS gene expression observed for this time point (Figure 2, frame A).

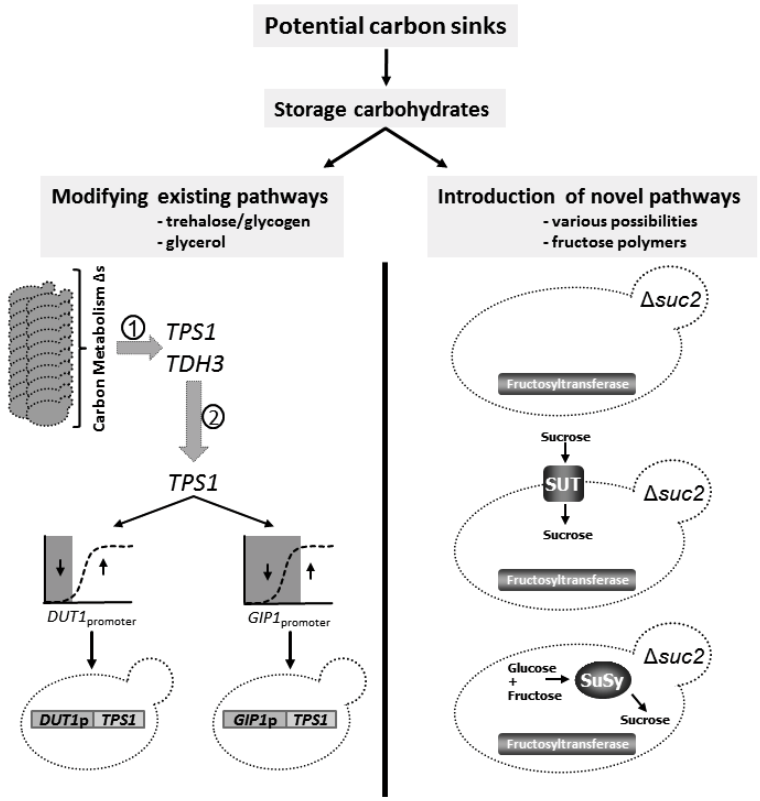


Figure 1. The strategies considering potential carbon sinks as an alternative to ethanol. In the first strategy, the modification of existing yeast pathways was investigated. Sixty-six yeast deletion mutants were screened for lower alcohol levels (1). *TPS1* and *TDH3* were identified as the most promising candidates from this screen. Further over-expression analysis eliminated *TDH3*, encoding a trehalose-phosphate synthase, as candidate for expression modification. Expression was altered to either be induced during early exponential (under the control of the *DUT1* promoter) or early stationary exponential growth (under control of the *GIP1* promoter). A second strategy investigated heterologous expression of sugar polymerizing enzymes in order to introduce novel pathways that would divert carbon away from glycolysis. This strategy used three separate yeast strains as base to express a bacterial fructosyltransferase (FTF), which catalyzes the formation of fructose polymers. The base strains included i) an invertase null mutant (*suc2*Δ) and also the same strain expressing a ii) sucrose transporter (SUT) or iii) sucrose synthase (SuSy), which would respectively import and synthesize the FTF substrate sucrose.

Table 1. Deletion strains from the EuroScarf collection used to screen for lower ethanol yield under simulated wine fermentation conditions. Up arrows (↑) are used to indicate increases in either the ethanol yield or residual sugars of at the end of alcoholic fermentation for each of the inoculated mutant yeast while down arrows (↓) indicate a reduced residual sugar level or final ethanol yield. No significant change is indicated by 'nc'.

	Standard name	Systematic name	Ethanol yield	Residual sugars
Glycolysis				
hexokinase I	*HXK1*	YFR053c	nc	↓
hexokinase II	*HXK2*	YGL253w	↑	↓
phosphofructokinase I	*PFK1*	YGR240c	nc	↑
phosphofructokinase II	*PFK2*	YMR205c	↑	↑
glyceraldehyde-3-phosphate dehydrogenase I	*TDH1*	YJL052w	↑	nc
glyceraldehyde-3-phosphate dehydrogenase II	*TDH2*	YJR009c	↑	nc
glyceraldehyde-3-phosphate dehydrogenase III	*TDH3*	YGR192c	↓	↓
pyruvate kinase II	*PYK2*	YOR347c	nc	nc
Anapleurotic reactions				
pyruvate decarboxylase I	*PDC1*	YLR044c	nc	↓
pyruvate decarboxylase V	*PDC5*	YLR134w	nc	↓
pyruvate decarboxylase VI	*PDC6*	YGR087c	↓	↓
pyruvate carboxylase 1	*PYC1*	YGL062w	↑	↓
pyruvate carboxylase 2	*PYC2*	YBR218c	↑	↓
Phosphoenolpyruvate carboxykinase	*PCK1*	YKR097w	↑	↓
Fermentation				
alcohol dehydrogenase II	*ADH2*	YMR303c	nc	nc
alcohol dehydrogenase III	*ADH3*	YMR083w	nc	↓
alcohol dehydrogenase IV	*ADH4*	YGL256w	nc	↓
alcohol dehydrogenase V	*ADH5*	YBR145w	nc	nc
adehyde dehydrogenase VI (major cytosplas-	*ALD6*	YPL061w	↓	↓
aldehyde dehydrogenase IV (major mitochon-	*ALD4*	YMR169c	nc	↓
aldehyde dehydrogenase V (minor mitochondri-	*ALD5*	YMR170c	↑	nc
aldehyde dehydrogenase VII	*ALD7*	YOR374w	nc	↓
Trehalose metabolism				
trehalose-6-phosphate synthase in TPS complex	*TPS1*	YBR126c	↓	↓
trehalose-6-phosphate phosphatase	*TPS2*	YDR074w	nc	↑
trehalose synthase long chain in TPS complex	*TSL1*	YML100w	↑	↓
TPS regulatory unit	*TPS3*	YMR261c	↑	↓
neutral trehalase	*NTH1*	YDR001c	↑	nc
acid trehalase	*ATH1*	YPR026w	↑	↓
Glycogen metabolism				
glycogen synthase initiator	*GLG2*	YJL137c	↑	↓
glycogen synthase1	*GSY1*	YFR015c	nc	↓
glycogen synthase2	*GSY2*	YLR258w	↑	↓
glycogen branching enzyme	*GLC3*	YEL011w	nc	↓
sporulation specific glycoamylase	*SGA1*	YIL099w	↑	↓
glycogen phoshorylase 1	*GPH1*	YPR160w	↑	↓
glycogen debranching enzyme1	*GDB1*	YPR184w	nc	↓
HP and OPPP				
phosphoglucomutase 1	*PGM1*	YKL127w	nc	↓
phosphoglucomutase 2	*PGM2*	YMR105c	↑	↓
homologous to udp-glc pyrophosphoylase		YHL012W	↑	↓
glucose-6-phosphate dehydrogenase	*ZWF1*	YNL241c	↑	↓
Transketolase	*TKL1*	YPR074c	↑	↓
Glycerol metabolism				
glycerol kinase1	*GUT1*	YHL032c	nc	↓
glycerol kinase2	*GUT2*	YIL155c	nc	↓
glycerol-3-phosphate dehyrdogenase1	*GPD1*	YDL022w	↑	↓
glycerol-3-phosphate dehyrdogenase2	*GPD2*	YOL059w	↑	nc

Table 1. Continued

	Standard name	Systematic name	Ethanol yield	Residual sugars
TCA metabolism				
citrate synthase 1	*CIT1*	YNR001c	↑	↓
citrate synthase 2	*CIT2*	YCR005c	↑	↓
citrate synthase 3	*CIT3*	YPR001w	↑	↓
aconitase 1	*ACO1*	YLR304c	↑	↓
aconitase 2	*ACO2*	YJL200C	↑	↓
isocitrate dehydrogenase NAD+ subunit1	*IDH1*	YNL037c	nc	nc
isocitrate dehydrogenase NAD+ subunit2	*IDH2*	YOR136w	↑	↓
a-ketoglutarate dehydrogenase complex	*KGD1*	YIL125w	nc	↓
dihydrolipoyl transsuccinylase a-kgdh com-	*KGD2*	YDR148c	nc	↓
a-subunit of succinyl coA ligase	*LSC1*	YOR142w	nc	↓
b-subunit of succinyl coA ligase	*LSC2*	YGR244c	↑	↓
Diploids				
glucose-6-phosphate isomerase	*PGI1*	YBR196c	nc	nc
Aldolase	*FBA1*	YKL060c	nc	nc
Triosephosphte isomerase	*TPI1*	YDR050c	nc	nc
3-phosphoglycerate kinase	*PGK1*	YCR012w	nc	↓
phosphoglycerate mutase	*GPM1*	YKL152c	↓	↓
enolase I	*ENO1*	YGR254w	↑	↑
pyruvate kinase I	*PYK1*	YAL038w	nc	nc
pyruvate decarboxylase	*PDC2*	YDR081c	nc	nc
udp-glucose pyrophosphorylase	*UGP1*	YKL035w	nc	↓

This increase appears to be maintained throughout fermentation as increases in trehalose levels for this strain are statistically significant at all subsequent sampling points. The increases in trehalose levels in these strains show that carbon was likely redirected to this alternative carbon sink in the transformed strains, accounting for the decrease in ethanol yield (Figure 2, frame B). Furthermore, the increased trehalose levels may have inhibited hexokinase activity, thus restricting associated flux through glycolysis. This would explain the slightly reduced fermentation rate and slightly higher residual sugar levels in fermentations conducted with these strains (data not shown).

3.4. Primary fermentation kinetics of TPS1 overexpressing VIN13 strains

Samples of the fermentation must were taken at various time-points during fermentation to determine the fermentation kinetics of transformed strains. The DUT-TPS and GIP-TPS test strains show significantly reduced ethanol levels as well as reduced ethanol yields at the end of fermentation (Figure 2, frame B). It appears that expression of *TPS1* both under control of the *DUT1* promoter during the early exponential growth phase as well as the *GIP2* promoter in early stationary phase has a significant metabolic impact in terms of decreasing total ethanol and ethanol yield (Figure 2, frame B).

There is also a slight, though not statistically significant increase in glycerol production observed for all transformed strains (data not shown). Another positive outcome was the fact that no increase in acetic acid levels for either of the test strains compared to their empty plasmid controls was observed (data not shown). This suggests that the slight overexpression of the TPS1 gene by these specific promoters can successfully shift carbon flux with minimal impact on redox balance and without any negative impact in terms of the production of unwanted fermentation end-products.

3.5. Levan, a bacterial fructose polymer, has potential as an alternative carbon sink in yeast

To assess the feasibility of using levan as an alternative carbon sink, three different genetic platforms where constructed to test fructosyltransferase functionality in yeast and also the ability to produce and accumulate levan inside the yeast cells. The broad aim with these approaches is to divert carbon from glycolysis and produce sugar polymers within the cells, where the produced fructan would be sequestered from the fermentation. Such a strategy would, in principle, enable the production of wines with reduced amounts of alcohol by shifting carbon flux, even if slightly, to levan production. The levansucrase, M1FT, from *Leuconostoc mesenteroides* was chosen to perform the sugar polymerization reactions. M1FT specifically presented an attractive target for expression, since the enzyme has recently been successfully expressed in *Pichia pastoris* (Kang *et al.* 2011). The three yeast backgrounds that were constructed for M1FT expression

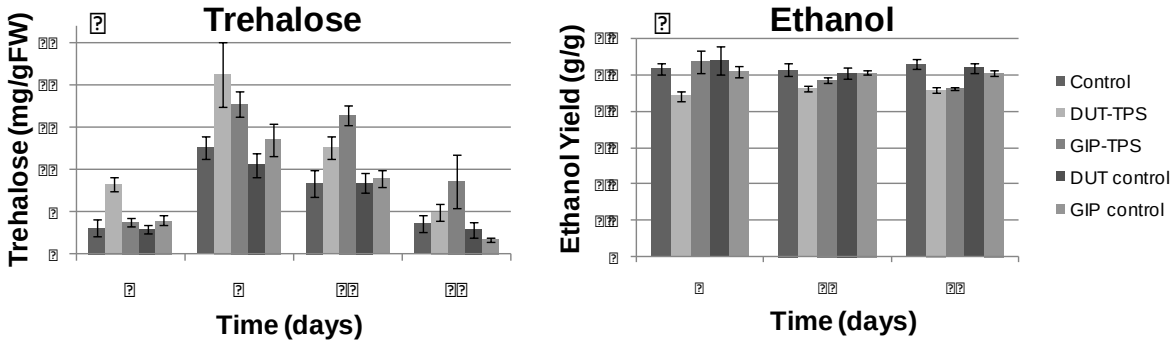


Figure 2. Trehalose levels (normalized relative to cell biomass) at different stages of wine fermentation (Frame A). Ethanol yields as determined at three different timepoints during fermentation are depicted in frame B. These figures have been adapted from Rossouw *et al*. (2013). Values are the average of three biological repeats ± standard deviation.

included an invertase (*suc2*Δ) null mutant and also two different approaches to produce and accumulate intracellular sucrose, the substrate and fructose donor, in the same background. In the latter two strains, sucrose accumulation was achieved by expressing either a potato sucrose synthase (SuSy) or a sucrose transporter (SUT) from spinach in the Δ*suc2* genetic background. The results indicate that the levansucrase from *L. mesenteroides*, M1FT, is functional when expressed in yeast and also able to catalyse the synthesis of levan from sucrose. A comparison of levan production in the different strains in different media conditions is presented in Table 1. Levan production by M1FT was detected in association with the yeast cells when expressed in BY4742Δ*suc2* cultures, when grown in the presence of sucrose. Removal of the secretion signal (M1FTΔsp) in the same background almost completely eliminates the production of levan. Levan production by M1FT was also confirmed in both the sucrose accumulation strains, when yeast cultures were grown in minimal media containing the required carbon source combinations (glucose and sucrose for SUT and glucose + fructose for the SuSy strain). In both cases, a clear increase in the amount of intracellular levan produced is visible upon removal of the predicted signal sequence (M1FTΔsp). In addition, expression of the full-length enzyme, with the intact signal peptide, resulted in the accumulation of a small amount of levan in the growth medium when cultures were grown in rich medium containing sucrose (YPDS). Together, these findings indicate that the M1FT signal peptide is functional and at least in part able to direct secretion of the enzyme.

The data generated when growing the sucrose accumulation strains in rich media that would support high growth and metabolic yields provided promising results (YPDS and YPDS ^{Fermentative} in Table 1). In these conditions M1FT produced large quantities of extracellular levan and also accumulated levan inside the cells when expressed in the SUT strains. In rich media (YPDS), intracellular production was observed both with and without the presence of the secretion signal when co-expressed with SUT. This clearly indicates that M1FT is functional when expressed in the SUT strains grown in rich media. However, when considering the expression of M1FT and M1FTΔsp in the BY4742Δ*suc2*-SuSy background, the levan production that is observed in minimal media could not be repeated when the cells were grown in rich media (YPDS) or when the strains were used to perform alcoholic fermentations at higher sugar concentrations and anaerobic conditions (100 g/L total sugar in either minimal or rich media). In fact, no detectable sucrose production by SuSy could be confirmed in the same conditions. The absence of sucrose, and consequently levan, during fermentative growth could be explained by *S. cerevisiae's* known preference to consume glucose at higher rates during the early phases of alcoholic fermentation. Fructose is generally used at slower rates and also later in the fermentation (Berthels *et al*. 2004). This would result in a reduced availability of fructose to be used for sucrose synthesis by SuSy. In addition, preliminary data indicate that SuSy has a limited ability to use fructose 6-phosphate as a substrate for sucrose synthesis and requires unphosphorylated fructose in order to function optimally (data not shown). Furthermore, the known predisposition of *S. cerevisiae* and other Crabtree positive yeasts to increase the rate of glycolysis in the presence of higher concentrations of sugar, would theoretically limit the heterologously expressed SuSy's access to the substrates (Pronk *et al*. 1996). Similarly, the higher growth rates associated with cultures grown in rich media compared to minimal media are also known to result in increases in metabolic rates, especially through glycolysis (Pronk *et al*. 1996). The optimized functioning of the yeast's metabolism may under these conditions rapidly direct fructose towards glycolytic metabolism. Together, these findings provide the foundation future efforts, which could potentially provide an alternative sink for carbon accumulation if access of SuSy to fructose, be-

fore entry into glycolysis, could sufficiently be optimized.

4. Conclusions

Screening of the yeast deletion library for strains showing altered ethanol yields proved to be a suitable strategy for the identification of target genes for flux modification in central carbohydrate metabolism. The findings indicated that key steps in multiple parts of this network of reactions are responsible for regulation of flux and final accumulated metabolite pools. One of the strongest candidates emerging from this study was the *TPS1* gene. Over-expression of this gene was carried out in an industrial yeast genetic background, with the added novelty of growth-stage specific promoters. Results from fermentations conducted with these strains show successful (moderate and stage-specific) over-expression of the *TPS1* gene and higher intracellular trehalose levels in transformed strains. The increase in trehalose was complemented by a reduced final ethanol and ethanol yield in these strains, thus proving to be a successful commercial wine yeast engineering strategy targeting alternative carbon pools as a means for lowering ethanol levels. Importantly, the transformed strains did not show any adverse phenotypic features such as decreased fermentation efficacy, or unwanted by-product formation, confirming the suitability of the controlled promoter strategy for genetic engineering strategies in wine yeast. The current study was thus successful in terms of validating the screening method for the identification of genes that affect carbohydrate flux, identification of deletion strains that alter final fermented ethanol yield, and provision of a platform for application to industrial yeast tailored for lower ethanol fermentations. The present study can be extended to include other potential targets identified in the initial mutant screens.

An alternative, heterologous strategy was followed to investigate the possibility of directing carbon flux away from glycolysis to the production of the bacterial fructose polymer, levan. In principle, this would result in the production of less ethanol from the same initial amount of fermentable sugar. The results show that such an option could indeed be a possibility, since levan production was effectively achieved by expressing the levensucrase, M1FT, in yeast strains modified to accumulate sucrose. Levan production was, nevertheless, dependent on growth conditions and could not be duplicated in fermentative conditions where glycolytic flux would be optimal. The work does, however, lay the foundation for future studies and presents appealing opportunities to optimize a system that would effectively divert carbon towards sugar polymer production and away from ethanol formation.

Table 1. Levan production by engineered yeast strains in minimal and rich media

Yeast strain	Media	Levan production	
		Intracellular*	Extracellular**
BY4742Δ*suc2*-M1FT	SCDS	≤ 0.05µg/mg	N.D.
BY4742Δ*suc2*-M1FTΔsp	SCDS	N.D.	N.D.
BY4742Δ*suc2*-M1FT	YPDS	N.D.	≥ 10 mg/L
BY4742Δ*suc2*-M1FTΔsp	YPDS	N.D.	N.D.
BY4742 Δ*suc2* SUT-M1FT	SCDS	≤ 0.05µg/mg	N.D.
BY4742 Δ*suc2* SUT-M1FTΔsp	SCDS	0.100 µg/mg	N.D.
BY4742 Δ*suc2* SUT-M1FT	YPDS	~50 µg/mg	5 g/L
BY4742 Δ*suc2* SUT-M1FTΔsp	YPDS	≤ 0.05µg/mg	N.D.
BY4742 Δ*suc2* SUT-M1FT	YPDS^{Fermentative}	N.D.	N.D.
BY4742 Δ*suc2* SUT-M1FTΔsp	YPDS^{Fermentative}	0.100 µg/mg	N.D.
BY4742 Δ*suc2* SuSy-M1FT	SCGF	≤ 0.05µg/mg	N.D.
BY4742 Δ*suc2* SuSy-M1FTΔsp	SCGF	0.100 µg/mg	N.D.
BY4742 Δ*suc2* SuSy-M1FT	YPGF	N.D.	N.D.
BY4742 Δ*suc2* SuSy-M1FTΔsp	YPGS	N.D.	N.D.
BY4742 Δ*suc2* SuSy-M1FT	YPGF^{Fermentative}	N.D.	N.D.
BY4742 Δ*suc2* SuSy-M1FTΔsp	YPGF^{Fermentative}	N.D.	N.D.

N.D. = Not detectable on TLC; YP^{Fermentative} = rich media with 100g/L total sugar
*µg levan/mg wet weight; ** levan/L culture medium

5. References

Banguela A, Arrieta JG, Rodríguez R, Trujillo LE, Menéndez C, *et al*. (2011) High levan accumulation in transgenic tobacco plants expressing the *Gluconacetobacter diazotrophicus* levansucrase gene. *J. Biotechnol*., **154**, 93–98

Belisario-Sánchez YY, Taboada-Rodríguez A, Marín-Iniesta F, Iguaz-Gainza A, López-Gómez A (2011) Aroma recovery in wine dealcoholization by SCC distillation. *Food Bioprocess Technol*., 1-11.

Bell W, Sun W, Hohmann S, Wera S, Reinders A, De Virgilio C, Wiemken A, Thevelein JM **(1998)** Composition and functional analysis of the *Saccharomyces cerevisiae* trehalose synthase complex. J. Biol. Chem., 27, 33311-33319.

Bely M, Sablayrolles JM, Barre P (1990) Automatic detection of assimilable nitrogen deficiencies during alcoholic fermentation in enological conditions. *J. Ferm. Bioeng.*, **70**, 246-252.

Berthels NJ, Cordero Otero RR, Bauer FF, Thevelein JM, Pretorius IS (2004). Discrepancy in glucose and fructose utilisation during fermentation by *Saccharomyces cerevisiae* wine yeast strains. *FEMS Yeast Res*., **4**, 683–689.

Bui K, Dick R, Moulin G, Galzy P (1986) A reverse osmosis for the production of low ethanol content wine. *Am. J. Enol. Vitic*., **37**, 297-300.

Catarino M, Mendes A (2011) Dealcoholizing wine by membrane separation processes. *Innovative Food Sci. Emerging Tech.*, **12**, 330-337.

de Barros Lopes M, Eglinton J, Henschke P, Høj P, Pretorius I (2003) The connection between yeast and alcohol: managing the double-edged sword of bottled sunshine. Aust. New Zealand Wine Ind. J., **18**, 17-22.

García-Martín N, Perez-Magariño S, Ortega-Heras M, González-Huerta C, Mihnea M, González-Sanjosé ML *et al* (2010) Sugar reduction in musts with nanofiltration membranes to obtain low alcohol-content wines. *Separation and Purification Technology*, **76**, 158-170.

Gómez-Plaza E, López-Nicolás J, López-Roca J, Martínez-Cutillas A (1999) Dealcoholization of wine. behaviour of the aroma components during the process. *LWT-food science and technology*, **32**, 384-386.

Guth H, Sies A (2002) Flavour of wines: towards an understanding by reconstitution experiments and an analysis of ethanol's effect on odour activity of key compounds *Proc. 11th Aust. Wine. Ind. Tech. Conf.* Adelaide, Australia, pp. 4–7.

Hoffmann D (1990) The European wine market up to the year 2000 – trends and marketing efforts. *In: Proc. 9th Int. Oen. Symp*. Cascais, Portugal, pp. 138-153.

Kang HK, Seo MY, Seo ES, Kim D, Chung SY, *et al*. (2005) Cloning and expression of levansucrase from *Leuconostoc mesenteroides* B-512 FMC in *Escherichia coli*. *Biochim Biophys Acta*, **1727**, 5–15.

Lafraya Á, Sanz-Aparicio J, Polaina J, Marín-Navarro J (2011) Fructo-oligosaccharide synthesis by mutant versions of *Saccharomyces cerevisiae* invertase. *Applied and environmental microbiology*, **77**, 6148–6157

Lilly M, Bauer FF, Styger G, Lambrechts MG, Pretorius IS (2006) The effect of increased breanched-chain amino acid transaminase activity in yeast on the production of higher alcohols and on the flavour profiles of wine and distillates. *FEMS Yeast Res.*, **6**, 726-743.

Pickering G, Heatherbell D, Barnes M (1998) Optimising glucose conversion in the production of reduced alcohol wine using glucose oxidase. *Food Res Int*., **31**, 685-692.

Pickering GJ (2000) Low-and reduced-alcohol wine: *A review. J. Wine Res*, **11**, 129-144.

Pronk JT, Yde Steensma H, Van Dijken JP (1996) Pyruvate metabolism in *Saccharomyces cerevisiae*. *Yeast*, **12**, 1607–1633.

Ramakers C, Ruijter JM, Deprez RH, Moorman AF (2003) Assumption-free analysis of quantitative real-time polymerase chain reaction (PCR) data. *Neurosci Lett*, **339**, 62-66.

Riesmeier JW, Willmitzer L, Frommer WB (1992) Isolation and characterization of a sucrose carrier cDNA from spinach by functional expression in yeast. *EMBO J*., **11**, 4705–4713.

Remize F, Roustan J, Sablayrolles J, Barre P, Dequin S (1999) Glycerol overproduction by engineered *Saccharomyces cerevisiae* wine yeast strains leads to substantial changes in by-product formation and to a stimulation of fermentation rate in stationary phase. *Appl Environ Microbiol*., **65**, 143-149.

Remize F, Andrieu E, Dequin S (2000) Engineering of the pyruvate dehydrogenase bypass in *Saccharomyces cerevisiae*: Role of the cytosolic Mg2 and mitochondrial K acetaldehyde dehydrogenases Ald6p and Ald4p in acetate formation during alcoholic fermentation. *Appl. Environ. Microbiol.*, **66**, 3151-3159.

Rossouw D, Heyns EH, Setati ME, Bosch S, Bauer FF (2013) Adjusting trehalose metabolism to modify ethanol yields in wine yeast strains. Appl Environ Microbiol doi: 10.1128/AEM.00964-13.

Salanoubat M, Belliard G (1987) Molecular cloning and sequencing of sucrose synthase cDNA from potato (*Solanum tuberosum* L.): preliminary characterization of sucrose synthase mRNA distribution. *Gene*, **60**, 47–56.

Schmitt ME, BrownTA, Trumpower BL (1990) A rapid and simple method for preparation of RNA from *Saccharomyces cerevisiae*. *Nucl. Acids Res*., **18**, 3091–3092.

Scotti PA, Chambert R, Petit-Glatron MF (1994) Extracellular levansucrase of *Bacillus subtilis* produced in yeast remains in the cell in its precursor form. *Yeast*, **10**, 29–38.

Scotti PA, Praestegaard M, Chambert R, Petit-Glatron MF (1996) The targeting of *Bacillus subtilis* levansucrase in yeast is correlated to both the hydrophobicity of the signal peptide and the net charge of the N-terminus mature part. *Yeast*, **12**, 953–963.

Scudamore-Smith P, Moran J (1997) A growing market for reduced alcohol wines. *Wine Ind. J*., **12**,165–167.

Varela C, Kutyna DR, Solomon MR, Black CA, Borneman A, Henschke PA, Pretorius IS, Chambers PJ (2012) Evaluation of gene modification strategies for the development of low-alcohol-wine yeasts. *Appl. Environ. Microbiol.*, **78**, 6068-6077.

Wenzel TJ, Migliazza A, Steensma HY, van den Berg JA (1992) Efficient selection of phleomycin- resistant *Saccharomyces cerevisiae* transformants. *Yeast*, **8**, 667-668.

Wright A, Pyle D (1996) An investigation into the use of the spinning cone column for *in situ* ethanol removal from a yeast broth. *Process Biochemistry*, **31**, 651-658.

Yoshikawa Y, Matsumoto K, Nagata K, Sato T (1994) Extraction of trehalose from thermally-treated bakers' yeast. *Biosci. Biotech Biochem*., **58**, 1226-123

1st International Symposium

Alcohol level reduction in wine

OENOVITI INTERNATIONAL Network

Session III - Potential reduction in alcohol levels strategies & technological practices & processes

OIV rules and implications concerning reduction of alcohol levels

Jean-Claude Ruf

International Organisation of Vine and Wine (OIV), 18 rue d'Aguesseau, 75008 Paris, France

Abstract: As Intergovernmental, scientific and technical reference Organisation for wine and viticultural products, the OIV has among its objectives to contribute to international harmonisation of existing practices and standards and, as necessary, to the preparation of new international standards in order to improve the conditions for producing and marketing vine and wine products, and to help ensure that the interests of consumers are taken into account. In particular, since 2004 when the OIV has, for the first time, recognised the principle of the de-alcoholisation of wine under certain conditions, the Member-States of the OIV have revised the oenological practices in this area to allow the development of different products with reduced alcohol content or with low alcohol content. At the same time Member-States of the OIV agreed to adopt definitions that cover these types of product which are already on the market.

Keywords: wine, de-alcoholisation, enological practices, regulation, wine consumers

The wine industry is facing changes and technological innovation has developed new solutions, some involving grape growing techniques and some involving oenological practices including de-alcoholisation processes or partial removal of alcohol. The adoption of such technologies offers opportunities to develop new products.

It is important for the vitivinicultural sector to consider a response to market signals suggesting emerging consumer interest in products containing less alcohol than has been traditionally associated with this sector. This interest may well be due, in part, to global efforts by governments to address irresponsible and harmful levels of alcohol consumption.

Notwithstanding the positive contribution of the vitivinicultural sector to human social and cultural interaction, the spirit of continuous improvement demands that we support innovative attempts to respond to these market and regulatory signals. It can be stipulated that the product is completely free of alcohol, or, alternatively, that the alcohol level of the product has been reduced below the amount contained at some earlier stage in its production. It is therefore important to define the following suggested product categories, with a view to providing enhanced choice for wine consumers and niche markets for economic operators in the vitivinicultural sector.

As the intergovernmental, scientific and technical reference Organisation for wine and viticultural products, the OIV has among its objectives to contribute to international harmonisation of existing practices and standards and, as necessary, to the preparation of new international standards in order to improve the conditions for producing and marketing vine and wine products, and to help ensure that the interests of consumers are taken into account.

Since 2004, the member-States of the OIV have adopted a resolution to obtain partially dealcoholised wine by a process to reduce the ethanol content of wine. However, in this case the alcoholic strength must not be reduced by more than 2% alc. vol.

The group of products resulting from the process of de-alcoholisation or the reduction or removal of alcohol from wine is very heterogeneous in terms of presentation and alcohol content. In addition, due to the differences in regulations regarding the definition of wine, the classification of alcoholic products, and the minimum alcohol level, new products are present in different markets.

For the wine definition, there are several minimum actual alcoholic strengths in different countries and also depending on grapevine products categories whereas the OIV only establishes a unique minimum actual alcoholic strength of 8.5 % vol., with the flexibility to be

Corresponding author : jruf@oiv.int

reduced to 7 % vol. For example in the European Union the limit is established at 8.5% vol with derogation at 4,5% vol for certain Geographical Indications and recently Australia has reduced the limit from 8% vol to 4.5% vol. For Argentina and China the limit is based at 7% vol. whereas some other countries there is no minimum specified.

In this framework, in 2012, the General Assembly of the OIV adopted unanimously new resolutions that address both the expectations of the vitivinicultural sector and a growing appetite of consumers for low-alcohol or directly dealcoholised beverages of vitivinicultural origin.

Resolutions OIV-ECO 432-2012 and OIV-ECO 433-2012 update the International Code of Oenological Practices with the inclusion of two new definitions for “beverage obtained by de-alcoholisation of wine” and “beverage obtained by partial de-alcoholisation of wine”.

Resolutions OIV-OENO 394A-2012 and OIV-OENO 394B-2012, on the other hand, specify the separation techniques that can be used either to dealcoholise wines or to correct the alcohol content of wines, respectively.

1. Correction of alcohol content vs de-alcoholisation

Through the adoption of resolutions OIV-OENO 394A-2012 and OIV-OENO 394B-2012, OIV Member-States have specified the conditions for reducing the alcohol content of wine, differentiating between a correction of the alcohol content and a de-alcoholisation of the wine.

Correcting the alcohol content of a particular wine, which means to reduce an envisaged excessive level of ethanol to improve its taste balance, is allowed with a maximum reduction of 20% of the initial alcohol content. Products obtained through this practice must still conform to the definition of wine, and especially keep the minimum alcoholic strength of wines, if they are to be presented as such.

Otherwise, if the alcohol content of the wine is reduced by more than 20%, it will fall under a de-alcoholisation process, which means to remove part or almost all of the ethanol content in wine in order to develop vitivinicultural products with low or reduced alcohol content.

This procedure is also allowed but the resulting product shall not be presented as wine, since it will not comply with the established definition of wine.

The separative techniques that can be used to achieve any of these goals are: partial vacuum evaporation, membrane techniques and distillation. This process must not be used on wines with any other organoleptic defects and the elimination of alcohol in wine must not be done in conjunction with a modification in the sugar content in the corresponding musts.

In addition, these processes consisting in separating must or wine into several fractions having different chemical composition must comply with the objectives and prescriptions indicated in the OIV resolutions Oeno 373A-/2010 and Oeno 373B-2010.

The objectives can be achieved by different techniques alone or in combination and in particular the membrane techniques, the evaporative techniques (such as distillation and vacuum distillation) or other separative techniques.

Some prescriptions are detailed indicating that:

a) The wine or must to be treated must comply with OIV’s definitions and limits.

b) These techniques cannot be used to cover fraudulent acts

c) Fractions, untreated or treated by oenological practices approved by the OIV must be blended exclusively with must or wine fractions, obtained by separative techniques, derived from the same original product. Fractions used as wine based products as defined in the International Code of Oenological Practices are the only exception.

d) Recombination must occur within the shortest possible time and in the same place when it is possible.

e) The techniques, membranes and equipment used, and the practices implemented in the additional procedures must comply with the provisions stated in the OIV International Code of oenological practices

f) Treatments of the fractions must comply with the OIV International Code of oenological practices

For the application of membranes techniques including the process of de-alcoholisation, there are different types of membrane techniques alone or in combination depending on the

sought after objectives, including microfiltration, ultrafiltration, nanofiltration, membrane contactor, reverse osmosis, electromembranes processes and other membrane techniques.

For the elaboration of products with alcohol reduced, it is also possible to reduce the sugar level of must before the fermentation. In this context and based on scientific and technical data, the OIV has adopted also some resolution covering the reduction of the sugar content in musts which describes the objectives and the prescriptions for achieving those objectives (Resolution OIV-OENO 450A-2012). At the same time, a specific application on the reduction of sugar content in musts through membrane coupling was adopted (Resolution OIV-OENO 450B-2012). This practice consists in extracting the sugar from a must through membrane coupling combining microfiltration or ultrafiltration with nanofiltration or reverse osmosis. Various detailed provisions also accompany this practice.

In general term, the reduction of the sugar content in musts excludes the de-alcoholisation of the wines from which they originate and must not be used in conjunction with the enrichment techniques for musts and wines

This process is limited because of the significant reduction of volume and the results of the separation techniques used and the treatment should be carried out on a volume of must that is determined according to the required result in terms of sugar content reduction. The objective of the first step is to prepare the must for the second concentration step and to filter out all the macromolecules smaller than the membrane's cut-off size, this step may be done by ultrafiltration. The ultrafiltrate obtained during the first step of the treatment is then concentrated by nanofiltration or reverse osmosis. The water and the organic acids filtered out by the nanofiltration process can be reintroduced into the treated must

2. Product definition

Through the adoption of resolutions OIV-ECO 432-2012 and OIV-ECO 433-2012, OIV Member States update the International Code of Oenological Practices with the inclusion of two new product definitions:

- "Beverage obtained by partial de-alcoholisation of wine" for products with ABV content comprised between the required minimum for wines and 0.5%,
- "Beverage obtained by de-alcoholisation of wine" for products with an ABV content below 0.5%.

The OIV is currently working to develop definitions for products that do not fall under the aforementioned resolutions, more particularly wines that have gone through an alcohol reduction of more than 20% but do still respect the minimum alcohol level for wine and special wine.

There is no definition of sales denominations but a footnote: indicating that "these definitions do not preclude the denominations "*dealcoholised wine*" and "*partially dealcoholised wine*" to be used respectively in case the legislation of each Member-States allows it."

3. The International Code of Oenological Practices

The OIV International Code of Oenological Practices constitutes a technical and legal reference document, aiming at a standardisation of products of the vitivinicultural sector that should serve as a foundation for the establishment of national or supra-national regulations and should be essential in international trade.

The OIV recommendations provide a basic standard on definitions and practices that the Member States can take into account to be adopted in their jurisdictions.

These OIV resolutions are needed as they will provide the basic standard, but they will be not enough to support the smooth development of this category and the smooth functioning of the internal market and the international trade:

Currently the OIV is working on the establishment of new definition in the International Code of oenological practices. Indeed, after the adoption of the definitions of "Beverage obtained by partial de-alcoholisation of wine" and "Beverage obtained by de-alcoholisation of wine" a whole range of potential products is not covered by such definitions. De-alcoholised products beyond 20% but respecting the minimum alcoholic strength of wine. These products currently exist on different the market and are labeled for example in Australia and New Zealand as "wine alcohol content reduced" or "wine".

In addition, the OIV is also identifying specific winemaking rules for the de-alcoholised products at stake which will be “as close as possible” to the existing winemaking rules for the conventional wines and sparkling wines. They should only use additives and processing aids approved for use in wine with some exception. For the elaboration of these products a number of additives and sweeteners seem to be needed for quality reasons to ensure microbiological stability and organoleptic properties

4. Conclusions

There is a growing demand for wine products with reduced alcoholic strength and wine producers are quite interested by this new possible segmentation of the offer. The OIV as intergovernmental organisation works in this area for harmonizing definition for alcohol low/free wine beverages and its oenological practices.

The lack of international harmonised definitions, denominations and practices could turn into barriers to trade and unfair competition, handicapping companies’ innovation and competitiveness in this area.

It is also important to ensure consistency and coherence with the winemaking rules, while allowing for appropriate flexibility need to facilitate technical innovation and offer quality products to the consumers.

Note : The opinions expressed in this document are those of the authors and do not reflect the views or the opinion of the OIV. Only the resolutions adopted by the Member States of the OIV have an official character.

5. References

International Organisation of Vine and Wine, 2010, Resolution OIV- OENO 373A-2012

International Organisation of Vine and Wine, 2010, Resolution OIV- OENO 373B-2012

International Organisation of Vine and Wine, 2012, Resolution OIV-ECO 432-2012

International Organisation of Vine and Wine, 2012, Resolution OIV-ECO 433-2012

International Organisation of Vine and Wine, 2012, Resolution OIV-OENO 394A-2012

International Organisation of Vine and Wine, 2012, Resolution OIV- OENO 394B-2012

International Organisation of Vine and Wine, 2012, Resolution OIV- OENO 450A-2012

International Organisation of Vine and Wine, 2012, Resolution OIV- OENO 450B-2012

Membrane contactor process to reduce ethanol in wine. Volatile compounds and stable isotope ratios changes

Roberto Ferrarini[1,2,*], Gian Maria Ciman[2], Laura Cugini[2], Carlo Gostoli[3], Marco Simoni[4] and Federica Camin[4]

[1]Department of Biotechnology, University of Verona, Via della Pieve 70, 37029 S. Floriano, Verona, Italy
[2]PerWine (Perfect Wine), Verona University spin off, Via della Pieve 70, 37029 S. Floriano, Verona, Italy
[3]Department of Civil, Chemical, Environmental and Materials Engineering – DICAM, University of Bologna, Via Terracini 28, 40131 Bologna, Italy
[4]Fondazione Edmund Mach, IASMA - Research and Innovation Centre, Via Mach 1 38010 S. Michele all'Adige, Trento, Italy

Abstract The reduction of ethanol level in wine is nowadays a notable topic in wine production. There are many different reasons: climate change, health and social matters, wine quality.
Many different techniques are available in wine industry to reduce high ethanol content in wine. Among theme contactor membranes, are certainly more efficient and common. We have studied the effect of this practice on the wine quality, aromas compounds and stable isotopes composition.
A pilot and industrial plant equipped with the membrane contactor system were used in the study of several white and red wines dealcoholisation. In both scale experiments we have observed changes for several classes of aroma compounds, even if these changes are not in perfect correlation with sensorial evaluation carried out. In addition is observed a variability in aromas variations for each aroma compound and wine variety.
We have in particularly studied the modifications on isotopes ratios. We have tested the process using waters with different $\delta^{18}O$ ratios, or wines, in different osmotic conditions for both compartments. Modifications of up to 1‰ for 2 %v/v and of up to 4‰ for 8 %v/v ethanol removal were encountered. It is very important to note that the $\delta^{18}O$ ratio changes are not only due by a water osmotic transfer through the membrane. The $\delta^{18}O$ decrease, is even depending by the $\delta^{18}O$ value of the extracting water solution. These variations must be taken into account when these parameters are evaluated to considered dealcoholised wine traceability.

Keywords: membrane contactor, wine dealcoholisation, aroma compounds, stable isotope ratios, isotopic diffusion.

1. Introduction

All over the world, in wine regions, the increase of alcohol level in wine is observed.

This problem, due to different reasons: climate change, viticulture techniques improvement and low yield, have an important influence to increase sugar content in grapes, even in temperate regions.

In addition, it is common over last few decades to reach a high grapes ripening, to have full body wine with local character. For these reasons it is easy to have very high sugar content in grape juice and consequently high ethanol level in wine.

Ethanol excess, that nowadays could be often more than 15%v/v, represent a problem for wine quality and balance, as well as social and healty benefits.

Therefore the reduction of ethanol level in wine is nowadays a very important issue that involves all the main wine producing countries, in this reason European regulation (EC Reg. 606/2009) which allows the reduction of the ethanol level of up to 2 % vol. prompted new interest on this matter.

Many different approaches have been applied to produce wine with a lower ethanol content, even though they can be grouped in three main strategies: the reduction of the sugar level of the grapes in the vineyard (Bindon *et al.*, 2008; Valenti *et al.*, 2011), lowering the ethanol produced during fermentation (De Risi *et al.*, 1997; Kutyna *et*

*Corresponding author. E-mail: roberto.ferrarini@univr.it

al., 2010; Larsson *et al.*, 1998) and finally applying technological protocols to either reduce the sugar content in the grape juice or to decrease the ethanol level in the final wine (Diban *et al.*, 2008; Pickering 2000; Pilipovik and Riverol, 2005; Varavuth *et al.*, 2009).

Many of these approaches show significant limitations when applied to the industrial scale.

Operations on the ethanol level of the final wine appear more versatile, more reliable and more adapt for the scale-up at industrial level (Pickering, 2000), though several problems have been encountered in developing procedures for the ethanol level reduction.

Among different techniques that are used to reduce ethanol content in wine, it seems that contactor membrane has the best performance to preserve wine quality: the hydrophobic membrane is placed between the wine and the extractant (typically water); the driving force is the vapour pressure difference due to the different ethanol concentration on the two sides of the membrane. The physical phenomena occurring is also wrongly called "osmotic distillation" as the compounds extracted are actually migrating through the membrane pores in a gas physical state (Hogan *et al.*, 1998).

In reality is not a distillation, however an evaporation process, and according to I.U.P.A.C. (International Union of Pure and Applied Chemistry, 1996) the process is defined as a Perstraction (separation process in which membrane permeation and extraction phenomena occur by contacting the downstream with an extracting solvent).

This technique could provoke a depletion of aromatic compounds with low boiling point (Fedrizzi *et al.*, 2013; Diban *et al.*, 2008; Varavuth *et al.*, 2009; Liguori *et al.*, 2012).

In model solution another phenomenon is observed: water transfer (Michaels 1993; Michaels et al. 1998) due to osmotic pressure caused by ethanol. In addition, all the technological approaches involving a distillation/evaporation step might cause a modification of the stable isotope ratios of H, C and O in wine as a consequence of an isotopic fractionation (Jancso and Van Hook, 1974). The analysis of the site-specific ratio D/H in the methylic [(D/H)I] and methylenic [(D/H)II] sites of ethanol is performed by using a site-specific natural isotopic fractionation and nuclear magnetic resonance. The evaluation of the $^{13}C/^{12}C$ (expressed in $\delta^{13}C$ ‰) of ethanol and $^{18}O/^{16}O$ ($\delta^{18}O$ ‰) of water ratios is determined with an Isotope Ratio Mass Spectrometer. Since 1990, as authorised by European law (i.e., EC Reg. 2676/90, 822/97, 440/03, now OIV methods MA-E-AS311-05-ENRRMN, MA-E-AS312-06-ETHANO, MA-E-AS2-09-MOU018), such procedures are officially used to determine wine frauds such as watering and chaptalisation.

The wine sample is defined "not authentic" if its isotopic values do not fall into the natural range of variability determined every year on the basis of reference samples officially collected and analysed in each member state (EC Reg. 555/2008). The possible variation of isotopic ratios in wine due to oenological practices such as dealcoholisation must be therefore verified and quantified in order to avoid false positives (incorrect judgment of watering or chaptalisation for authentic wine).

Several authors have noticed the isotopic fractionation phenomenon during the wine dealcoholisation process with contactor membrane (Barbeito *et al.*, 2009; Ferrarini, 2011; Fedrizzi *et al.*, 2013), however depth studies regarding the parameters which influence it, are missing; therefore, because of the criticality which could emerge during the dealcoholisation treatment of wines for the loss of the wine isotopic traceability and for the evaluation of possible "watering" phenomena, due to water transfer in wine compartment, an ample experimentation with pilot plant has been realized for the study of ratio modifications of wine stable isotopes during the treatment with contactor membrane.

In this work some results of this experimentation (isotopic ratios changement) and the impact of membrane contactor on the aroma profile of Italian wines, dealcoholised in industrial plants, are reported.

2. Materials and Methods

2.1. Industrial dealcoholisation plant

The samples used were commercial wines provided from several wineries involved in the project. The dealcoholisation process was carried out with different wines: one Yellow Muscat, three Verdicchio, one Soave, two Sfursat, one Chianti, one Valpolicella, one Incrocio Manzoni and two Sauvignon blanc.

The contactor membrane apparatus involves the treatment of the wine with a hydrophobic membrane which, placed between the product and an extractive solution (water), creates a gaseous layer (gaseous membrane) through which most volatile compounds pass (Hogan *et al.*, 1998); among these compounds, the most representative is ethanol. The difference between the ethanol vapour pressure in the wine and the extractive solution permits the ethanol transfer in a vapour state, through the gaseous membrane which then is dissolved in the extractant. The membranes used in this work were provided by Ju.Cla.S. (Verona, Italy); this is made by polypropylene hollow fibers which had a surface of 20 m^2. The flow rate of wine and water was about 0.2 ms^{-1}. 1000 L of wine and 500 L of water were used in the industrial plant. All the treatments have been carried out at room temperature (20 °C). The vaporising efficiency of the membrane is between 0.1 and 0.2 $Lm^{-2}h^{-1}$ of anhydrous alcohol at 20 °C.

2.1. Industrial dealcoholisation plant

The pilot plant used for the experimentation was supplied by Ju.Cla.S. - Vason VR - I. This is constituted by:

- Contactor Membrane
- Treatment compartment
- Extractive compartment
- Connection pipes

The contactor membrane is made by a hollow fibers module of polipropylen placed into inox vessel (membrane characteristics: pore 0.04 µm, thickness δ 40 µm; module characteristics: internal area 1.4 m^2). The flow rates and the pressures during different dealcoholisation processes have been maintained constant: 420 L/h and 0.6 bar for the treatment compartment, 150 L/h and 0.4 bar for the extractive compartment. After the application of the technique, an evaporative efficiency of the membrane has been observed between 0.1 and 0.2 $l/m^2/h$ (expressed as anhydrous alcohol) at 20 °C.

All experimentations were carried out on laboratory scale at room temperature (20 °C), operating with 10-15 L of different solutions as shown below and with a ratio of 1:1 between the product to and the extractant solution.

The water and the product to be treated have been placed in recirculation in batch.

In order to evaluate the different isotopic fractionation phenomena and the transfer of matter due to the osmotic pressure, the experimentation has been done using:

- water coming from must concentration plants, obtained from Central-Southern Italy (+4.8 - +3.9 $\delta^{18}O$ ‰) and northern Italy (+0.3 $\delta^{18}O$ ‰) grapes Must water
- demineralized local (Verona, I) tap water,
- Valpolicella wine,
- Food Grade glycerol (ACEF-I),
- ethanol 95% vol of viticultural source.

The experimental protocols with pilot plant were:

Test 1

Treatment compartment: 10 L must water ($\delta^{18}O$ = +4.80).

Extractive compartment: 10 L demineralized tap water $\delta^{18}O$ = -8.30).

Samples:

- t0 start process,
- t1 1 hour,
- t2 2 hour,
- t3 3 hour
- …
- t8 8 hour (end process).

The samples were taken in both compartment.

Test 2

Treatment compartment: 14 must water 14% vol ethanol $\delta^{18}O$ = +3.90).

Extractive compartment: 14 L demineralized tap water ($\delta^{18}O$ = -8.40).

Samples:

- t0 start process,
- t1 15 min,
- t2 30 min,
- t3 45 min,
- t4 60 min,
- t5 90 min,
- t6 120 min,
- t7 180 min (end process).

The samples were taken in both compartment.

Test 3

Treatment compartment: 13.5 L must water 14% vol ethanol $\delta^{18}O$ = +3.90).

Extractive compartment: 13.5 L demineralized tap water with 15.5 wt %

glycerol ($\delta^{18}O$ = -8.40). The osmotic pressure of this solution is the same as a hydroalcoholic solution at 10.5% vol.

Samples:

- t0 start process,
- t1 15 min,
- t2 30 min,
- t3 45 min,
- t4 60 min,
- t5 90 min,
- t6 135 min,
- t7 200 min (end process).

The samples were taken in both compartment.

Test 4

Treatment compartment: 15 L wine 14.3% vol ethanol ($\delta^{18}O$ = +1.70).

Extractive compartment: 15 L demineralized tap water ($\delta^{18}O$ = -8.30).

Samples:

- t0 start process,
- t1 15 min,
- t2 30 min,
- t3 45 min,
- t4 60 min,
- t5 90 min,
- t6 120 min,
- t7 180 min (end process).

The samples were taken in both compartment.

Test 5

Treatment compartment: 15 L wine 14.3% vol ethanol ($\delta^{18}O$ = +1.80).

Extractive compartment: 15 L demineralized tap water with 20.69 wt % glycerol ($\delta^{18}O$ = -8.50). The osmotic pressure of this solution is the same as a hydroalcoholic solution at 14.3 % vol.

Samples:

- t0 start process,
- t1 15 min,
- t2 30 min,
- t3 45 min,
- t4 60 min,
- t5 90 min,
- t6 120 min,
- t7 180 min,
- t8 240 min (end process).

The samples were taken in both compartment.

Test 6

Treatment compartment: 14 L must water 14.3% vol ethanol ($\delta^{18}O$ = +0.30)

Extractive compartment: 14 L demineralized tap water with 20.69 wt % glycerol ($\delta^{18}O$ = -8.70). The osmotic pressure of this solution is the same as a hydroalcoholic solution at 14.3% vol.

Samples:

- t0 start process,
- t1 15 min,
- t2 30 min,
- t3 45 min,
- t4 60 min,
- t5 90 min,
- t6 120 min,
- t7 180 min,
- t8 210 min (end process).

The samples were taken in both compartment.

2.2. Analysis

All samples of dealcoholized wines and the solutions used in pilot plant protocols have been analyzed in triple, employing the following methods.

- Alcohol analysis

Analysis of alcohol content was carried out by applying the reference method (G.U. CEE 2676/90).

- Stable Isotope Ratios Analysis

The stable isotope ratios in wine and water extractive solution were carried out according to the official methods described in the OIV methods MA-E-AS311-05-ENRRMN, MA-E-AS312-06-ETHANO,MA-E-AS2-09-OU018.

For measurement of $^{13}C/^{12}C$ in ethanol and of $^{18}O/^{16}O$ in water we used an IRMS (SIRA II, VGISOGAS Fisons, Rodano, Italy), interfaced with an elemental analyser (NA 1500, Carlo Erba, Milan, Italy) and with a CO_2 equilibrator (Isoprep 18, VG Fisons), respectively. Ethanol was distilled using a distillation system that guarantee a recovery of more than 95 % of ethanol (Ing. Bullio, Milan, Italy).

The ratios $^{13}C/^{12}C$ and $^{18}O/^{16}O$ were expressed in δ‰ against international standards (Vienna—Pee Dee Belemnite for $\delta^{13}C$,Vienna—Standard Mean Ocean Water for $\delta^{18}O$) according to the equation δ ‰ = [(R_{Sample} / $R_{Reference}$) - 1] × 1000, where R is the

ratio of the heavy to light stable isotope in the sample (R_{Sample}) and in the international reference material ($R_{Reference}$). The isotopic values were calculated against working in-house standards (ethanol and water), calibrated against international reference materials: fuel oil NBS-22 (IAEA, Vienna, Autria) and sugar IAEA-CH-6 (IAEA) for $\delta^{13}C$ and V-SMOW and V-SLAP (IAEA) for $\delta^{18}O$. The uncertainty of measurements was 0.3 ‰ for both parameters.

Wine aroma analysis

Analysis of aroma compounds was carried out by applying a method previously published (Fedrizzi *et al.*, 2011). Recovery of the analytes from a solid phase extraction (SPE) cartridge was followed by gas chromatography—mass spectrometry (GC-MS) analysis. The SPE method was performed with an Aspec XL Sample Processor (Gilson Inc., Middleton, USA). The cartridges (1 g ENV+ cartridges; Isolute, IST Ltd., Mid Glamorgan, UK) were conditioned with methanol (10 mL) and MilliQ water (10 mL). The wine samples (76 mL) diluted 1:4 v/v with MilliQ water and added with 1-heptanol as internal standard (500 μgL^{-1}), were eluted through the cartridge. The cartridge was rinsed with 10 mL of MilliQ water and the free aroma compounds were recovered with 9 mL of dichloromethane. The solution was dried with Na_2SO_4 and concentrated to 0.4 mL under nitrogen. GC-MS analyses were performed on a 6890 N NetworkGC System coupled with a 5978B inert XL EI/CI MS(Agilent Technologies, Milano, Italy), equipped with a HPWAXPEG fused silica capillary column (60 m×320 µmi.d.×0.25 µm film thickness; Agilent Technologies). MS conditions were: electron impact energy and MS source temperature 70 eV and 230 °C, respectively. GC injector temperature was 250 °C and helium was used as the carrier gas (flow, 1.2 $mL \bullet min^{-1}$).

Column temperature program was: 50 °C (4 min), 4 $°C \bullet min^{-1}$ to 240 °C, 240 °C (16 min).

Recognition of the analyte was achieved by injecting the pure reference standards and using the NIST library.

Tableau 1 - Losses (percentage) of main aroma compounds classes (not glycosylate-free) during the dealcoholisation of different white (w) and red (r) Italian wines at ethanol removal levels up to −2 % vol. in an industrial-scale plant. Dealcoholization process carry out at 14 - 18 °C, except for Sauvignon blanc B and Incrocio Manzoni which have been worked at 4 - 8 °C.

Wine	Alcohol romoval (%)	Ethyl Esters	Acetates*	Norisoprenoids	Terpene alcohols	β-phenyl-ethyl alcohol	C6	Oak lactones	3-MH + 3-MHA
Sfursat A (r)	- 1,65	39	36	15	15	35**	12	24	nd
Sfursat B (r)	- 1,33	13	10	22	22	30**	22	18	nd
Chianti (r)	- 1,70	20	20	1	2	5**	10	10	nd
Valpolicella (r)	- 1,00	27	38	8	8	17**	14	15	nd
Verdicchio A (w)	- 2,00	8	44	1	4	4	12		nd
Verdicchio B (w)	- 2,00	14	36	3	16	2	9	nr	nd
Verdicchio C (w)	- 2,00	12	56	8	38	2	15	nr	nd
Soave (w)	- 2,00	25	36	2	20	6	12	nr	nd
Moscato giallo (w)	- 2,00	33	30	0***	23	7	0	nr	nd
Sauvignon blanc A (w)	- 2,00	nd	nd	nd	nd	nd	nd	nr	26
Sauvignon blanc B (w)	- 2,00	13	22	2	2	3	- 4,2	nr	nd
Incrocio Manzoni (w)	- 2,00	7	17	4	- 2	- 6	5	nr	nd
Average	**- 1,81**	**19**	**31**	**6**	**13**	**5**	**10**	**17**	**26**
Standard dev.	- 0,33	11	16	7	12	12	7	6	-
Min	- 2,00	7	10	0	- 2	-6	-4	10	-
Max	- 1,00	39	56	22	38	35	22	24	-
Median	-	14	36	3	15	5	12	17	-

nd = not determined, nr = not available, * Except ethyl acetate, ** Values lower than 10 mg/L, *** Only β-damascenone

3. **Results and discussion**

3.1 Aromas

Tableau 1 shows the results of various dealcoholization experiences of different italian red and white wines typology, carried out with industrial plant. Except β-phenyl-ethylalcohol and Oaklactone, the data analysis of aromatic compounds are reported expressed not individually but as sum of the aromatic

classes; furthermore it should be considered that the expressed data do not underline the real sensorial impact of the technique, because the presence of different aromatic compounds in wines was already under perception threshold.

The results obtained about the wines are quite important as it is noticeable that a significant number of aroma compounds are depleted during the dealcoholisation procedure; the extreme data variability should be underlined, as it pointed out from standard deviation.

The esters and, particularly, the acetates are reduced during the dealcoholisation. We observed a decrease of ethyl esters (C5, C4-C10) varying between 7 and 39%, tendencially a few higher for the MW-higher esters; we could consider a decrease of about 20 % for a 2 proof decrease, mostly evaluating the usually higher ester, the ethyl octanoate.

As for the acetates also important in contributing to the fruity scent above all in young white wines, both the most representing one, the isoamyl acetate, and the total sum of them have a level reduction of about 36%.

We considered some norisoprenoids which are possible complementing scent in the wine just as young products, as the β-damascenone and β-ionone, or by progressed aging as TDN (1,1,3-trimethyl-1,2-dihydronaphthalene), vitispiranes (VTP), actinidols and relevant ethyl ethers. The relevant concentrations of the ethyl ethers are quite constant, while those compounds can have an important decrease as for TDN until about 40% or a little more reduced variation (-15%), as for VTP (both contained in Sfursat wines in moderate amounts).

Instead, free monoterpenes alcohols and organic acids (not reported data) are only partially lost in the process.

For the β-phenyl-ethyl alcohol it can observe a small decrease (average 5%), but also a little increase, because of the concentration due to ethanol loss.

In the case of C6-alcohols, a little decrease can also be observed at about 10%; only in the case of the Muscat treatment, we measured an approximate constance of the values.

The Oak Lactones, a compound derived mainly from the barrels and relevant co-operage techniques, undergoes a decrease of 17% and that suggests the dealcoholisation intervention before to insert the wine in barrique.

About the decrease of the typical Sauvignon blanc tiolic compounds, it has been noticed a decrement of 26%.

As aroma interesting trace-compounds grouped as ‘phenols’ like eugenol, guaiacol, o- and p-cresol and phenol, when properly evaluated as a sum in our wines, even a possible decrease of about 20% can be estimated (not reported data).

In addition, it is possible to notice for all classes of considered compounds, that the main loss occurs in the first fraction (−2% vol.), while in the following fractions the aroma profile remains almost constant (not reported data).That could suggest, in order to limit the aroma loss due to the process, to treat with a strong dealcoholisation only a wine fraction, mixed with the not treated product, which reduces the alcohol level until the desired value. Furthermore, it is interesting to underline that the dealcoholisation treatments with contactor membrane at low temperatures cause losses of free aromatic compounds definitely lower than the treatments made at normal winery temperatures (14-18 °C); as the data prove about Sauvignon blanc B and Incrocio Manzoni, which have been worked at 4-8 °C.

All aromatic glycosylate precursors (not reported data) undergo an increase with dealcoholisation, because of the concentration due to decrease from alcohol loss.

These results appeared generally agreement with those previously published (Diban *et al.*, 2008; Varavuth *et al.*, 2009), though the examples, reported in the literature, either considered a model wine solution or took into account a number of compounds not significant for evaluating the effect of this technique in a complex matrix such as wine. The different behavior showed by the different classes of compounds account for their physical chemical diversity, for the different concentration in the wine matrix and also for their different affinity to the membrane, that, together with the removal variability of different aromatic compounds and with the strong sensorial interaction due to ethanol decrease, portends complex impacts of the technique on wines sensorial profiles with a specific behavior for the individual dealcoholated product by “directly contactor”.

3.2 Aromas

Different experimentations have been made with a laboratory pilot plant using model solutions. In particular in tests 2 and 3 it has been worked with a hydroalcoholic solution at 14% v/v ($\delta^{18}O$ +3.90) in the treatment compartment; the extractant of test 2 was constituted by demineralized tap water ($\delta^{18}O$ -8.40), while in test 3 it was constituted by demineralized tap water with 15.5 wt % of glycerol ($\delta^{18}O$ -8.40), in order to create an osmotic pressure as well as a hydroalcoholic solution at 10.5% v/v. The $\delta^{18}O$ value of the solution in the treatment and extractive compartment tends to equilibrium following a kinetic linked to isotope concentrations; the lower is the $\delta^{18}O$ of extractant, the higher will be the variation of $\delta^{18}O$ in wine at the end of dealcoholisation process. Therefore, the difference between $\delta^{18}O$ of treatment compartment and $\delta^{18}O$ of extractive compartment is crucial.

In test 2, where the osmotic pressure is always higher in treatment compartment solution (simulation of a real oenological condition), after removing 2% v/v of ethanol, the $\delta^{18}O$ value of wine alike solution shifts from +3.90 to +3.32 (0.58 drop), with an effect on $\delta^{18}O$ ratio comparable at an "hypothetical" watering of 4.7%.

In test 3, for a 2% v/v of removed ethanol, the wine alike solution $\delta^{18}O$ value shifts from +3.9 to +3.46 (0.44 drop), with an effect on $\delta^{18}O$ ratio comparable at an hypothetical watering of 3.6%.

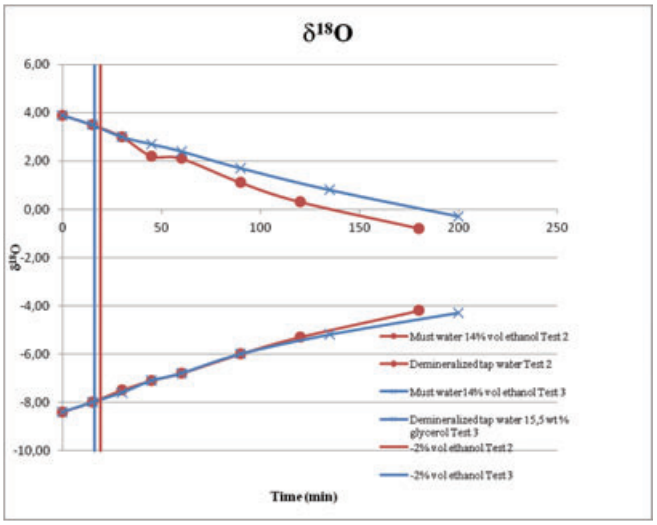


Figure 1 - $\delta^{18}O$ variation during the dealcoholisation process of Test 2 and Test 3

Compared to the previous case (test 2) where $\Delta\pi$ was notable, the osmotic pressure balancing reduces the "hypothetical" watering at 1.1% (4.7 – 3.6). This underlines that most of isotopic fractionation is not due to water migration from extractant to wine caused by osmotic transfer, but it is due to an isotopic diffusion controlled by Fick's law or more likely by Knudsen's law, as this last one rules when porosity ranges from 2 to 40 nm.

In these two experimentations ethanol transfer kinetic was also evaluated (not reported data); it has been proved to be certainly higher than kinetics which control water isotopes migration.

Moreover, glycerol in extractant solution does not decrease ethanol transfer for a 2 % v/v of removed ethanol.

Regarding $\delta^{18}O$ and alcohol values of both compartment solutions during dealcoholisation process, it seems that different osmotic pressure in extractive compartment has low influence on isotopic exchanges, whereas it can slow down alcohol transfer kinetic towards extractive solution (not reported data), according with other authors (Michaels, A.S International Publication Number WO 93/22036 WIPO 1993).

Fig. 2 describes variations of isotopic ratios during test 1, in which has been made

"contact" between two different $\delta^{18}O$ waters; in this case matter transfers due to osmotic pressure difference among solutions of both compartments are excluded.

Even in lack of osmotic phenomena, an important isotopic fractionation happens with behavior like previous experimentation (Test 2). This again means that isotopic fractionation during dealcoholisation process occurs, mostly, because of isotopes diffusion.

Table 2 shows the data obtained in the laboratory experimentation regarding the isotopic fractionation of $\delta^{13}C$ and the reduction of ethanol content in different osmotic pressure conditions.

Looking at the treatment compartment, data shows a progressive increase of the ethanol fraction richer of the heavier isotope (^{13}C which has a vapour pressure lower than the ^{12}C) during the early phases of dealcoholisation.

Therefore there is a prevailing transfer of low molecular weight ethanol towards the extractive compartment. Near to the ethyl alcohol concentration balance point between the two compartments, the transfer driver of ethanol (considering both isotopes) due to its partial pressure difference between the two compartment tends to be null, whereas concerning the heavier isotope concentration difference between the two compartments favours its diffusion in the extractive compartment taking to the $\delta^{13}C$ decreasing up to the initial values in case of high dealcoholisation rates.

This follows a more rapid kinetic with the maximum increase of $\delta^{13}C$ when the extractant is constituted of water (Test 2 and 4), a situation similar to the contactor membrane dealcoholisation directly applied on wine;

In these conditions, and dealcoholizing up to 2% vol. EtOH, the increase of $\delta^{13}C$ ratio changes from 1 (in case of EtOH ratio of -26.8, test 4) to 1,28 (in case of EtOH ratio of -14.6, test 2).

Instead, when it has been worked with an exctractant assembled by must water and glycerol at 20,69 wt % (Test 5 and 6), equivalent to the osmotic pressure, which is created by an hydroalcoholic solution at 14.3% v/v, the kinetic of $\delta^{13}C$ ratio modification was slowed. Also the maximum increase of the $\Delta\,\delta^{13}C$ has taken lower values and it has reached early compared with test 4, where the extractant is only tap water.

Test 3 underlines an intermediate kinetic compared with previous test, inasmuch the glycerol concentration (15.5 wt %) in the treatment compartment is the same as hydroalcoholic solution at 10.5% v/v.

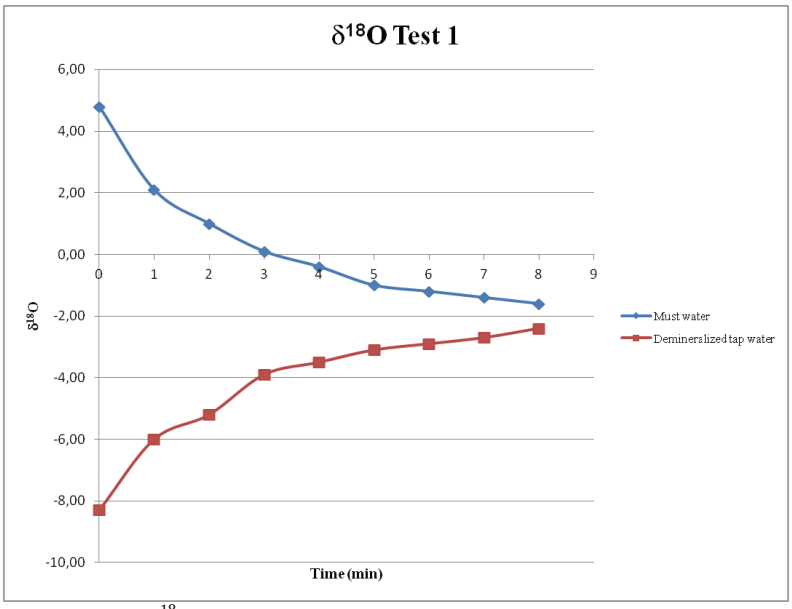
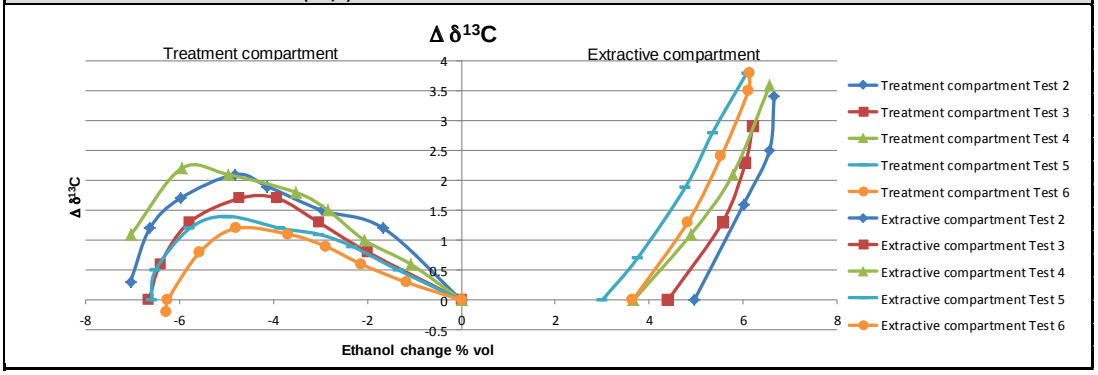


Figure 2 - $\delta^{18}O$ variation during the dealcoholisation process of Test 1

Tableau 2 – Effect of $\delta^{13}C$ isotopic fractionation and of ethanol content reduction in different conditions of osmotic pressure during the treatment with contactor membrane. In the charts, the results in terms of alcohol concentration decrease (treatment compartment) and increase (extractive compartment) are shown.

Test 2	Time (min)	0	15	30	45	60	90	120	180	-
	Δ $\delta^{13}C$ treatment compartment	0	1.2	1.5	1.9	2.1	1.7	1.2	0.3	-
	Δ $\delta^{13}C$ extractive compartment	-	-	-	-	-	1.6	2.5	3.4	-
	Ethanol lost (% v/v)	0	-1.66	-2.97	-4.13	-4.81	-5.986	-6.632	-7.046	-
Test 3	Time (min)	0	15	30	45	60	90	135	200	-
	Δ $\delta^{13}C$ treatment compartment	0	0.8	1.3	1.7	1.7	1.3	0.6	0	-
	Δ $\delta^{13}C$ extractive compartment	-	-	-	-	-	1.3	2.3	2.9	-
	Ethanol lost (% v/v)	0	-1.99	-3.04	-3.92	-4.72	-5.79	-6.41	-6.65	-
Test 4	Time (min)	0	15	30	45	60	90	120	180	-
	Δ $\delta^{13}C$ treatment compartment	0	0.6	1	1.5	1.8	2.1	2.2	1.1	-
	Δ $\delta^{13}C$ extractive compartment	-	-	-	-	-	1.1	2.1	3.6	-
	Ethanol lost (% v/v)	0	-1.08	-2.06	-2.84	-3.52	-4.97	-5.96	-7.03	-
Test 5	Time (min)	0	15	30	45	60	90	120	180	240
	Δ $\delta^{13}C$ treatment compartment	0	0.5	0.9	1.1	1.2	1.4	1.2	0.5	0
	Δ $\delta^{13}C$ extractive compartment	-	-	-	-	0.7	1.9	2.8	3.8	ND
	Ethanol lost (% v/v)	0	-1.34	-2.33	-3.04	-3.85	-4.99	-5.78	-6.51	-6.6
Test 6	Time (min)	0	15	30	45	60	90	120	180	210
	Δ $\delta^{13}C$ treatment compartment	0	0.3	0.6	0.9	1.1	1.2	0.8	0	-0.2
	Δ $\delta^{13}C$ extractive compartment	-	-	-	-	-	1.3	2.4	3.5	3.8
	Ethanol lost (% v/v)	0	-1.18	-2.13	-2.9	-3.68	-4.79	-5.57	-6.25	-6.28



4. Conclusions

Due to the climatic change and also to the current social needs towards alcohol intake, reducing wine alcohol content is often necessary, especially in the production of "typical" wines. There are several possibilities, especially for wine dealcoholisation, but every different possible technique presents some economic, qualitative, or management issues.

Some tests made in industrial plants by "contactor" membrane technology show that the technique can be used directly on the wine, in the case of operating for less than 2 percent alcohol reduction as required by Community legislation.

This system allows money savings and easy usage, however, the process of dealcoholisation could present some problems, as:

- Removal of aromatic compounds. The phenomenon lies more with fermentation aromas from esters and, above all, acetates which are particularly abundant in young wines and, however, decrease during wine storage, so it is necessary to work with this technique as soon as possible after alcoholic fermentation and it is better to dealcoholise a small part of the whole wine with great intensity. It is also important to operate at low temperatures, which greatly limit the loss of free volatile compounds. Besides, operating on process parameters (i.e. "contactor" surface and porosity, treatment time, temperature, extractor volume) it is possible to minimize losses of free aromatic compounds, making almost unnecessary the implementation of other technological solutions such as, for example, combination of contactor technique with other membrane techniques (OI and NF); furthermore these solutions make the process considerably more complex and expensive and, above all, not completely effective, for example, esters and, in particular, acetate rejection of NF membranes is only partial rejected (Ferrarini, 2011); at the same time, dealcoholisation with a contactor membrane on the NF permeate does not exceed critical

isotope fractionation studied (Fedrizzi *et al.*, 2013, Ferrarini, 2011).

- Impact on the wines sensory profile. The high variability in removal of various aromatic compounds, as well as the simultaneous increase of other compounds (Fedrizzi, 2011) and the strong sensorial interaction due to ethanol decrease, may cause complex changes of sensory profiles of the wines with the result of specific behavior for each dealcoholated product through "direct contactor". This is partly forecasted by "Sweet spot" laboratory test. Moreover, various experiments showed that, on sensory level, the decrease of alcohol amount, with more volatile compounds feeling, can compensate the loss of these volatile fractions.

- Possible wine dilution due to water migration from the extractant compartment. Several experiences have demonstrated that watering due to osmotic phenomenon of water recall in wine compartment is limited and comparable to water introduction in wine caused by the use of oenological adjuvants in aqueous dispersion (eg bentonite); $\delta^{18}O$ value drops, which would indicate much more intense dilution, is mainly caused by "isotopic diffusivity" phenomenon, that involves ^{18}O water molecules migration from wine compartment to extractant compartment.

- Loss of isotopic tracking. During previous experiences (Fedrizzi *et al.*, 2013) of contactor membrane dealcoholisation conducted with industrial plants a decrease of up to 1.1 ‰ for wine water $\delta^{18}O$ and increase of 1.1 ‰ $\delta^{13}C$ for ethanol are observed in the course of wine alcohol reduction of legal 2% vol., and these data were confirmed by results obtained in laboratory experiments reported in this paper.

The $\delta^{18}O$ decrease seems to depend on $\delta^{18}O$ value of extracting water solution. These variations must be taken into account when these parameters are considered for evaluating watering or chaptalisation of dealcoholized wine, especially for wines having an isotopic composition at the limit of the range of natural variability before dealcoholisation. Anyway, verification of a dealcoholized wine can be undertaken by the official controlling body as all these kinds of procedures are required to be registered by the operator (EU Reg 606/2009).

Besides, this issue can not be overcome by technical solutions previously proposed as the wine permeate obtained from NF dealcoholisation (Ferrarini, 2011).

Therefore, the biggest issue in contactor membrane dealcoholisation treatment may be considered isotopic fractionation, which can also occur in other "evaporative" dealcoholisation techniques .

However, in case of contactor membrane, isotopic transfers are controlled by different "Drivers": Δ vapor pressure of the various water and ethanol isotopes and their Δ relative vapor pressure, Δ osmotic pressure created mainly by ethanol, all of them more or less related.

Finally, this work shows that isotopic ratios variations and, in particular, $\delta^{18}O$ value modification, which is a "sensitive" parameter in wine dealcoholisation, is caused by "isotopic diffusivity", a phenomenon which has been never reported in literature. It can be interpreted by Fick's or Knudsen's law, depending on contactor membrane charateristics (pore diameters).

Therefore, in the future, these test data, only presented partially here, will be used for their modeling in order to better quantify/understand matter transfers and isotopic ratios change ought to various phenomena and in order to evaluate any intervention on the process for restrict or overcome issues emerged.

Acknowlegements : Thanks to JU.CLA.S. (Verona-I), for pilot plant and technological support provided, and Dr. Enrico Bocca (Vason Group-I) for the collaboration in the realization of industrial experience.

This work was realized with Veneto Region support as part of the Rural Development Programme 2007-2013 for the Veneto, mis. 124 project "LOWALCOL." The experiment was authorized as an exception to EC legislation by the Ministry of Agriculture, Food and Forestry (RU 0008075-09/11/20003).

4. References

Barbeito, M. E., Quini, M. Murgo, C., Stocco, N., & Guzman, J. (2009) Fraccionamiento isotopico del agua del vino desalcoholizado mediante osmosis inversa en la fase de separacion del alcohol a traves de un sistema de membrana perextractiva. *OIV - CII TECHNO* 03.2009-20.

Bindon, K. A., Dry, P. R., & Loveys, B. R. (2008). The interactive effect of pruning level and irrigation strategy on grape berry ripening and composition in Vitis vinifera L. cv. Shiraz. *South African Journal of Enology and Viticulture*, **29**, 71–78.

De Risi, J. L., Iyer, V. R., & Brown, P. O. (1997). Exploring the metabolic and genetic control of gene expression on a genomic scale. *Science, 278* (5338), 680–686.

Diban, N., Athes, V., Bes, M., & Souchon, I. (2008). Ethanol and aroma compounds transfer study for partial dealcoholisation of wine using membrane contactor. *Journal of Membrane Science*, **311**, 136–146.

Ferrarini, R. (2011) Alcohol management and production of alcohol reduced wines. *Proceedings of the 16th International Oenology Symposium, Bolzano* 16-18 maggio 2011, 43-49.

Fedrizzi, B., Zapparoli, G., Finato, F., Tosi, E., Turri, A., Azzolini, M., et al. (2011). Model aging and oxidation effects on varietal fermentative, and sulfur compounds in a dry botrytized red wine. *Journal of Agricultural and Food Chemistry*, **59**, 1804–1813.

Fedrizzi, B., Nicolis, E., Camin F., Bocca E., Barbieri P., Finato F., & Ferrarini. R. (2013) Stable isotope ratios and aroma profile changes induced due to innovative wine dealcoholisation approaches. *Food and Bioprocess Technology*, doi: 10.1007/s11947-013-1068-x

Hogan, P. A., Canning, R. P., Peterson, P. A., Johnson, R. A., & Michaels, A. S. (1998). A new option: osmotic distillation. *Chemical and Engineering Progress*, **49**.

Jancso, G., & Van Hook, W. A. (1974). Isotopic fractionation of water during evaporation. *Chemical Review*, **7**, 545–678.

Kutyna, D. R., Varela, C., Henschke, P. A., Chambers, P. J., & Stanley, G. A. (2010). Microbiological approaches to lowering ethanol concentration in wine. *Trends in Food Science and Technology*, **21**, 6, 293–302.

I.U.P.A.C. - International Union of Pure and Applied Chemistry (1996). Terminology for membranes and membrane processes. www.iupac.org/

Larsson, C., Pahlman, I. L., Ansell, R., Rigoulet, M., Adler, L., & Gustafsson, L. (1998). The importance of the glycerol 3-phosphate shuttle during aerobic growth of *Saccharomyces cerevisiae*. Yeast, **14**, 4, 347–357.

Liguori, L., Russo, P., Albanese, D., & Di Matteo, M. (2012). Effect of process parameters on partial dealcoholization of wine by osmotic distillation. *Food and Bioprocess Technology*. doi:10.1007/ s11947-012-0856-z.

Michaels, A.S.(1993). Membrane Extraction Process; Patent application, International Publication Number WO 93/22036 WIPO 1993.

Michaels, A.S., Canning, R.P., & Hogan, P. (1998). Methods for dealcoholization employing perstration, US Patent no. 5,817,359.

Pickering, G. J. (2000). Low- and reduced-alcohol wine: a review. *Journal of Wine Research*, **11**(2), 129–144.

Pilipovik, M. V., & Riverol, C. (2005). Assessing dealcoholisation systems based on reverse osmosis *Journal of Food Engineering*, **68**, 437–441.

Valenti, L., Mattivi, F., Bravi, M., Dell'Orto, M., & Ghiglieno, I. (2011). Pruning and shade control the sugar level. *Informatore Agrario*, **67** (13), 22–24.

Varavuth, S., Jiraratananon, R., & Atchariyawut, S. (2009). Experimental study on dealcoholization of wine by osmotic distillation process. *Separation and Purification Technology*, **66**, 313-32

Alcohol reduction in wine by nanofiltration. Some comparisons with reverse osmosis technique

Gonçalves F. [1], Ribeiro R. [2], Neves L. [2], Lemperle T. [2], Lança M. [2], Ricardo da Silva J. [2] and Laureano O.[2*]

[1]Enofisis Estudos Enológicos Lda. Instituto Superior de Agronomia, Tapada da Ajuda, Ed. Inovisa, 1349-017 Lisboa, Portugal
[2]Centro de Botânica Aplica à Agricultura, Instituto Superior de Agronomia, Tapada da Ajuda, 1349-017 Lisboa, Portugal

Abstract: The Increasing alcohol content in wines recorded in recent years, as well as greater consumer concern in relation to alcohol, leads to the need to develop technologies for wine dealcoholization. The use of nanofiltration (NF) membranes for wine dealcoholization is assessed. We studied three application of nanofiltration in reducing the alcohol content: reduction of alcoholic strength by 1-2 % and comparison with reverse osmosis (RO); production of new wines with low-alcohol content 5-8 % and dealcoholized wine(<0.5 %). In the first application, a wine with 16 % of alcohol was reduced to 14 % of alcohol by both RO and NF. The results indicate that with the exception of alcohol physico-chemical composition of the wine has remained largely unchanged. The wines treated by NF showed lower decrease in anthocyanins and their volatile acidity was reduced. Two rosé wines were dealcoholized to 7 % and 0.3 %. A decrease in total and volatile acidity was observed along with the alcohol reduction. The total phenols and anthocyanins contents suffered a reduction that seems to be independent form the extent of dealcoholization. In both wines there are no negative impacts on the color, aroma and flavor of wines.

Keywords: Dealcoholization, nanofiltration, reverse osmosis, membranes, alcohol

1. Introduction

During the last decades an increase in the alcohol content of wines was observed. This relates to improvements in viticulture and climate changes that led to a progressive increase in the sugar content of grapes. Moreover, consumers have shown preference for flavorful wines obtained from more mature grapes (Meilon *et al.*, 2010). This general trend had consequences on the wines alcohol level, with most wines between 14 and 16% v/v alcohol (Moutounet *et al.*, 2007).

This trend of increasing alcohol content led to the development of techniques to control or reduce the alcohol content of wine. A slight decrease in alcohol content (1-2 % alcohol) can produce wines with alcohol levels more acceptable and balanced, with full aromatic potential and a good phenolic ripeness. There is also a growing consumer concern with aspects of health and consumer habits that emphasize the need to reduce the alcohol content of wine. This reduction may not be restricted to the correction of the alcoholic strength by 1-2 % alcohol, but be taken further, with the aim of creating wines with low alcohol content (5-8 % v/v), or even wines no alcohol (<0.5 % v/v). This new type of wine has the health benefits associated with wine consumption, but with lower alcohol intake. The achievement of these goals requires a technology capable of reducing of the alcohol content of wine but maintaining the physico-chemical and sensory characteristics of wines.

Several approaches have been proposed to reduce alcohol in wines (Pickering, 2000). Most of the first wine dealcoholization processes were based on evaporation of the ethanol through techniques such as distillation, evaporation under reduced pressure, evaporation by contact with a counter-flow gaseous current, etc. However, these techniques remove volatile aromatic compounds together with the ethanol, leading to a product with poor aromatic intensity of little interest. A solution for this problem consists of a second distillation operation, to separate the aromatic compounds from the ethanol, which are then returned to the beverage.

Recently a technology based on spinning cone column has been used to dealcoholize wine. Here, the aromas are first removed from wine which is then dealcoholized and the aromas returned to the wine. Although these solutions reduce the aroma loss, this manipulation of the aromatic substances may have a negative effect on the aromatic profile of the original beverage.

Corresponding author : olgalaureano@isa.utl.pt

Another approach is the use of membrane separation process. One of the most used processes is reverse osmosis (RO) (Bui, 1986). These membranes allow the permeation of water and ethanol by means of high pressures, 60 to 80 bar. Two currents are obtained from the original beverage: one of permeate containing water and ethanol, and one of retentate with the remaining compounds, macromolecules, salts, etc. The decrease in volume resulting from permeation is compensated by adding water to the retentate (wine). Because of the low ethanol content in the RO permeate, the quantities of permeate removed can be significant which leads to addition of a large amount of water to the retentate. The reverse osmosis membranes require the use of very high pressures, usually higher than 60 bar, which, in addition to considerable energy consumption, brings about possible changes of the organoleptic properties of the wine.

Another membrane process similar to RO that can be used to wine alcohol removal is nanofiltration (NF). Nanofiltration membranes allow higher permeation flows than reverse osmosis membranes and higher permeation of solutes such as ethanol and salts. The use of NF membranes to remove ethanol appears to be advantageous over reverse osmosis since they allow a higher ethanol permeation flow and consequently a lower permeation volume is necessary. Another advantage of the use of this type of membranes is that the permeate is richer in ethanol than that obtained using RO membranes, resulting in a lower difference of osmotic pressures between the retentate and permeate, and so lower working pressures are necessary (Gonçalves, 2003).

These membranes, contrary to reverse osmosis, allow permeation of some salts. Permeation of some ions can be an advantage, as in the case of the acetate ion, as it can be eliminated from the beverage. Due to their large molecular weight polyphenols and macromolecules are retained in the retentate, so the body and flavor of wine are unaffected (Gonçalves, 2008).

Here we evaluated the use of nanofiltration for the reduction of alcohol levels in wine and compare with a more established technology: reverse osmosis. We intend also to evaluate the use of nanofiltration to produce low alcohol (5-8 % v/v) and dealcoholized wines (<0.5 % v/v). The wines dealcoholized by NF are characterized in terms of physico-chemical and sensory changes.

2. Material and methods

2.1. RO and NF experiments

The flow diagram of the equipment used for NF and RO dealcoholization assays is presented in Figure 1.

The wine is first pumped from a tank by means of a high pressure pump and passes through a heat-exchanger, cooled with water for temperature control, and then enters the membrane modules. Two spiral wound 4" membrane modules in series were used. Here two streams are obtained: one of retentate wich is recirculated into the wine tank and another of permeate that is removed continuously from the system.

The RO assays were performed using Vaslin-Bucher membranes type X at 70 bar transmembrane pressure. The NF assays were performed using ALNF97 membranes froma Alfa-Laval under 30 bar transmembrane pressure.

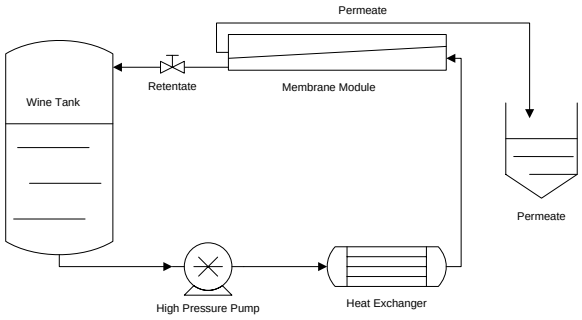


Figure 1. Scheme of the experimental set-up used for RO and NF assays.

2.2. Analytical methods

Alcohol concentrations were determined by ebulliometry for levels above 4 % (v/v) and for levels below 4 % (v/v) distillation/oxidation and titration by ferrous ammonium sulfate as described by Ribéreau–Gayon *et al.* (1972) was used. Analysis of pH, total acidity, total tartaric acid, total malic acid, and volatile acidity were done according to the methods prescribed by the International Organization of Wine and Vine (OIV) (2009). Finally, analysis of total phenols and the characterization of color were conducted as specified by Somers and Evans (1977). A *Unicam, UV4, UV/Vis* Spectrophotometer was used to obtain the measurements of total phenols and the characterization of color.

3. Results and discussion

A red wine with high alcohol content, 16 %, was used to evaluate the use of RO and NF for alcohol correction. Two samples of the same wine were dealcoholized by RO and NF, aiming a final alcohol degree of 14 %. The analytical results of the original and dealcoholized wines are presented in Table 1.

As can be seen from the results there is a reduction in most of the analytical parameters measured. The reduction of total acidity is most pronounced in the wine treated by NF (5 %) than by RO (1%). Along with the reduction of total acidity there is a pro-

nounced reduction of the volatile acidity in the wine treated by NF (14 %). This is related, as expected, to the fact that NF membranes allow a higher passage of ions, especially acetate ions, than RO.

Although the total phenols content suffers a minor change in both wines treated by NF and RO, there is an appreciable loss of anthocyanins. This decreased is more pronounced in the wine treated by RO (26 %) than by NF (9 %). The loss of anthocyanins is certainly not due to permeation through the membrane as its molecular mass is very high. The most probable cause seems to be adsorption on the membrane surface. In fact, during the cleaning of the membranes, after the wine dealcoholization assay, a colored solution was obtained, indicating the presence of phenolic material adsorbed on the membrane.

Table 1. Comparison between the same wine dealcoholized by NF and RO.
Results from Lança, 2010.

	Original	Dealc. NF	Dealc. RO
Alcohol (%)	16.0	14.2	14.1
Total acidity (g/L)	5.87	5.55	5.81
Volat. acidity (g/L)	0.80	0.69	0.82
pH	3.39	3.38	3.33
Color intensity	10.9	11.5	12.1
Hue	0.60	0.57	0.58
Total anth. (mg/L)	404.3	366.2	298.8
Total phenols (AU)	59.8	58.0	58.0

Despite this change in anthocyanins content, the color intensity and hue of the wine remain almost unchanged. There even an increase of the color intensity in the wines treated that is probably related to the decrease in pH that also occurs. The sensory evaluation performed on the wines treated by NF and RO also revealed no differences in color. The results from sensory evaluation of the wines showed a preference by the wine treated by NF over the original wine and the wine treated by RO.

The use of nanofiltration for the production of low-alcohol wine (7 % alcohol) and dealcoholized wine (<0.5 % alcohol) was also evaluated. In order to obtain these low alcohol values the treatment by NF is more intense, i.e. requires more passages by the membrane and therefore more pronounced changes in wine are expected. The analytical results of two different rosé wines prior and after the dealcoholization process are presented in Table 2.

The results in Table 2, show for both wines a reduction of the total and volatile acidity. The reduction of these two parameters is more pronounced as the alcohol reduction increases. The reduction of volatile acidity is very pronounced, being of 34 % for the low-alcohol wine assay and 86 % for the dealcoholized wine assay. This is due to the larger number of passages and consequent permeation of wine acids, namely acetic acid.

Table 2. Analytical results of two rosé wines dealcoholized by NF.
[1] Results from Ribeiro, 2007; [2] Results from Lemperle, 2010.

	Low-alcohol [1]		Dealcoholized [2]	
	Original	Dealc.	Original	Dealc.
Alcohol (%)	14.7	7.2	11.8	0.3
Total acidity (g/L)	4.0	3.7	4.7	3.6
Volat. acidity (g/L)	0.38	0.25	0.36	0.06
pH	3.56	3.41	3.29	2.99
Color intensity	3.36	3.33	2.47	2.39
Hue	-	-	0.69	0.64
Total anth. (mg/L)	159.9	131.9	184	149
Total phenols (AU)	16.7	13.7	15.9	12.4

As found in the results from Table 1 there is also a reduction of total phenols and anthocyanins in the wines treated. In opposition to the results regarding the decrease of acidity, the reduction of total phenols and anthocyanins seems to be independent of the extent of the NF treatment. The observed reduction of total phenols was 18 % for low-alcohol wine and 22 % for dealcoholized wine. Regarding anthocyanins the observed reductions were 18% for low-alcohol wine and 19 % for dealcoholized wine. Once again the values of the color intensity and hue remained almost unchanged.

Sensory evaluation was performed for both wines. Compared to their original wine, the dealcoholized wines showed no significant changes in color and the aromatic profile remained unchanged. The major differences found were related to the absence of alcohol and the wines were found more acidic despite de reduction of acidity measured. The dealcoholised wine obtained was compared with commercial samples of dealcoholized wines obtained by different technologies, showing better overall evaluation.

4. Conclusions

The use of nanofiltration for wine alcohol removal proved to be an effective technique. For wine alcohol correction when compared to reverse osmosis, the use of nanofiltration presents some advantages, namely the reduction of volatile acidity and a minor loss of anthocyanins.

Nanofiltration also allows a more extended removal of alcohol allowing the production of low-alcohol wines and dealcoholized wine. There are some changes on the physico-chemical composition of the wine, namely reduction of both total and volatile acidity and polyphenols. Nevertheless the wines maintained their color characteristics and aroma profile.

5. References

Bui K., Dick R., Moulin G. and Galzy P., 1986. A Reverse Osmosis for the Production of Low Ethanol Content Wine. *American Journal of Enology and Viticulture*, **37**, 4, 297-300

Gonçalves F., 2002. Optimização da Clarificação e Estabilização Tartárica de Vinhos por Processos de Membranas. Influência das Macromoléculas e da Composição Iónica. Tese de Doutoramento em Engenharia Química, Instituto Superior Técnico / Universidade Técnica de Lisboa.

Gonçalves F. and Pinho M., 2003. Processo integrado de nanofiltração para redução do teor alcoólico de bebidas. Patente de Invenção Nacional Nº102976.

Gonçalves F., 2008. Produção de vinho com baixo teor alcoólico. 1º Infowine Fórum – Congresso técnico-científico da VINIDEAS – "Inovação e Excelência" – Aromas, textura e consumidores ... sensações à prova!, Vila Real.

Lança M., 2011. Desalcoolização parcial de vinhos por nanofiltração e osmose inversa. *Master thesis of Master of Viticulture and Enology*, Instituto Superior de Agronomia / Universidade Técnica de Lisboa.

Lemperle T., 2010. Evaluation of Nanofiltration membranes for the production of a dealcoholized beverage at 0,5% (v/v). *Master Thesis of "European Master of Enology and Viticulture"*.

Meillon S., Dugas V., Urbano C. and Schlich P., 2010. Preference and Acceptability of Partially Dealcoholized White and Red Wines by Consumers and Professionals, *American Journal of Enology and Viticulture*, **61**, 1, 42-52.

Moutounet M., Bes M. and Escudier J., 2007. Las tecnologías de elaboración de vinos con bajo nivel de etanol. *ACE – Revista de Enología*, **84**.

Neves L., 2008. *Optimização da desalcoolização de vinhos rosados*. Relatório do trabalho de fim de curso de Engenharia Alimentar, Especialidade em Viticultura e Enologia, Instituto Superior de Agronomia/Universidade Técnica de Lisboa.

Pickering G.J., 2000. Low- and Reduced-alcohol Wine: A Review. *Journal of Wine Research*, **11**, 2, 129-144

Ribeiro R., 2007. Desalcoolização de vinho por nanofiltração: optimização da pressão de trabalho. Relatório do trabalho de fim de curso de Engenharia Alimentar, Especialidade em Viticultura e Enologia, Instituto Superior de Agronomia/Universidade Técnica de Lisboa.

Ribéreau-Gayon P., Dubourdieu D., Donèche B., Lonvaud A., 2006. *Handbook of Enology: Volume 1 The Microbiology of Wine and Vinifications. 2nd Edition*. John Wiley & Sons Ltd. ISBN-13: 978-0-470-01034-1, pag 459.

Somers T. and Evans M., 1977. Spectral evaluation of young red wines: Anthocyanin equilibria, total phenolics, free and molecular SO_2, chemical age". *Journal of the Science of Food and Agriculture*, **28**, 3, 279-287.

Alcohol reduction by osmotic distillation: system and result

Stassi Alberto*[1], Dulac Philippe [2] and Filippo Melandri [2]

[1]R&D Deparment, Diemme Enologia S.P.A. Via Bedazzo, 19, 48022 Lugo (RA), Italy
[2]Diemme Enologia S.P.A. Via Bedazzo, 19, 48022 Lugo (RA), Italy

Abstract: Osmotic Distillation, also called, Evaporative Perstraction, is a new separation process in which a liquid mixture containing a "volatile" component is contacted with a microporous, non-liquid-wettable membrane whose opposite surface is exposed to a second liquid phase capable of "absorbing" that component. In the case of polar liquids (for example an aqueous solution or an hydroalcholic solution) non-liquid-wettable membrane means a hydrophobic membrane, made in PP, PTFE o PVDF. This technology can be applied to the reduction of the alcohol content of wine.

Keywords: Osmotic Distillation, Evaporative Perstraction, Alcohl reduction, wine

1. Introduction

Since they have small diameter pores (< 1 μm) hydrophobic membranes cannot be crossed / wetted by aqueous solutions or polar solutions until the system pressure exceeds a threshold value. Said pressure value depends on the wetting liquid characteristics, system temperature, and membrane properties in that it depends on the material of which the membrane is made, the treatments to which it has been subjected, and the pores diameter.

Below the mentioned "intrusion pressure" threshold hydrophobic membranes (non-wettable) make it possible to "separate" two liquid masses, also miscible masses such as, for example, a hydroalcoholic solution and an aqueous solution. The interphase, which is composed of the "void" volume of the membrane pores (effectively the interphase is double) can however be crossed by gases and vapors by simple diffusion.

One of application of Osmotic Distillation is the reduction of the alcohol content of wine. In this process the ethanol contained in a wine stream, is transfer to a water stream in a contactor device, that is a device which contain the hydrophobic membrane and have two separate inlets and outlets which allow feed the contactor with two streams.

This transfer of ethanol is based on his spontaneous vaporization, which takes place just in correspondence of the porosity of the membrane (figure 1). The vapor of ethanol may migrate into this void volume of the membrane to reach the opposite side of the membrane where they condense on the water stream. If the liquid boundary layers on the both sides of membrane are "removed", the mass transfer continues until, after infinite time, water and wine streams have the same concentration on ethanol. The driving force of this migration is therefore the difference of concentration on ethanol in the both (water and wine) stream.

Also the water may vaporize and migrate as vapor on the empty volume of membrane, but the water is less volatile than ethanol. At room temperature (20 °C) the ethanol content of vapor in equilibrium with hydro alcoholic solution having 13 % v/v of ethanol (0.065 mole/mole), is more or less 55 % v/v (0.379 mole/mole) (figure 2).

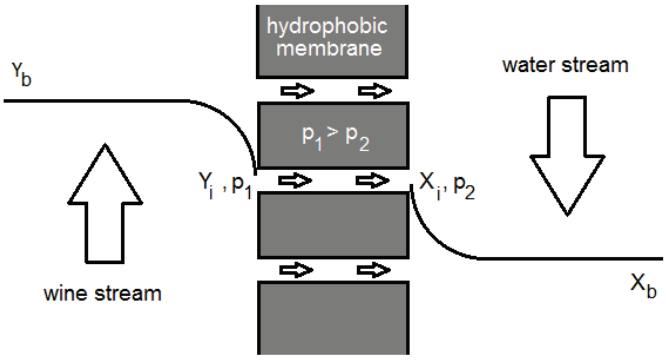


Figure 1 - Mass transfer of ethanol in osmotic distillation

Corresponding author : stassi@diemme-enologia.com

With regard to the membrane geometry or type, the market offers flat (parallel plate reactors) or tubular elements, capillary elements and hollow fiber elements.

The capillary fiber and hollow fiber types are indisputably the simplest to make and manage and are therefore to be preferred with respect to flat membranes.

2. Principle of osmotic distillation

The Osmotic Distillation separation process can be treated as a liquid/liquid (L/L) extraction between two liquid phases that are "virtually" immiscible with one another. Conventionally, the liquid phase from which the solute (wine or hydroalcoholic solution) is to be separated is called the "feed" and its derivatives are called "raffinates", while the liquid phase (water) that receives the volatile solute is called the "solvent" or "extractant" and its derivatives are called "extracts".

Before examining the thermodynamics or kinetics of the process in numerical (quantitative) terms a synopsis of several basic notions will prove helpful.

3. Expression of concentration, volumes and flow rate

In general the ethanol concentration of a hydroalcoholic solution is expressed as a relative volumetric percentage, i.e. as a quantity in volume of ethanol (EtOH) contained in 100 volumes of wine or hydroalcoholic solution:

$$Y_{EtOH} = 100 \frac{V_{EtOH}}{V_{wine}}$$

where: Y_{EtOH} is the percentage volumetric concentration, V_{EtOH} is the volume of Ethanol contained in the hydroalcoholic solution or wine V_{wine} is the total volume of wine or hydroalcoholic solution considered

Since during the dealcoholation process the volumes of the "feed" phase (wine) and that of the "extractant" phase undergo significant changes (caused by the separation process) it is more convenient to express the alcohol concentration not as a relative volumetric percentage but as an "absolute fraction by volume" defined by the ratio between the ethanol contained in a given volume of hydroalcoholic solution and the volume of said solution after removing the ethanol:

$$y_{EtOHl} = \frac{V_{EtOH}}{V_{Sol} - V_{EtOH}}$$

In the case of feed phase:

$$y_{EtOHl} = \frac{V_{EtOH}}{V_{wine} - V_{EtOH}}$$

where: y_{EtOH} is the concentration of ethanol in feed express as absolute fraction by volume. The absolute fractions and the percentage fractions can be correlated together using the following formulas:

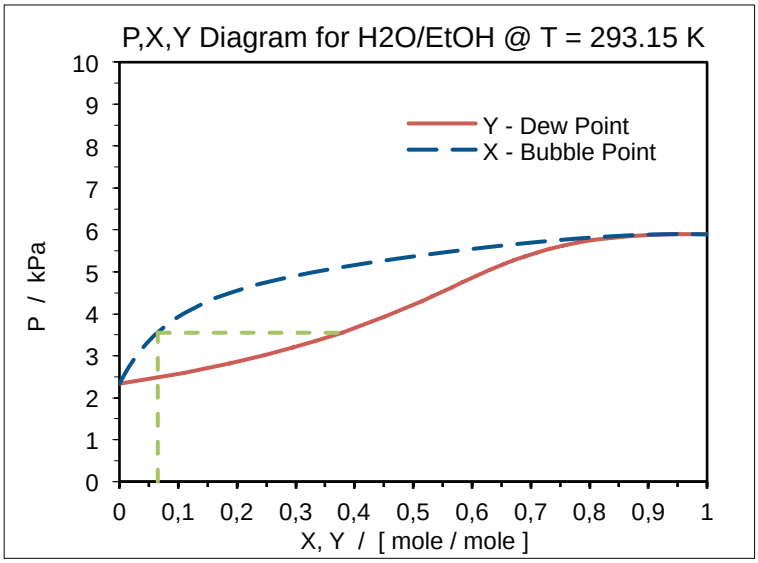


Figure 2. P,X,Y phase equilibrium diagram for binary system Ethanol/water at 295.15 K (20°C) - The Dew Point and Bubble Point curve are calculated from binary equilibrium data.

$$y_{EtOH} = \frac{Y_{EtOH}}{1 - Y_{EtOH}}$$

$$Y_{EtOH} = \frac{y_{EtOH}}{1 + y_{EtOH}}$$

likewise for the extracts

$$x_{EtOHl} = \frac{V'_{EtOH}}{V_{sol} - V'_{EtOH}}$$

$$x_{EtOH} = \frac{X_{EtOH}}{1 - X_{EtOH}}$$

$$X_{EtOH} = \frac{x_{EtOH}}{1 + x_{EtOH}}$$

where: X_{EtOH} is the percentage volumetric concentration, V'_{EtOH} is the volume of Ethanol contained in the "extract" solution V_{sol} is the total volume of extract solution considered and x_{EtOH} is the concentration of ethanol in extract solution express as absolute fraction by volume.

If in order to describe the composition of a liquid phase absolute fractions are employed in place of relative fractions, it will be necessary to express the volumes of extractant or raffinate as volumes of hydroalcoholic solution without alcohol:

$V_{RT} = V_{wine} - V_{EtOH}$ is the volume of hydroalcoholic solution to be deacoholized, without alcohol,

V_{Ex} is the initial volume of the extractant solution (without alcohol)

The advantage of this representation lies in the fact that if the mass transfer process is limited exclusively to volatile substances (e.g. ethanol), and hence the two phases "behave" as if they were "virtually immiscible", the quantities of V_{RT} and V_{EX} are independent of their composition and they remain constant throughout the process.

The same consideration can thus be extended to include the flow rates, which will be expressed as volume quantity (without alcohol) per unit time.

4. Phase Equilibrum

The driving force of the dealcoholation process is the concentration difference of ethanol between the liquid phase to be dealcoholized (raffinate) and the liquid extractant phase (extract). Since the two phases are both hydroalcoholic solutions, the condition of equilibrium is achieved when the respective ethanol concentrations are identical.

In more stringent terms, i.e. from the thermodynamic standpoint and with constant T and p, the equilibrium condition requires that the chemical potentials of the volatile solute in the two phases be identical.

Since the solute may vaporize, the chemical potential in the two phases will be equal when the fugacity of the vapor phases is identical. For each component "i" and for each phase present in the system (liquid or vapor) the chemical potential is given by:

$$\mu_i = RT \ln f_i + \theta(T)$$

where μ_I is the chemical potential, R is the ideal constant of the gases, T is the absolute temperature (expressed in K), f_i is the fugacity of component "i" which expresses the greater or lesser tendency of the component to transit to the vapor phase.$\theta(T)$ is a constant.

In conditions of equilibrium the chemical potential of component "i" in the liquid phase will be identical to that assumed by the same component in the vapor phase and at constant Temperature and pressure conditions this translates into equality of the two fugacities. Hence, for component "i" we will have:

$$f_i^L = f_i^V$$

and

$$\gamma_i \, x_i \, f^{\circ L}_i = y_i \, \phi_i \, P$$

where $f_i^{\circ L}$ is the fugacity of component "i" in pure form and in the liquid phase, x_i is the concentration of component "i" in the liquid phase, γ_ι is the activity coefficient of component "i" in the liquid phase, ϕ is the fugacity coefficient of component "i" in the vapor phase y_i is the concentration of component "i" in the vapor phase P is the system pressure.

We can however introduce a simplification: if we consider that our system is composed of the two liquid phases separated by a hydropho-

bic membrane, we can assert that both the phases can be assimilated to two hydroalcoholic solutions that share (in a manner of speaking) the same vapor contained in the membrane pores.

The chemical potential of the three phases (raffinate/vapor/extract) will be identical only if the two phases have the same composition. Disregarding the temperature and pressure difference at the sides of the membrane, and in equilibrium:

$$\gamma_{iL(RT)}\ x_{i(RT)} p_i^O = y_i P = \gamma_{iL(EX)}\ x_{i(EX)} p_i^O$$

this means that if

$$x_{i(RT)} = x_{i(EX)}$$

then

$$\gamma_{iL(RT)} = \gamma_{iL(EX)}$$

5. Effect of temperature

The temperature of the liquid phases in dealcoholation is a fundamental process parameter because it influences the viscosity of the phases (and hence the diffusivity of the gaseous or solubilized components) and the solubility of the gases and vapors in the liquids (Henry's coefficient) and because it determines the ethanol vapor pressure.

To calculate the ethanol vapor pressure at different temperatures we can use the Antoine equation, which is derived from the Clausius–Clapeyron relation.

$$\log10(P) = A - (B / (T + C))$$

where: P is the pressure in bar, T is the temperature in K

Figure 3 shows a pressure / temperature graph in which the vapor pressure values are plotted for the three components from 0 °C to T = 50°C and calculated utilizing the aforementioned Antoine equation. From the graph we can see that the vapor pressure for all three components depends exponentially on the temperature: for each 10 degree temperature rise the vapor pressure value doubles. (e.g. for ethanol at T = 10 °C p = 0.031 bar; at T = 20 °C p = 0.058 bar). Therefore, a temperature rise increases vapor production and hence tends to promote mass transfer, in a proportional manner.

On the other hand, the influence of temperature on the vapor composition is less significant: figure 4 shows the concentrations of the liquid phase (X) and vapor phase (Y) at the condition of phase equilibrium and at temperatures between 303.15 K (30 °C) and 343.15 k (70 °C) for the ethanol and water binary system. From the figure it can be seen that in the range of concentrations typical of Osmotic Distillation (i.e. for molar fractions between 0.5 and 0.1 mole/mole) the vapor composition at equilibrium remains almost unchanged at the three temperatures.

6. Single stage extraction (direct osmotic distillation)

The simplest configuration that can be created to perform dealcoholation by osmotic distillation consists of the single-stage batch type, which requires the reactor containing the hydrophobic membrane to be fed with complete recirculation of the extracts and raffinates until the raffinate concentration reaches the desired value. Figure 5 shows a schematic representation of the layout of a single stage direct osmotic distillation unit, while figure 6 shows the AlcolFlex 15 unit manufactured by Diemme Enologia S.p.a

Direct Osmotic Distillation offers the following benefits:

- The unit is simple to build.
- The process is cost-effective (no heat exchange or pressurization required).
- The product is not subjected to mechanical, thermal or chemical stress.
- The membrane functions in ideal conditions (low pressure and low temperature).
- The system can function even with wine alcohol content > 17%.

The volume loss of the treated wine is identical to the volume of alcohol extracted On the downside, the system has the following drawbacks:

- Process efficiency depends on the alcohol content of the wine and the extraction water.
- Process efficiency is medium-low, although when correctly sized it is possible to achieve high average extraction rates with values even higher than 100 l/h of absolute alcohol and with limited water consumption.
- At the end of the process the extraction water concentration is never higher than 6.5 % v/v in alcohol.
- Risk of wine oxygenation (suitable countermeasures required).

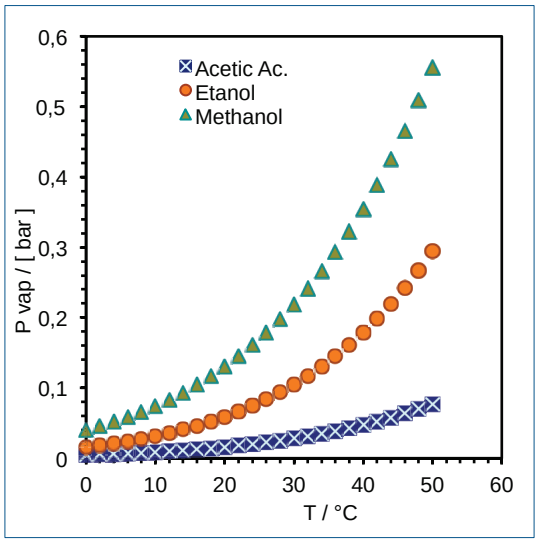


Figure 3. Vapor pressure of ethanol, methanol and acetic acid calculated with the Antoine equation for temperatures between T = 0°C and T = 50°C.

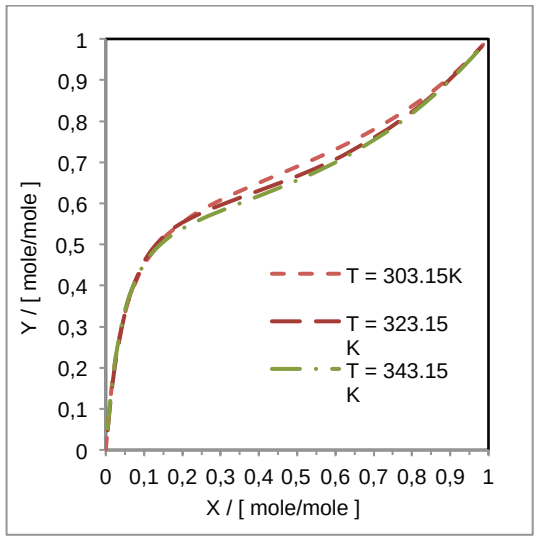


Figure 4. Diagram of the composition of the Liquid phase (X) and Vapor phase (Y) for the Ethanol/Water binary system at phase equilibrium and at the temperatures of 303.15 K, 323.15K and 343.15K.
X and Y are expressed as molar fraction.

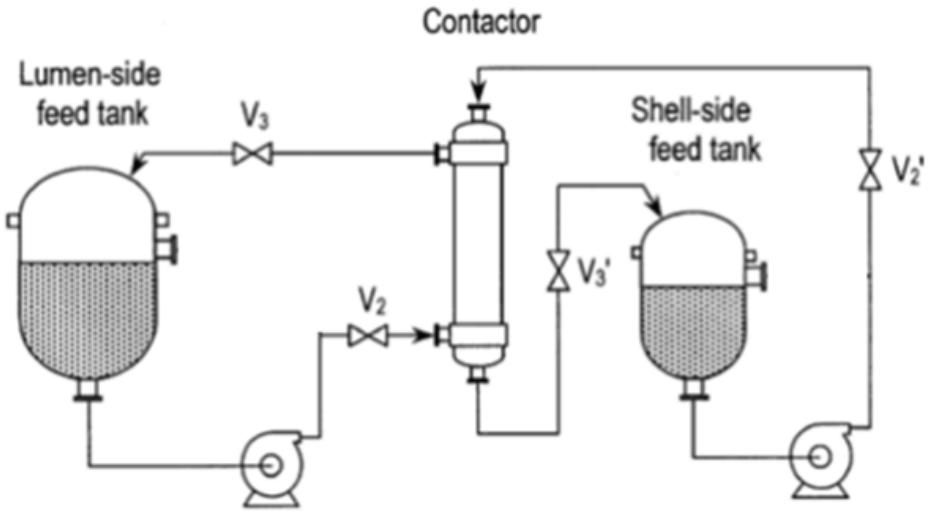


Figure 5. Layout of a single stage dealcoholation plant by direct Osmotic Distillation.

7. Optimization of osmotic distillation

Optimization of the direct Osmotic Distillation process consists of minimizing the consumption of extractant in order to recover a hydroalcoholic solution of the maximum possible concentration as the final extract. Procedure:

Consider we are to treat a mass V_{wine} of wine having ethanol concentration y° (expressed as an absolute volume fraction) with volume V_{EX} of water having initial ethanol concentration of zero x° = 0 (express as absolute volume fraction). At the end of the process we can collect a raffinate of composition y_{final} and an extract of composition x_{final}.

We can now perform a partial mass balance by assuming that all the ethanol released by the raffinates is collected in the extracts, and we obtain:

$$V_{RT}(y^\circ - y) = V_{EX}(x - x^\circ) \qquad (1)$$

where: $V_{RT} = V_{wine} - V_{EtOH}$ is the volume of hydroalcoholic solution to be deacoholized, without alcohol, V_{Ex} is the initial volume of the extractant solution, without alcohol, y°, x° are the initial absolute volume fractions of raffinates and extracts; y and x are the final absolute volume fractions of raffinates and extracts.

Figure 6. Direct direct Osmotic Distillation unit AlcolFlex 15

In addition to facilitating the overall calculations, equation (1) allows us to make several practical observations: let us assume that we are to perform the process for a time sufficient to achieve phase equilibrium (identical concentration of ethanol in the raffinate and extract phases). The partial mass balance between the initial state and the equilibrium state, in the absence of losses or accumulations, is:

$$V_{RT}\left(y^{\circ}-y^{*}\right) = V_{EX}\left(x^{*}-x^{\circ}\right)$$

with phases in equilibrium y* = x* and using an extractant solution with zero initial ethanol contents (x° = 0), the equation is reduced to:

$$V_{RT}\left(y^{\circ}-y^{*}\right) = V_{EX}\,y^{*}$$

From this equation we can therefore calculate either the minimum quantity of extractant required to achieve a given level of dealcoholation of a wine mass of volume V_{RT}, or we can also calculate the concentration at equilibrium of a system composed of pre-established volumes of feed and extractant:

$$V_{EX}\ \min = \frac{y^{\circ}-y_{fin}}{y_{fin}}V_{RT}$$

$$y^{*} = \frac{V_{RT}}{\left(V_{EX}+V_{RT}\right)}y^{\circ} = \frac{1}{R+1}y^{\circ}$$

We can obtain the same information by adopting the method of the McCabe-Thiele diagram (modified), which involves constructing a diagram in which the x axis shows the concentration of extracts and the y axis shows the composition of raffinates (wine). Finally, this diagram shows the "equilibrium curve" which, in the case of the dealcoholation process under consideration, is a line with a uniform slope (y* = x*).

With this representation each point of the graph, and hence each pair of coordinates (x,y), that is above the equilibrium curve will show a possible state of our system and it is derived from the mass balance equation (equation 1).

If we consider a system composed of V_{EX} of extractant phase and V_{RT} of raffinate phase, the initial conditions of our plant will be expressed by the coordinates (y° and x°).

From this point we can plot the "operating curve" which has a slope equivalent to the reverse of $R = V_{EX}/V_{RT}$, which will intersect the equilibrium curve at coordinates y* and x* which, as we have seen, depend in turn on y° and on R.

Each point of the foregoing curve represents the compositions of the extract and raffinate phases that can be obtained during the process conducted with proportions of raffinate and extractant phase defined by R.

For example, point A of the coordinates (x1,y1) represents the raffinate and extract compositions obtainable by setting an R ratio of 1 and starting from an initial composition defined by x° and y° after a given extraction time.

One of the benefits of this representation is that it allows simple calculation of the extent to which the concentration of the extracts will increase by altering the value of the R ratio, considering pre-established alcohol content reductions.

By way of example, figure 7 shows three operating curves plotted starting from the same starting conditions (same initial composition) but referred to treatments performed by setting different values of R (i.e. different V_{EX}/V_{RT} ratios) of between 1 and 0.2. From a comparison of the three operating curves we can rapidly display the effects of an increase in the R ratio on the concentration of extracts, which will be progressively lower, but also the effects on the driving force of the process which, as mentioned earlier, is the difference between the current wine concentration and the concentration at equilibrium (y - y*).

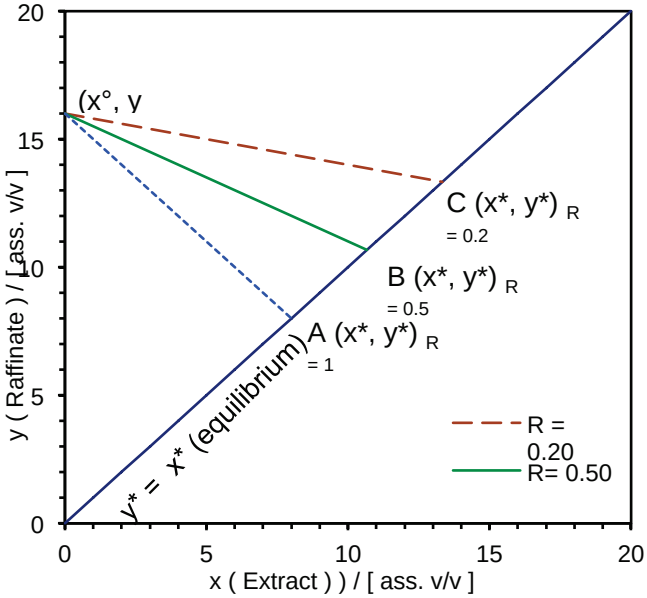


Figure 7. McCabe Thiele diagram for single-stage dealcoholation

8. Experimental tests

All experimental tests were conducted in batch mode, i.e. by recirculating both the extractant phase (extract) and the phase to be extracted (raffinate) until reaching a preset alcohol content using both a bench-top unit and an AlcolFlex 15 perstraction unit manufactured by Diemme Enologia S.p.a. (figure 5).

During each test small samples of wine in extraction and of extractant phase are collected to measure the alcohol content using the Anton Parr Alcolyzer instrument.

9. Interpretation of dealcoholation process kinetic curves

For the first check the values of the alcohol concentrations of the extracts and raffinates collected during each test was entered in the alcohol concentration of extracts (x) vs alcohol concentration of raffinates (y) graph; both concentrations are expressed in absolute form (figure 8).

The points relative to the foregoing concentrations are arranged on a line with slope equivalent to the reverse of $R = V_{Ex}/ V_{Rt}$, thus confirming the matters outlined in the paragraph concerning the graphic representation of the single stage system.

In contrast, by representing the experimental data of Ethanol concentrations of raffinates and extracts in the alcohol concentration vs time graphm we can obtain the characteristic kinetic curves that can be schematically broken down into two separate stages (figure 9):

- Process "activation" stage (lag time)
- Run-down stage (arrival at equilibrium)

The first stage or 'lag time' is the start-up stage; in large size plants the duration of this stage depends on the mixing of the extracts and raffinates in the corresponding recirculation tanks.

For laboratory units, which perform osmotic distillation with reduced volumes and hence with perfect mixing of raffinates and extracts, this stage is a phenomenon comparable to phenomena of permeance of gases and vapors in polymers (e.g. in packaging films) and it indicates that the process calls requires the development of "interaction" between solute and membrane. For example, we can consider that mass transfer starts only once the membrane is saturated with vapor.

Once the "latency stage" is terminated the process proceeds with a typical kinematic trend that, in the concentration vs time g raph (figure 8) is represented by a curve with horizontal asymptote both for the raffinates and for the extracts. The horizontal asymptote towards which the two curves tend to move is the equilibrium concentration, which is given by

$$y^* = \frac{1}{R+1} y^\circ$$

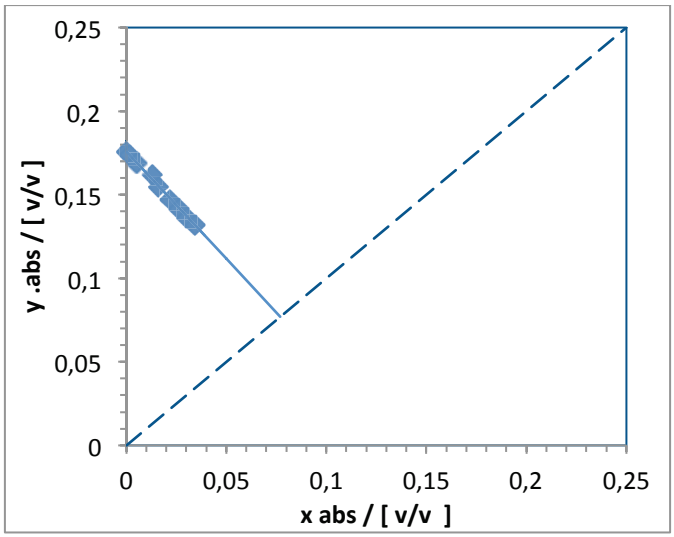


Figure 8. Representation of the alcohol concentration of the samples of extracts and raffinates collected during an experimental dealcoholation test in the McCabe Thiele diagram.

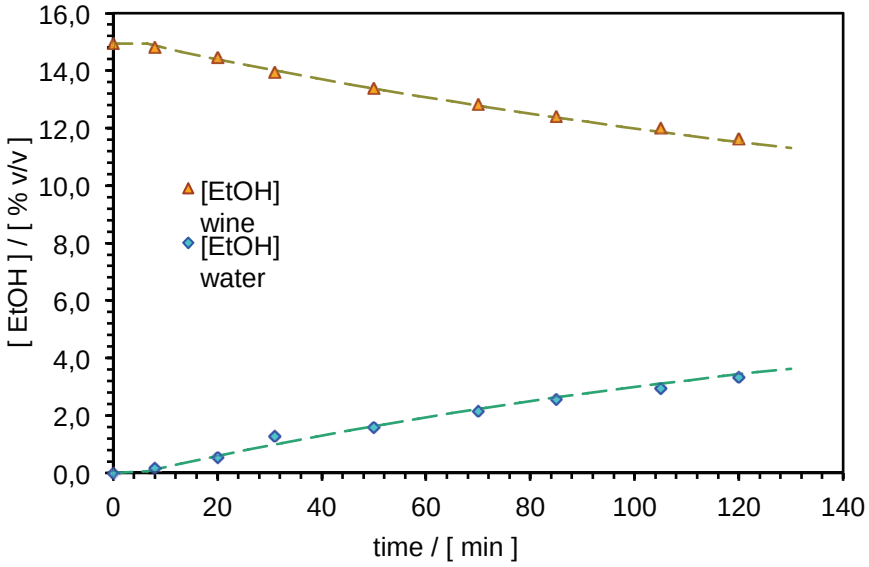


Figure 9. Dealcoholation of Sangiovese wine.
Experimental conditions: T = 18 °C, V°wine 550 dm^3 ; extractant Water V°EX = 600 dm^3

Where: y*is the equilibrium concentration of the system expressed as an absolute fraction, R is the Volume Ratio, which is given by R = V_{EX}/V_{RT} both expressed as without alcohol , y°is the initial concentration in ethanol of the raffinate, expressed as an absolute fraction.

Processing the data associated with the ethanol concentrations of samples collected during experimental tests it is calculated that, in the case of a single stage process, the rate of alcohol reduction can be expressed by the following equation:

$$N = \frac{dy}{d\theta}\,\rho_{EtOH}\,V_{RT} = KA\left(y - y^*\right) \quad (2)$$

where: N is the net flow of ethanol expressed as a variation of the mass of ethanol in time, ρ_{EtOH} is the ethanol density, V_{RT} is the volume of raffinate without alcohol, y is the concentration in ethanol in the wine expressed as an absolute fraction by volume, y* is the concentration in Ethanol in the wine at equilibrium with the extractant phase expressed as an absolute fraction by volume. K is the overall mass transfer

coefficient expressed as the exchanged ethanol mass per unit of time and of exchange surface area, A is the membrane exchange surface area and θis the extraction time

By integration between the time θ = 0 where y = y° and the time θ where y = $y_{(\theta)}$ and introducing the lag time as a translation on the y axis, we obtain:

$$y_{(\theta)} = y^* + (y^\circ - y^*) \cdot \exp\left[-\frac{KA}{\rho_{EtOH} V_{RT}}\left(\theta + \theta_{lagtime}\right)\right] \quad (3)$$

This equation allows us to correlate the ethanol concentration of the raffinate and extract phases of the samples collected during the process with a good level of approximation (figure 8), but above all it allows us to calculate a highly significant kinetic value constituted by the overall mass transfer coefficient K, which will show the contributions to the extraction process of operating parameters such as temperature, flow rate of raffinates and extracts, the nature of the solutes and the characteristics of the liquid phases and of the membrane (e.g. porosity).

The correlation between K and temperature is a point of particular interest: by computing the experimental data concerning different dealcoholation temperature conditions it can be calculated that:

$$K = K^\circ \exp(-A/T).$$

or

$$\ln(K) = \ln(K^\circ) - A/T$$

Representing ln(K) vs (1/T) in a semi-log graph we can obtain a straight line whose angular coefficient (A) can be interpreted, using Arrhenius' equation, as the ratio between the "process activation energy" and the gases constant, suitably expressed.

Figure 10 shows the values of ln(K) obtained by processing experimental data of treatments conducted at different temperatures using a bench-top unit. The alignment of the points is extremely good and the calculated angular coefficient is -4742.1, a value that is very close to the given value of the ratio between the vaporization enthalpy at the standard boiling point of 351.45 K and the ideal gas constant, i.e. $\Delta H_{vap}/R$ = -4638.0.

In the case of larger Direct Osmotic Distillation systems the angular coefficient value deviates from the value derived from the enthalpy of vaporization. Figure 11 shows, in the semi-log graph (ln K versus 1/T), the values of the overall mass transfer coefficient calculated for the dealcoholation tests performed with an AlcoFlex 15 dealcoholation unit.

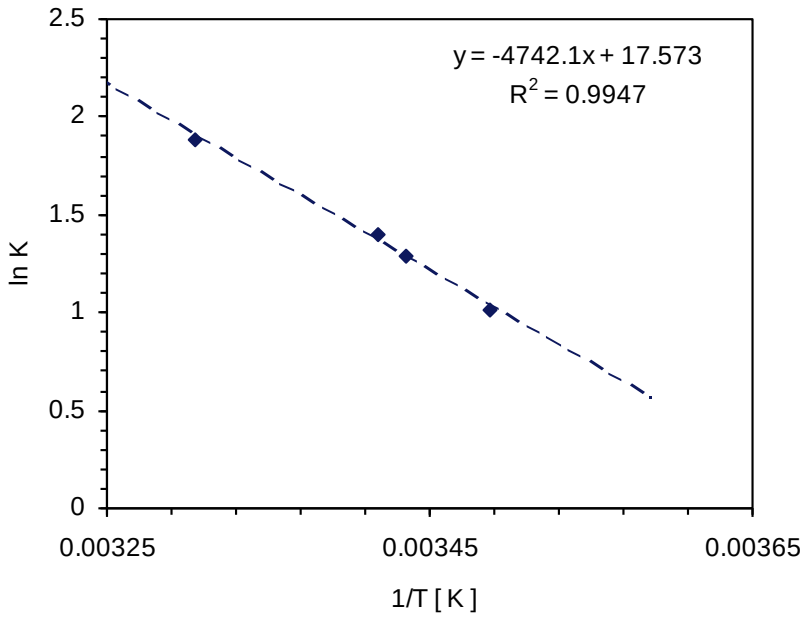


Figure 10. Correlation between overall mass transfer coefficients and temperature (T= 15-30°C) for a lab- scale unit

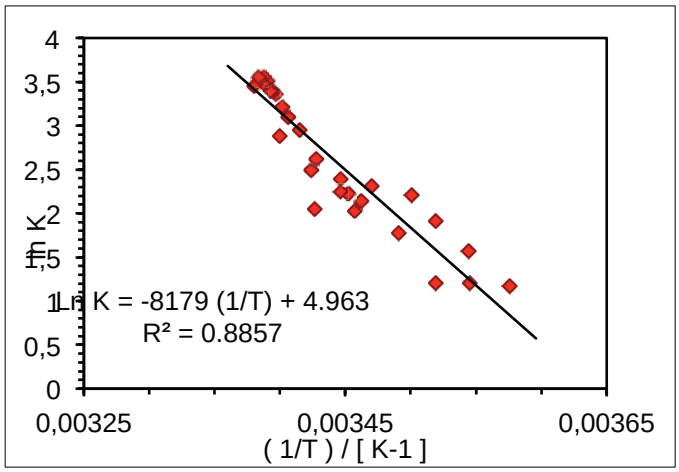


Figure 11. Correlation between overall mass transfer coefficients and temperature (T= 15-30°C) for AlcoFlex 15 Osmotic Distillation system

10. References

S. Carrà, V. Ragaini, L. Zanderighi- "Operazioni di trasferimento di massa – Principi chimico fisici ed applicazioni" - Ed C. Manfredi Editore Milano, 1969.

R. Treybal - Mass-transfer Operations - Third Edition – Mc-Graw Hill International Editions, 1981.

Review of processing technology to reduce alcohol levels in wines

Bithika Saha, Peter Torley, John W. Blackmann and Leigh M. Schmidtke*

National Wine and Grape Industry Centre, School of Agricultural and Wine Science, Charles Sturt University, Locked Bag 588, Wagga Wagga, New South Wales, 2678, Australia

Abstract: Lower ethanol content wines are becoming an important style in the range of beverages offered for sale by many wineries as consumers become more attuned to societal attitudes that govern alcohol consumption. The removal of ethanol using various engineering solutions is an important approach for the production of beverages that are more acceptable for certain consumers. Common approaches for ethanol removal include the spinning cone column, reverse osmosis, osmotic distillation, nanofiltration and evaporative perstraction. Each approach has specific advantages and disadvantages and the best approach for ethanol removal from wine is largely determined by the required ethanol reduction, production volumes, capital and operating costs. This review highlights the important features of the commonly used processing techniques for removal of ethanol from wine.

Keywords: alcohol reduction, osmotic distillation, reverse osmosis, spinning cone

1. Introduction

The production of wines with reduced ethanol concentration is an important aspect of wine production that has gained considerable attention over the past 10 years or so. Consumer demand for wines that are perceived as healthier, more favourable excise rates for lower alcohol products and changing attitudes of consumers regarding the social consequences of excessive ethanol consumption are just some of the attitude drivers for lowering ethanol levels in wine. Significant consumer demand is apparent for wines with lower ethanol levels.

Producers may also wish to marginally reduce alcohol concentrations (e.g. 1 or 2% reduction) in wine to correct balance and maintain consistency of style from one vintage to another. Depending upon the required adjustment to ethanol concentration a portion of the base wine may be extensively dealcoholised and used for blending purposes thereby avoiding the requirement to treat the entire wine blend.

A range of methods have been reported in the literature that have application for the removal or moderation of ethanol in wine (Schmidtke *et al.*, 2012). Certain techniques are somewhat theoretical and have limited practical application since the wine matrix is substantially modified, e.g. the use of glucose oxidase to convert fermentable carbohydrate to gluconic acid (Pickering *et al.*, 1999); require considerable energy inputs such as freeze thawing of juice or wine (Vella, 1984); or involve highly specialized plant and equipment with little opportunity for other winery applications such as supercritical liquid extraction (Seidlitz *et al.*, 1991).

Viticultural approaches such as rootstock selection, manipulation of vine leaf to crop ratio (Stoll *et al.*, 2009; Etchebarne *et al.*, 2010), optimizing varietal selection for specific climatic conditions, early grape harvest (Kontoudakis *et al.*, 2011) and altering vineyard management practices which impact and moderate the imbalance between grape sugar accumulation and the development of grape derived flavour precursors are attracting interest and offer solutions that are suitable for moderating ethanol levels to some degree. Moreover, these are very acceptable approaches from a consumer perspective.

After harvest, grape juice can be nanofiltered to concentrate fermentable carbohydrate, with the by-product being a juice with lower potential alcohol that may also been employed for the production of reduced alcoholic wines (García-Martín *et al.*, 2010).

Corresponding author : lschmidtke@csu.edu.au

The selection of novel or modified yeast strains, or manipulating fermentation conditions, so that carbohydrate is biochemically diverted through alternative pathways rather than glycolysis and subsequent ethanol production are also approaches that have potential to moderate the final ethanol concentration in wine (Kutyna *et al.*, 2010). Post fermentation, the most common winery production techniques utilized for ethanol removal rely upon membrane processes and/or thermal distillation. It is feasible to remove ethanol during fermentation using non-membrane techniques without substantial loss of yeast metabolic activity (Wright & Pyle, 1996; Aguera *et al.*, 2010), however the added complexity of operations during the peak labour demands often means that technological approaches to ethanol removal are applied post fermentation.

This review will focus on the commonly used processing technological approaches for removal of fermentable carbohydrate or ethanol from wine, namely membrane based and thermal distillation techniques.

2. Membrane Based Technologies

Membrane based technologies have become a popular tool for wine processing due in part to the flexible configuration of equipment, size, portability and, compared to other technologies, the relatively modest capital requirements for establishing this capacity within wineries. All membrane processes result in the partitioning of the wine into permeate (i.e. passes through the membrane), and retentate (i.e. retained in the feed) streams. Several membrane based techniques have been developed for ethanol removal from beverages. These rely upon different driving forces to transport ethanol across a semi-permeable barrier. The driving forces are: transmembrane pressure (reverse osmosis), partial pressure differential (pervaporation) or a vapour pressure gradient (osmotic distillation). Each technique relies upon the molecular permeation of ethanol from the feed stock with high concentration to a stripping phase with low concentration.

A significant advantage of membrane based technologies is the relatively low cost of operations, and with most operations taking place at low to moderate temperatures, thermal damage to wine aroma by chemical reactions or aroma degradation is limited. Besides this, membranes have been developed that are reasonably selective for ethanol, limiting the loss of volatile aroma compounds from the beverage (Catarino *et al.*, 2007). Disadvantages of membrane technologies are the capital investment required (e.g. membranes, housings etc) and aroma losses may still occur during the membrane processes that affect wine sensory properties. In the case of reverse osmosis there is the added disadvantage of needing to incorporate a second process to separate aroma compounds and water from ethanol in the reverse osmosis permeate (Meier, 1992; Pyle, 1994; Massot *et al.*, 2008). The removal of ethanol from wine using membrane process can be a relatively slow process that requires the base wine to ‘pass’ the membrane numerous times in order to achieve the desired adjustment of ethanol concentration. A comparison of the basic approaches for ethanol removal using reverse osmosis, osmotic distillation and pervaporation is shown in figure 1, and a summary of these methods appears in table 1.

2.1. Reverse Osmosis process

The basic operating principle of reverse osmosis (RO) units is the application of high pressure to the wine so that specific substances will migrate across a semi-permeable membrane from high to low concentration. Dealcoholisation of wine by RO is performed at high pressures to obtain sufficient permeation rates. The application of such high pressures inevitably leads to increases of temperature at the membrane surface. Prevention of excessive temperature exposure of the wine during operations necessitates the use of ancillary heat exchangers. RO is a widely used process for ethanol removal from wine and was the first membrane based technology commercially used for ethanol removal from beverages (Meier, 1992). There are two main modes of RO operations: dead end and cross-flow. In dead end mode, the feed stock flows directly towards the filter, the permeate passes through the RO membrane, and the retentate remains on the feed side of the membrane throughout the dealcoholisation process. In cross-flow mode, the feed material flows tangentially to the membrane surface, and a portion of the feed material selectively passes through the membrane (permeate). The remainder of the feed material remains on the feed side of the membrane (retentate). The retentate is swept away by the inflow of fresh feed material,

and is collected continuously at the downstream side of the reverse osmosis unit.

The membranes typically used for RO ethanol removal are asymmetric, flat sheet membranes composed of cellulose acetate or cellulose triacetate.

Fine hollow fibres of aromatic poly amides or cellulose triacetate and thin film composites, where an extremely fine layer of a highly hydrophilic polymer has been placed on a microporous support, usually made from polysulphone, are also utilised (Noble & Stern, 1995).

Table 1. Membrane separation processes of relevance for moderating ethanol concentration

Separation Process	Approximate size range	Separation mechanism	Driving force	Application	Selected References
Nanofiltration	0.5-5 nm	Sieving and charge effects	Pressure	Controlling juice sugar concentration	[A, B]
Reverse osmosis	0.1-1 nm	Transfer through a semi-permeable membrane due to pressure	Trans Membrane Pressure	Ethanol removal	[A, C]
Osmotic distillation (evaporative perstraction/membrane contator)	0.03-0.5 µm	Transport of volatile component	Vapour pressure gradient	Controlling juice sugar concentrations Ethanol removal	[F, G]
Pervaporation	Nonporous permselective membrane	Partial vaporization	Difference in partial pressure	Dealcoholization of wine Aroma recovery	[D, E]

A: (Cuperus & Nijhuis, 1993); B: (Echavarría *et al.*, 2011); C: (Catarino *et al.*, 2007); D: (Diban *et al.*, 2008) E: (Hogan *et al.*, 1998); F: (Karlsson & Trägårdh, 1996); G: (Catarino & Mendes, 2011)

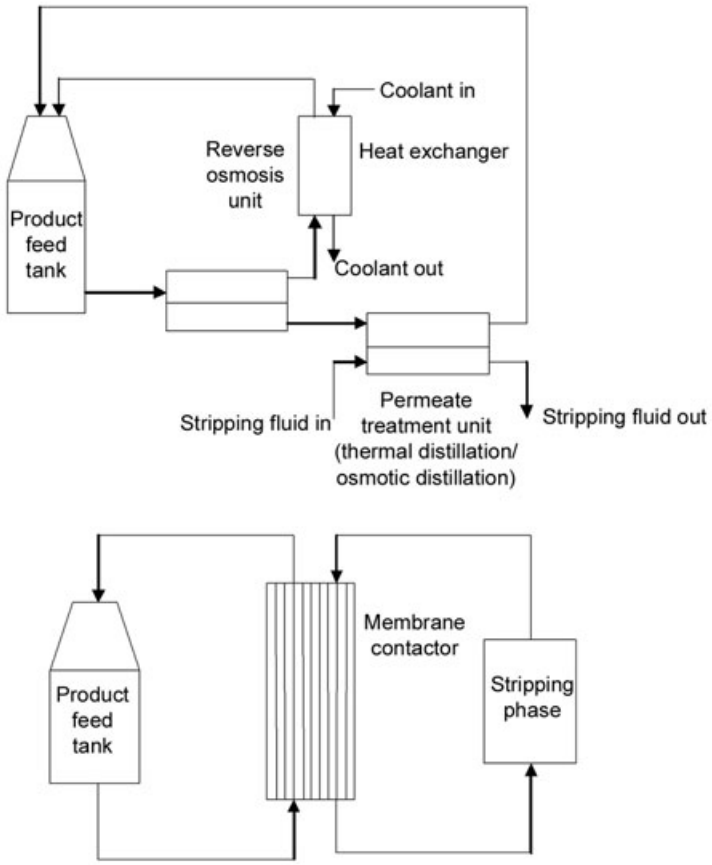


Figure 1. Schematic figure comparing the basic approaches of reverse osmosis (top) and osmotic distillation (bottom) separation approaches for removal of ethanol in wine.

Composite membranes with a high strength polymer supporting layer are most commonly used. These arrangements provide the necessary permeation rates, selectivity and have the capacity to be cleaned and back-flushed to remove any contaminating material that has collected upon the membrane surface. Cellulose acetate or cellulose triacetate thin film membranes are commonly used for ethanol removal. These membranes have a high water and ethanol permeability and good rejection of compounds with high molecular weight such as proteins, polyphenols and carbohydrates. For the ethanol reduction, the best membrane should have the highest permeate flux and lowest ethanol rejection.

Operating parameters such as feed pressure, temperature and flow rates, influence the efficacy of ethanol removal and it is therefore necessary to determine the optimum condition for ethanol permeation and retention of other wine components (Catarino *et al.,* 2007). The effects of these process parameters are summarized as:

- Increasing feed pressure results in higher solvent permeation and thus higher ethanol flux, but also increases the permeation of aroma compounds.
- Ethanol and aroma compound permeation also increases with temperature.

Several membrane configurations have been developed including flat sheet (plate and frame), tubular, hollow fibre and spiral wound. The spiral wound configuration is amongst the most efficient in terms of space. It is essentially a large flat membrane that has been rolled around a hollow retentate collection tube with separate alternating membrane layers. These are separated by a feed spacer, allowing the feed to enter the membrane housing with the flow directed longitudinally. The space between the membranes is directed to the retentate collection tube.

Concentration polarization is an inevitable consequence of membrane separation processes in which a reversible and direct decline of permeate flux across the membrane occurs. This phenomena arises as a build up in the concentration of retained molecules on the pressure side of membrane creating additional resistance to solvent permeation (Cuperus & Nijhuis, 1993). The detrimental effects of concentration polarization and fouling is greater in dead end filtration in comparison to cross flow devices in which accumulated retentate is removed from the membrane surface with the flow of feed stock. Regular back flushing during operations can alleviate to some degree the effects of membrane fouling and restore membrane performance.

The RO permeate stream is usually around 0.7-1.5 % v/v ethanol and therefore contains substantial wine derived water. The consequence of water removal is significant as concentration of wine components arises during processing. Wine must be restored to the original water concentration and this is usually accomplished by the continuous treatment of the permeate to separate the water and ethanol components, with the water redirected back to the treated wine during operations. Permeate treatment using downstream processes of thermal distillation, membrane contactor or pervaporative extraction are required to obtain a high ethanol by-product and water fractions (Massot *et al.,* 2008). The relative low ethanol concentration in the permeate therefore dictates that all of the wine must be 'passed' across the membrane several times to achieve significant alcohol reduction which increases the overall processing time and exposure of the wine to the process.

Reverse osmosis does has advantages over the other dealcoholization process. It has low energy consumption, minimizes thermal degradation of aroma compounds and so preserves the sensory characteristics of wine along with ethanol removal (Catarino *et al.,* 2007; Labanda *et al.,* 2009). On the other hand, some researchers argue that the production of low ethanol beverages by reverse osmosis units is not commercially feasible because the production cost and energy consumption increases with incremental increases in osmotic pressure (Pilipovik & Riverol, 2005).

2.2. Osmotic distillation

Osmotic distillation (OD), also referred to as isothermal membrane distillation or evaporative perstraction, is a promising membrane based technique for low to moderate rates of ethanol removal from beverages. It can be used to reduce the ethanol content in alcoholic beverages with minimal effect on the organoleptic properties of the product. The driving force of the

process is the partial or vapour pressure differences between volatile solute feed and stripping solutions (Varavuth *et al.*, 2009). In OD the feed stock is circulated through a hydrophobic hollow fibre membrane contactor with the stripping liquid flowing on the opposing side of the membrane. In the removal of ethanol from wine, the stripping fluid is degassed water with hydrophobic membranes (polypropylene or polyvinylidenefluoride) employed to create the pressure differential (Varavuth *et al.*, 2009; Diban *et al.*, 2013). A significant advantage of OD is that most processing can be conducted at ambient temperatures, without the requirement for high pressure. This helps allow the process to be relatively cost effective.

Two streams flow through a hydrophobic hollow fibre membrane contactor, with volatile compounds moving from the liquid with high vapour pressure into that of lower vapour pressure. Microporous hydrophobic membranes create a vapour gap between the two liquid phases. Volatile compounds from the feed (high concentration) solution are free to migrate, by convection or diffusion, to the stripping solution. This configuration can avoid the aqueous solution penetrating into the pores (Diban *et al.*, 2008). Therefore the application of a closed loop OD without downstream processing of the permeate can clearly result in the removal of ethanol from wine until an equilibrium is achieved and the desired ethanol reduction is achieved (Hogan *et al.*, 1998). Replacement of the stripping water and batching the OD process can result in the complete removal of ethanol from wine although significant aroma losses also occur when wine is processed in this manner (Liguori *et al.*, 2013).

Common applications of osmotic distillation are the concentration of fruit juices (Varavuth *et al.*, 2009), and ethanol removal from wine or beer (Hogan *et al.*, 1998; Liguori *et al.*, 2013). OD is suitable for ethanol removal from wine since:

- ethanol is the most concentrated volatile component and the most rapidly diffusing species across the membrane
- the vapour pressure of the aroma compounds is low and so the osmotic distillation flux is also low

The rate of ethanol removal from the feed stream can be manipulated by varying processing conditions (e.g. feed velocity, stripping solution velocity and temperature), however water flux from the strip side of the membrane to that of the feed may also arise (Varavuth *et al.*, 2009). However, some aroma losses do occur with increasing operating time and temperature. For low rates of ethanol (<2 % v/v) removal from wine, OD is reported to have a relatively modest impact upon the concentration of aliphatic acids, monoterpenes and some alcohols. However ethyl esters were substantially reduced (Fedrizzi *et al.*, 2013) and these observations are generally in agreement with previous results (Diban *et al.*, 2008; Varavuth *et al.*, 2009; Diban *et al.*, 2013).

2.3. Pervaporation

Pervaporation is a separation technique in which a liquid feed is separated by partial vaporization through a nonporous selectively permeable membrane. The feed mixture flows on one side of membrane and part of the feed vaporizes passing through the membrane to the opposite side where a carrier gas, or vacuum, removes the permeate whereupon it is condensed to liquid. An important aspect of pervaporation is a phase transfer of the diffusing compounds from the feed into the permeate. Mass transfer in pervaporation occurs in three consecutive steps (Karlsson & Trägårdh, 1996):

- Selective absorption into the membrane at the feed side;
- Selective diffusion through the membrane;
- Desorption into the carrier gas on the permeate side.

The membrane composition used for pervaporation will determine the overall selectivity of mass transfer and hence the application of the process. Typically membranes are dense non-porous and may be hydrophilic or hydrophobic (organophilic). Cellulose acetate or polyvinyl alcohol materials have hydrophilic properties and thus water permeation is favoured when these membrane are used. Conversely polydimethylsioxane, polyoctylmethylsiloxe or polytrimethylsilypropane membranes favour the permeation of organic substances (Catarino *et al.*, 2009). Hydrophobic pervaporation membranes can be used for dealcohlisation as etha-

nol permeates these membranes more readily than water (Brüschke, 1990). Unfortunately, aroma compounds are also organic and significant aroma losses may arise when pervaporation is applied to wine. The permeability of hydrophilic membranes is highest for water, intermediate for ethanol and low for aroma compounds. However, if the sweeping gas contains water vapour, the flux of water through the membrane is reduced, but ethanol is still passed across the membrane. Consequently a high proportion of ethanol can be separated from a water-ethanol mixture using a hydrophilic membrane. Pervaporation performance is affected by several factors including permeate pressure, feed concentrations, interactions between components in the feed, mass transfer resistance and most importantly temperature (Karlsson *et al.*, 1995). Pervaporation can be operated at low or ambient temperatures although most alcohol removal procedures have been conducted at temperatures at or exceeding 30°C (Tan *et al.*, 2005; Takács *et al.*, 2007).

The most obvious use for pervaporation is the recovery of aroma compounds from beverages and the techniques has been applied to both wine and beer before alcohol reduction using another process (Catarino & Mendes, 2011). The rate of pervaporation of the major classes of aroma compounds varies depending on operating conditions and so optimization of these parameters is necessary to achieve reduced ethanol wine with desirable aroma.

3. Spinning cone

The spinning cone column (SCC) has wide applications in the food and beverage industries for flavour recovery, to preserve the freshness or taste of processed food or drinks, and preparation of concentrates. The SCC can be operated with either liquid or slurry mixes and can therefore be readily used for ethanol removal during fermentation without a requirement for clarification or stabilization of the wine (Wright & Pyle, 1996). The column contains a rotating vertical central shaft fitted with upward facing cones alternating with sets of fixed downward conical baffles attached to the casing of the column (Pyle, 1994). The liquid feed enters at the top of the column and flows down, while the stripping gas (steam or inert gas) is fed into the base of the column and flows up (Pyle, 1994). To avoid regulatory issues in wine production the stripping vapour can be generated by redirecting a portion of the product discharge through a heater prior to reinjection into the base of the column. As the liquid flows downwards a thin film is created by the centrifugal force of the rotating cone and the liquid migrates to the top of the rotating vane whereupon it drops onto the underneath fixed cone and migrates back towards the centre of the column. This liquid flow path is repeated for the number of rotating and fixed cones thereby creating a large surface area to facilitate mass transfer of volatile components from liquid to the gaseous phase. The SCC operates under vacuum so volatile aroma components are transferred to the gas phase at relatively high vacuum and low temperatures (Harders & Sykes, 1999). Vapour phase turbulence is promoted by fins on the undersurface of the rotating cones and this enhances mass transfer whilst preventing pressure gradient formation within the column. Coupled with reasonable clearances between alternating cones, the fins also ensure that a constant pressure and therefore constant temperature is maintained through the entire length of the column. The cone arrangements and a schematic diagram illustrating the SCC setup for ethanol removal from wine is shown in figure 2.

Ethanol removal from wine using the SCC is typically a two-stage process. In the first stage, the more delicate aroma components are removed at moderate to high vacuum (~0.04 atmosphere) and low temperature (~26-28 °C) (Belisario-Sánchez *et al.*, 2009) with these components typically collected in a high strength ethanol stream that represents approximately one percent of the original wine volume. The second stage in which ethanol is removed from the base wine is conducted at a higher temperature, usually around 38 °C and results in an alcohol concentrate that is typically above 50 % v/v along with the dealcohlised base wine. The concentrated alcohol stream can therefore be used for alternative products without significant additional downstream processing and water is not removed from the original base wine.

It is possible to reduce the ethanol concentration from 15 % v/v to less than 1 % v/v using the SCC. Adjustment of the ethanol concentration in a blend to achieve sensory balance is usually conducted by extensively treating a portion of the final volume. The final dealcoholised wine is produced by blending the recovered aroma with the wine produced by the se-

cond stage (Pyle, 1994). Total residency time of the wine is between 10-20 seconds, for each pass of the feed stock, depending upon the size of the SCC and operating conditions with flavour and ethanol removal occurring in a single pass for each fraction.

A number of ancillary devices are required for the SCC; namely heat exchangers to warm the product feed to operating temperatures, pumps and condensers to collect the gaseous vapour and collect the removed fraction. In terms of cost the SCC has a relatively high requirement for capital outlay and operating expense, however the advantage of high throughput, flexible operating conditions, hygiene and application to the production of juice concentrate make the SCC a suitable technology for large wineries.

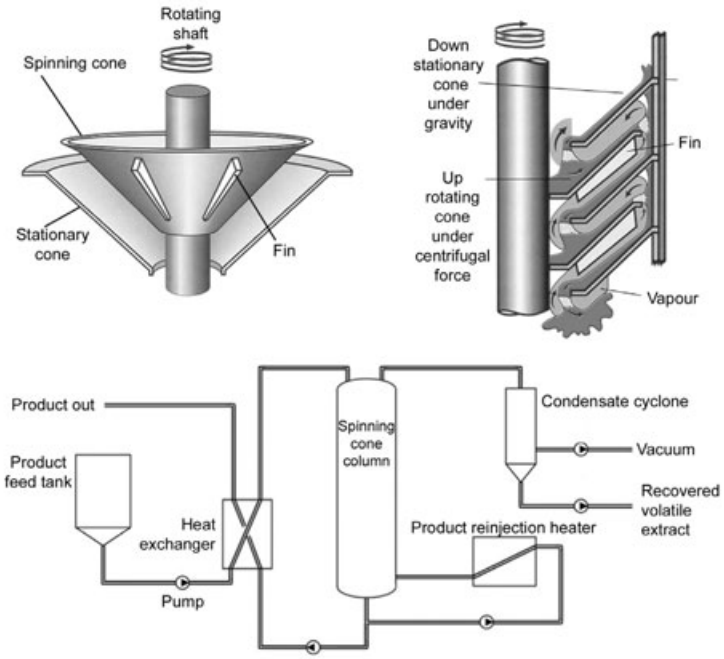


Figure 2. Rotating and fixed stationary cones produce a large surface area for liquid to vapour phase transfer.
Product reinjection to create the stripping vapour is used for wine processing. Courtesy Flavourtech, Lenehan Rd, Griffith, NSW.

4. Conclusion

The method chosen by wine producers to moderate ethanol levels are often determined by consideration of the important factors of wine style, production volume, the level of ethanol to be removed from the product, a desire to retain natural or organic association of the product, capital outlay, operating expenses, flexibility for use of equipment and staff training requirements. For many wine producers it will be important to implement strategies that target the entire wine production process, commencing with vineyard site and varietal selection, management practice, careful control of fermentation parameters along with judicial use of appropriate processing technologies for producing wines with lower ethanol concentrations. Several processing technologies are available for ethanol removal from wine; however, no single approach is likely to produce a significant alcohol reduction without substantial alteration to the sensory properties of the product. Such changes to the sensory properties of the product arise from aroma loss and alteration to mouth feel properties such as body, heat, sweetness and perception of bitterness and acidity. Importantly, the perceived change of aroma may not always be associated with the actual removal of aroma active compounds from the wine. The presence of absence of ethanol in wine substantially alters the polarity of the wine base and this impacts the volatility of aroma compounds (Aznar *et al.*, 2004; Villamor & Ross, 2013). The extent of sensory change in reduced alcohol wines is very much a factor of the presence or absence of specific classes of compound responsible for the varietal aroma and the extent of ethanol removal. The behaviour of different aroma compounds and aroma classes

with regard to retention or permeation through membranes, modification or degradation with thermal exposure and alterations to volatility remain largely unknown. It is likely that the best processing approach for ethanol removal from specific wines will therefore be determined by the varietal composition and with careful optimization of operating conditions such as temperatures, flow rates, stripping, condensation and aroma recovery rates. Clearly significant research into the behaviour of flavour and sensorially important compounds is required to optimize wine processing technologies for ethanol removal.

5. References

Aguera, E., Bes, M., Roy, A., Camarasa, C. & Sablayrolles, J.-M. (2010). Partial removal of ethanol during fermentation to obtain reduced-alcohol wines. *Am. J. Enol. Vitic., 61*(1), 53-60.

Aznar, M., Tsachaki, M., Linforth, R. S. T., Ferreira, V. & Taylor, A. J. (2004). Headspace analysis of volatile organic compounds from ethanolic systems by direct apci-ms. *Int. J. Mass spectrom., 239*(1), 17-25.

Belisario-Sánchez, Y. Y., Taboada-Rodríguez, A., Marín-Iniesta, F. & López-Gómez, A. (2009). Dealcoholized wines by spinning cone column distillation: Phenolic compounds and antioxidant activity measured by the 1,1-diphenyl-2-picrylhydrazyl method. *J. Agric. Food. Chem., 57*(15), 6770-6778.

Brüschke, H. E. A. (1990). Removal of ethanol from aqueous streams by pervaporation. *Desalination, 77*(0), 323-330.

Catarino, M., Ferreira, A. & Mendes, A. (2009). Study and optimization of aroma recovery from beer by pervaporation. *J. Membrane Sci., 341*(1–2), 51-59.

Catarino, M. & Mendes, A. (2011). Non-alcoholic beer—a new industrial process. *Separation and Purification Technology, 79*(3), 342-351.

Catarino, M., Mendes, A., Madeira, L. M. & Ferreira, A. (2007). Alcohol removal from beer by reverse osmosis. *Sep. Sci. Technol., 42*(13), 3011 - 3027.

Cuperus, F. P. & Nijhuis, H. H. (1993). Applications of membrane technology to food processing. *Trends Food Sci. Tech., 4*(9), 277-282.

Diban, N., Arruti, A., Barceló, A., Puxeu, M., Urtiaga, A. & Ortiz, I. (2013). Membrane dealcoholization of different wine varieties reducing aroma losses. Modeling and experimental validation. *Innovative Food Science & Emerging Technologies*(IN PRESS).

Diban, N., Athes, V., Bes, M. & Souchon, I. (2008). Ethanol and aroma compounds transfer study for partial dealcoholization of wine using membrane contactor. *J. Membrane Sci., 311*(1-2), 136-146.

Echavarría, A. P., Torras, C., Pagán, J. & Ibarz, A. (2011). Fruit juice processing and membrane technology application. *Food Eng Rev, 3*(3-4), 136-158.

Etchebarne, F., Ojeda, H. & Hunter, J. J. (2010). Leaf:Fruit ratio and vine water status effects on grenache noir (vitis vinifera l.) berry composition: Water, sugar, organic acids and cations. [Article]. *S. Afr. J. Enol. Viticul., 31*(2), 106-115.

Fedrizzi, B., Nicolis, E., Camin, F., Bocca, E., Carbognin, C., Scholz, M., Barbieri, P., Finato, F. & Ferrarini, R. (2013). Stable isotope ratios and aroma profile changes induced due to innovative wine dealcoholisation approaches. *Food and Bioprocess Technology, In Press*, 1-9.

García-Martín, N., Perez-Magariño, S., Ortega-Heras, M., González-Huerta, C., Mihnea, M., González-Sanjosé, M. L., Palacio, L., Prádanos, P. & Hernández, A. (2010). Sugar reduction in musts with nanofiltration membranes to obtain low alcohol-content wines. *Sep. Purif. Technol., 76*(2), 158-170.

Hogan, P. A., Canning, R. P., Peterson, P. A., Johnson, R. A. & Michaels, A. S. (1998). A new option: Osmotic distillation. *Chem. Eng. Prog., 94*(7), 49-61.

Karlsson, H. O. E., Loureiro, S. & Trägårdh, G. (1995). Aroma compound recovery with pervaporation — temperature effects during pervaporation of a muscat wine. *J. Food Eng., 26*(2), 177-191.

Karlsson, H. O. E. & Trägårdh, G. (1996). Applications of pervaporation in food processing. *Trends Food Sci. Tech., 7*(3), 78-83.

Kontoudakis, N., Esteruelas, M., Fort, F., Canals, J. M. & Zamora, F. (2011). Use of unripe grapes harvested during cluster thinning as a method for reducing alcohol content and ph of wine. *Aust. J. Grape Wine Res., 17*(2), 230-238.

Kutyna, D. R., Varela, C., Henschke, P. A., Chambers, P. J. & Stanley, G. A. (2010). Microbiological approaches to lowering ethanol concentration in wine. *Trends Food Sci. Tech., 21*(6), 293-302.

Labanda, J., Vichi, S., Llorens, J. & López-Tamames, E. (2009). Membrane separation technology for the reduction of alcoholic degree of a white model wine. *LWT Food Sci. Technol., 42*(8), 1390-1395.

Liguori, L., Russo, P., Albanese, D. & Di Matteo, M. (2013). Evolution of quality parameters during red wine dealcoholization by osmotic distillation. *Food Chem., 140*(1–2), 68-75.

Massot, A., Mietton-Peuchot, M., Peuchot, C. & Milisic, V. (2008). Nanofiltration and reverse osmosis in winemaking. *Desalination, 231*(1–3), 283-289.

Meier, P. M. (1992). The reverse osmosis process for wine dealcoholization. *The Australian and New Zealand Grapegrower and Winemaker, 348*, 9-10.

Noble, R. D. & Stern, S. A. (1995). *Membrane seperations technology: Principles and applications.*

Pickering, G. J., Heatherbell, D. A. & Barnes, M. F. (1999). The production of reduced-alcohol wine using glucose oxidase treated juice. Part i. Composition. *Am. J. Enol. Vitic., 50*(3), 291-298.

Pilipovik, M. V. & Riverol, C. (2005). Assessing dealcoholization systems based on reverse osmosis. *J. Food Eng., 69*(4), 437-441.

Pyle, L. (1994). Processed foods with natural flavour: The use of novel recovery technology. *Nutrition & Food Science, 94*(1), 12-14.

Schmidtke, L. M., Blackman, J. W. & Agboola, S. O. (2012). Production technologies for reduced alcoholic wines. *J. Food Sci., 77*(1), R25-R41.

Seidlitz, H., Lack, E. & Lackner, H. (1991). United States of America Patent No. 5034238. U. S. P. Application.

Stoll, M., Scheidweiler, M., Lafontaine, M. & Schultz, H. (2009). *Possibilities to reduce the velocity of berry maturation through various leaf area to fruit ratio modifications in vitis vinifera l. Riesling.* Paper presented at the Proceedings 16th International Giesco Symposium, Davis, California.

Takács, L., Vatai, G. & Korány, K. (2007). Production of alcohol free wine by pervaporation. *J. Food Eng., 78*(1), 118-125.

Tan, S., Li, L., Xiao, Z., Wu, Y. & Zhang, Z. (2005). Pervaporation of alcoholic beverages—the coupling

effects between ethanol and aroma compounds. *J. Membrane Sci.*, *264*(1–2), 129-136.

Varavuth, S., Jiraratananon, R. & Atchariyawut, S. (2009). Experimental study on dealcoholization of wine by osmotic distillation process. *Sep. Purif. Technol.*, *66*(2), 313-321.

Vella, P. (1984). United States of America Patent No. 4,468,407. U. S. Patent.

Villamor, R. R. & Ross, C. F. (2013). Wine matrix compounds affect perception of wine aromas. *Annual Review of Food Science and Technology*, *4*(1), 1-20.

Wright, A. J. & Pyle, D. L. (1996). An investigation into the use of the spinning cone column for in situ ethanol removal from a yeast broth. *Process Biochem.*, *31*(7), 651-658.

Alcohol level reduction in wine

OENOVITI INTERNATIONAL Network

Session IV - Sensory impact and consumers preferences of wines with alcohol levels reduction

Partial dealcoholization of red wines, sensory and composition quality

Maria Tiziana Lisanti*, Angelita Gambuti and Luigi Moio

Department of Agriculture, University of Naples Federico II, viale Italia, 83100, Avellino, Italy

Abstract: 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), by a polypropylene hollow fibre membrane contactor apparatus. Triangle sensory tests showed that both -2 % wines were not perceived as different from the standard wines, while both -5 % wines were different. Sensory profiles and overall quality ranking were obtained by a selected and trained panel. Dealcoholization caused a modification of the sensory profiles, and the greatest differences were found after 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 red wine. Concerning taste, both -5 % dealcoholized wines were more astringent than the correspondent untreated ones. Slighter differences were found for the other degrees of dealcoholization. The analysis of the volatile compounds, both free and glycoconjugated was performed by Solid Phase Extraction (SPE) and GC/MS analysis. While the composition of the glycoconjugated volatile fraction was almost not affected, many free compounds were decreased, most of all esters and alcohols, with an increasing amounts as the level of dealcoholization raised.

Keywords: partial dealcoholization, membrane contactor, sensory characteristics, free and bound volatiles

1. Introduction

The vinification of grapes at full maturation can produce rich, full bodied wines, with intense and complex flavour profiles. However, the juice obtained from such grapes may have very high sugar concentration, resulting in wines with an excessive concentration of ethanol. In addition to this, increases in average annual temperatures due to global warming, in some wine growing regions, exacerbate this problem. In parallel with the increase of the alcohol content of wines on the market, also the demand for reduced alcohol beverages has increased in recent years, as a result of health and social concerns about the risks related to the consumption of alcohol. For this reason, the production of wines with a low alcoholic content is currently one of the most important issues for the wine industry. Nowadays, the treatments aimed at reducing the ethanol level in finished wines are spreading more and more. Among dealcoholization techniques, membrane technologies are reported to allow the ethanol content to be reduced under mild conditions, thus preserving the sensory characteristic of the original product (Labanda *et al*., 2009). Among them, the hollow fiber membrane contactor technique is a novel and promising technology (Diban *et al*., 2008). In this technology, an aqueous phase containing the volatile components is circulated through a hydrophobic hollow fibre membrane contactor while a second aqueous phase, used as stripping liquid, flows along the downstream side of the membrane. The driving force of the process is the partial pressure or vapours pressure differences of the volatile solute in feed and stripping solutions. The important advantages of this process are: thermal damage of the wine components is avoided; little aroma and flavour loss; low energy consumption; temperature of process of 10-20 °C; and the use of water as stripping agent. The dealcoholization could negatively affect the organoleptic quality of the wine, leading in the worst cases to an unacceptability of the product, by altering the complex equilibrium among hundreds of organic compounds responsible for its taste, flavour and mouthfeel. The changes in the organoleptic characteristics of a dealcoholized wine can be due both to the reduction in ethanol content itself, playing a role in taste (Martin and Pangborn, 1970; Fischer and Noble, 1994), mouthfeel (Fontoin

Corresponding author : mt.lisanti@hotmail.it

et al., 2008; Pickering *et al*., 1998; Nurgel and Pickering, 2005) and olfactory properties of wine (Escudero *et al*., 2007; Goldner *et al*., 2009, Whiton and Zoecklein, 2000; Le Berre *et al*., 2007; Robinson *et al*., 2009); and to the losses in sensory active compounds, such as volatiles and polyphenols, during the process (Diban *et al*., 2008, 2013; Gambuti *et al*., 2011; Fedrizzi *et al*., 2013). In a first time, the European Commission fixed a limit of 2 % v/v for wine dealcoholization (Commission Regulation (EC) N 606/2009), recently this limit has been changed in 20 % of the initial ethanol content (Commission Regulation (UE) N 144/2013), thus allowing higher level of dealcoholization. However in some cases, as during very warmed vintages, there could be a necessity of higher reduction, thus the international debate, principally focused on the effect of the dealcoholization process on the sensory properties of wine, is still open. In this study, two red wines (cv. Aglianico) with different initial alcohol contents, 15.37 % v/v (Wine 1) and 13.28 % v/v (Wine 2), were partially dealcoholized at three levels (-2 %, -3 %, -5 % v/v). Sensory triangle tests were conducted in order to evaluate if dealcoholized wines differed from the untreated ones. Moreover, olfactory sensory profiles were obtained by a selected and trained panel. Base chemical characterization and the analysis of free and glycoconjugated volatile compounds, by Solid Phase Extraction (SPE) and GC/MS analysis, were also performed.

2. Materials and methods

2.1 Wine samples

Two red wines (*Vitis vinifera* cv. Aglianico), with a different initial alcoholic strength were studied (Wine 1 and Wine 2) (Taburno winery, Foglianise, Benevento, Italy). Each standard wine was partially dealcoholized of almost 2%, 3% and 5% v/v of ethanol. The exact alcoholic strengths of the wines were: Wine 1 =15.46±0.11, Wine 1 (-2) =13.54±0.05, Wine 1 (-3) =12.40±0.32, Wine 1 (-5) =10.84±0.14, Wine 2 =13.81±0.25, Wine 2 (-2) =11.65±0.18, Wine 2 (-3) =10.52±0.09, Wine 2 (-5)=8.83±0.12. After dealcoholization, sulphur dioxide content was adjusted in each wine sample to 40 mg/L of free SO_2, in order to compensate the losses observed after the process. In order to differentiate the effect of the membrane contactor technology from the effect of ethanol content on the perceived sensory differences, two samples called “reconstituted” (rec) were produced. Wine 1 (rec) and Wine 2 (rec) were made by adding ethanol to the -5 % dealcoholized wines up to the initial alcoholic strength. (final ethanol contents: Wine 1 (rec)= 15.35 ± 0.23; Wine 2 (rec)= 13.57 ± 0.18). Wines were then manually bottled and stored for four months in a cold and dark room at 10 °C.

2.2 Wine dealcoholization

Wines were partially dealcoholized by a hollow fiber membrane contactor apparatus (ALCOLESS PRIMO, Enolifes.r.l. Montemesola, Taranto, Italy). The membrane contactor employed was a Liqui-Cel® 4x28 Extra-flow module commercialized by CELGARD LLC (Charlotte, USA), equipped with microporous polypropylene hollow fiber membrane Celgard® X50. Feed (wine) and stripping (water) streams were fed to the module in cross flow direction. Wine and water flow rates were 35 L/min and 11 L/min, respectively, temperature was kept at 20 °C. Membrane operating pressures were: wine inlet pressure 1 bar; wine outlet pressure 0.6 bar; water inlet pressure 1 bar; water outlet pressure 0.2 bar. 100 Litres of each standard wine were reincirculated through the system and the content of ethanol in the treated wine was measured during the process, until the desired alcoholic degree was achieved.

2.3 Standard chemical analyses

Alcoholic strength by volume, reducing sugar, total acidity, pH, volatile acidity, dry extract, free and total SO_2 were determined according to the “OIV Compendium of international methods of wine and must analysis”. Each analysis was carried out in triplicate. Both wines did not show changes in base chemical parameters, except for a loss of free and total SO_2 in -3 and -5 wines (data not shown).

2.4 Sensory analysis

All the sensory analyses were conducted in individual sensory booths. The samples (30 mL) were presented at room temperature (18 °C) in black tulip-shaped glasses, covered with glass Petri dishes and coded with random three-digit codes. Unsalted crackers and room temperature water were provided to rinse mouth between samples. All wines were uncorked one hour before evaluation and checked for cork taint by the experimenter.

2.4.1 Triangle test

Partially dealcoholized and reconstituted wines were compared to the correspondent standard wine by triangle test, in order to evaluate the existence of perceptible sensorial differences. The panel was composed of 30 judges (16 females and 14 males, 22-65 years of age), recruited from the staff and the students of the Department of Food Science of the University of Naples "Federico II" and trained in performing the triangle test method. Wine 1 and Wine 2 were evaluated in separate sessions. For each session the judges were presented with five sets of samples (standard wine compared with -2 %, -3 %, -5 % and rec).

2.4.2 Sensory profiling

For standard wines and the correspondent partially dealcoholized ones the olfactory profiles were obtained. The sensory panel was composed of 12 judges (5 females and 7 males, 22-61 years of age) recruited from the staff and the students of the Department of Food Science of the University of Naples "Federico II", selected on the basis of their sensory performances and specifically trained in performing sensory profiling of red wine. Odour attributes were determined by consensus. Wines were evaluated in duplicate, both samples and descriptors on the sheets were presented in a randomized order. The intensity of the descriptors was rated using a 9-points scale (0=not detected, 1=weak, 2=medium, 3=strong, 4=very strong), half values being allowed.

2.4.3 Overall sensory quality ranking

The same panel used for the sensory profiling was asked to rank the wines of each set (Wine 1 and Wine 2) for their overall sensory quality, with the lowest rank (1) being given to the wine with the lowest overall sensory quality and the highest rank (4) to the wine with the highest overall sensory quality. For each wine the sum of ranks was calculated.

2.5 Extraction and analysis of free and bound volatiles

The extraction of free and glycosidically bound volatile compounds was carried out by means of C-18 SPE cartridges, according to the method proposed by Di Stefano (1991) and slightly modified (Lisanti *et al.*, 2013). All extractions were carried out in triplicate. Each organic extract (1.2 µL) was injected in splitless mode (injector port temperature 250 °C) into a Shimadzu GCMS-QP2010S gas chromatograph coupled to a quadrupole mass spectrometer (Kyoto, Japan). The GC/MS conditions are reported in Lisanti *et al.*, 2013. Relative concentrations of isolated compounds were expressed as a ratio of peak area against that of the internal standard (2-octanol).

2.6 Data analysis

Quantitative data relative to volatile compounds, standard chemical analyses and sensory profiles were submitted to one-way ANOVA and Tukey test (for both, differences of $p < 0.05$ were considered significant). When the assumptions for ANOVA did not hold, data were analysed by Kruskal-Wallis test and significant differences were established by using Notched Box Plots (differences of $p < 0.05$ were considered significant). Homogeneity of variances was evaluated by Levene's test ($\alpha=0.05$) and normal distribution of data by Shapiro-Wilk test ($\alpha=0.05$). All statistical analyses were performed using the Statgraphics plus (5-PC) statistical packet (Manugistics Group Inc., USA). The statistical significance of the results of the triangle test was evaluated by binomial test tables (O'Mahony, 1986). Results of overall sensory quality ranking test were analysed by the Kramer test tables (O'Mahony, 1986).

3. Results and discussion

3.1 Sensory analysis

3.1.1 Triangle test

Both -2 wines presented very slight differences, not significantly perceived by judges (Table 1). Both -5 wines were perceived as different from the standard wine, with the same significance. Concerning the dealcoholisation by 3% v/v, different results were obtained for the two wines. Wine 1 (-3) was not significantly distinguished from the correspondent standard wine, while the difference between Wine 2 and Wine 2 (-3) was significant. Expressing the dealcoholization level as a percentage of the initial alcohol content (decreasing %), instead of a percentage of the wine, it seems that a value of about 20 % decreasing could be considered the boundary between not perceivable and perceivable sensory modifications. This result supports the recent change of the dealcoholization limit by the EU, from 2% v/v to 20% of the

initial ethanol content (Commission Regulation (UE) N 144/2013). Triangle tests comparing (-5) wines and rec wines were performed in order to understand if sensory differences following dealcoholization were due to the lower ethanol level or to the loss of sensory active compounds. Both the rec wine was significantly perceived as different from the correspondent (-5) wine, thus suggesting that there was a loss of sensory active compounds during the process.

3.1.2 Sensory profiles

Figures 1 and 2 report the sensory profiles of Wine 1 and Wine 2, respectively, before and after the partial dealcoholization. For both wines, the most decreased olfactory attributes were "Cherry" and "Red fruits", at a higher extent with the increase of the level of dealcoholization, although only in the two -5 wines the reduction of their intensity was statistically significant. Also in the previous studies (Meillon *et al*., 2009, 2010) on wines dealcoholized by reverse osmosis, red fruits and blackcurrant odours were found to decrease after the dealcoholisation of red wines (Meillon *et al*., 2009). The subtraction of ethanol was expected to enhance the fruity odours, as ethanol was found to suppress fruitiness of red wines (Escudero *et al*., 2007; Goldner *et al.*, 2009) by reducing the headspace/wine partition coefficient of the responsible compounds. The suppression of fruity odours suggested that the process caused a loss of volatile compounds, according to the results of the triangle test of reconstituted wines. The highest number of significantly different odour descriptors was found for Wine 2 dealcoholized wines, in accordance with the triangle test. The significant increase of the intensity of the descriptor "Grass" in Wine 2 (-5) is in accordance with the previous studies, both considering only the ethanol content (the highest the ethanol level, the highest the intensity of herby attribute, Goldner *et al*., 2009) and considering a dealcoholization process by vacuum distillation and reverse osmosis, causing an increase of vegetative attributes, probably due to the removal of masking components, such as esters (Fischer, 1995). In Wine 2 the descriptor "Black pepper", characterizing Aglianico wine (Gambuti *et al.*, 2007), was significantly less intense in (-5) wine. In both experiments the judges perceived the off-flavour "Cooked" with an intensity significantly higher than standard wine in Wine 1 (-5) and Wine 2 (-3) and (-5). Considering that the dealcoholization apparatus was operated at 20°C, the off-flavour could be due to the lost of the masking effect of other olfactory notes, like the fruity ones. Concerning taste and mouthfeel, dealcoholization determined an increase of acidity, significant for Wine 1 (-3) and (-5) and for Wine 2 (-3), in agreement with previous studies (Fischer and Noble, 1994; Martin and Pangborn, 1970). The increase of bitterness found in Wine 2 (-3) and (-5) appears in contrast with literature in which an increase of ethanol content is reported to enhance the perception of bitterness in model solutions (Martin and Pangborn, 1970), white wines (Fischer and Noble, 1994) and wine model solutions (Fontoin *et al.*, 2008). However, as bitter taste of red wine is due to the interaction of phenolic compounds with specific human receptors (Drewnoski and Gomez-Carneros, 2000), it is possible that in red wines ethanol affects this specific binding. In both experiments dealcoholization caused a progressive increase of astringency, significant only for (-5) wines.

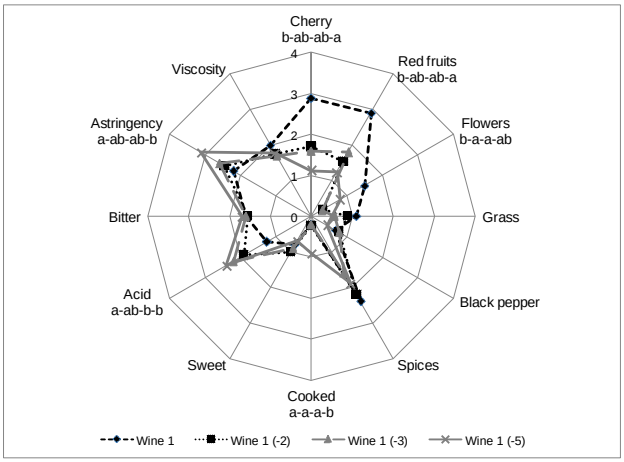


Figure 1. Sensory profiles of Wine 1 before and after the dealcoholisation treatments (letters under the descriptors indicate a statistically significant difference at $p<0.05$, in the order: untreated, (−2), (−3), (−5).

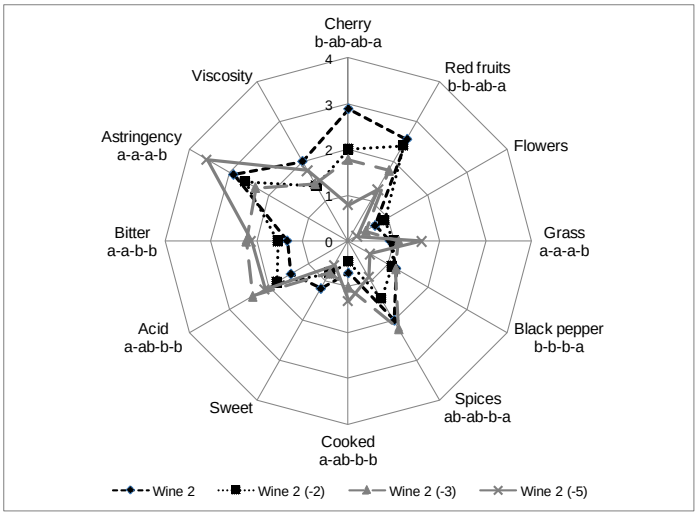


Figure 2. Sensory profiles of Wine 2 before and after the dealcoholisation treatments (letters under the descriptors indicate a statistically significant difference at $p<0.05$, in the order: untreated, (−2), (−3), (−5)

.

Table 1. Results of the sensory triangle test.

Triangle test	Answers (correct/total)	Significance [a]
Wine1 *vs* Wine1 (-2)	13/30	p=0.166
Wine1 *vs* Wine1 (-3)	12/30	p=0.276
Wine1 *vs* Wine1 (-5)	18/30	**p=0.002**
Wine1 *vs* Wine1 (rec)	16/30	**p=0.019**
Wine2 *vs* Wine2 (-2)	11/30	p=0.415
Wine2 *vs* Wine2 (-3)	15/30	**p=0.043**
Wine2 *vs* Wine2 (-5)	18/30	**p=0.002**
Wine2 *vs* Wine2 (rec)	17/30	**p=0.007**

vs=compared with

[a] Bold numbers = samples statistically different ($p<0.05$)

This result is in accordance with the previous works in which the suppressing effect of ethanol on the perceived intensity of astringency was shown (Gawel, 1998; Vidal *et al.*, 2004; Fontoin *et al.*, 2008), while a minimal effect was reported by Noble (1998). After a dealcoholization by reverse osmosis of red wines (namely Merlot dealcoholized of 1.5 and 3 % v/v and Syrah dealcoholized of 2, 4 and 5.5 % v/v), Meillon and coworkers (2009, 2010), by means of TDS, found an increase in the perception of astringency at the expense of heat sensation, as the alcohol content decreased. Recently, a direct role of ethanol in reducing the interactions between salivary proteins and tannins has been demonstrated, both in model solution and in red wine (Rinaldi *et al.*, 2011; Gambuti *et al.*, 2011) and can explain this finding.

3.1.3 Overall sensory quality ranking

Judges ranked the overall sensory quality of the wines of the experiment 1 as follows: Wine 1 (-2)> Wine 1 + Wine 1 (-3)> Wine 1 (-5). For the experiment 2 the ranking was the following: Wine 2> Wine 2 (-2) + Wine 2 (-3)> Wine 2 (-5). In both experiments (-5) wines had the lowest score and (-3) wines were located in the "medium" category, according to the extent of the modifications of sensory profiles. The non-dealcoholized Wine 2 was located in the "significantly higher" category, while for the experiment 1, surprisingly, Wine 1 (-2) was located in the "significantly higher" category and the standard wine in the "medium" category along with Wine 1 (-3). It seems that the dealcoholization of 2 alcoholic degrees of Wine 1 (the most alcoholic) resulted in a higher appreciation by judges, in spite of the slight decrease of several olfactory notes.

3.2 Analysis of volatile fraction

3.2.1 Free volatile fraction

A total of 37 compounds were identified and quantified, including esters, alcohols, terpenes, acids, phenols, and miscellaneous compounds (Table 2).

Esters

Among the determined volatiles, esters are responsible for the fruity aroma in wines, their origin being mainly fermentative. All esters were decreased in both wines, with % losses from 11 to 100 %, except ethyl vanillate in both

wines at all dealcoholization levels, and ethyl 2-methylbutanoate in Wine 2 (-3), which were not decreased. In a previous study, a loss of isoamyl acetate, ethyl hexanoate and ethyl octanoate was shown after a partial dealcoholization of 2 % v/v by PP hollow fibre membrane contactor, both in red wine and in wine model solution (Diban *et al*., 2008). Both in Wine 1 and in Wine 2, most of esters showed increasing % losses, as the level of dealcoholization grew. In Wine 1 (-2) the lowest decrease was found for β-phenylethyl acetate (11 %) and the highest for hexyl acetate (55 %). The same result was found both for Wine 1 (-3) (β-phenylethyl acetate 22 % and hexyl acetate 64 %) and for Wine 1 (-5) (β-phenylethyl acetate 36 % and hexyl acetate 100 %). Also after the dealcoholization of Wine 2, hexyl acetate was the most decreased among esters, while the lowest % losses were found for ethyl 2-methylbutanoate. According to the previous studies, berry fruit notes of red wines are related to the additive effect of several esters (Escudero *et al.*, 2007; Pineau *et al.*, 2009) rather than to a specific compound; therefore, the loss of esters after the dealcoholization process was quite definitely responsible for the decrease of "Cherry" and "Red fruits" olfactory notes, as found by sensory analysis

Table 2. Free volatile compounds determined in the wine samples and significant percentage decreases (p<0.05) after the dealcoholization.

Ret.Time	Compound	Wine1	Wine1 (-2)	Wine1 (-3)	Wine1 (-5)	Wine2	Wine2 (-2)	Wine2 (-3)	Wine2 (-5)
	ESTERS		**dec %**	**dec %**	**dec %**		**dec %**	**dec %**	**dec %**
16.392	Ethyl 2-methylbutanoate	0.042 ± 0.004	-26	-47	-64	0.013 ± 0.002	-30		-34
17.300	Ethyl 3-methylbutanoate	0.046 ± 0.004	-31	-47	-60	0.026 ± 0.002	-39	-53	-64
20.583	Isoamyl acetate	1.007 ± 0.012	-34	-46	-61	1.526 ± 0.058	-58	-57	-71
28.008	Ethyl hexanoate	0.457 ± 0.007	-43	-55	-52	0.449 ± 0.010	-49	-45	-63
30.600	Hexyl acetate	0.009 ± 0.001	-55	-64	-100	0.009 ± 0.001	-90	-83	-89
41.475	Ethyl octanoate	0.609 ± 0.027	-48	-55	-43	0.504 ± 0.016	-34	-36	-52
54.108	Ethyl decanoate	0.223 ± 0.016	-28	-37	-57	0.130 ± 0.005	-54	-52	-70
64.100	β-Phenylethyl acetate	0.263 ± 0.006	-11	-22	-36	0.359 ± 0.016	-38	-39	-53
102.733	Ethyl vanillate	0.099 ± 0.013				0.181 ± 0.011			
	ALCOHOLS								
18.642	2-Methyl-1-propanol	0.227 ± 0.015	-29	-38	-49	0.252 ± 0.012		-57	-71
22.050	1-Butanol	0.025 ± 0.005		-32	-44	0.015 ± 0.003			-48
26.858	3-Methyl-1-butanol	99.075 ± 4.771		-15	-24	103.008 ± 5.456	-14	-26	-41
33.608	4-Methyl-1-pentanol	0.085 ± 0.003		-18	-27	0.119 ± 0.015			-34
34.467	3-Methyl-1-pentanol	0.290 ± 0.005	-8	-17	-26	0.266 ± 0.008	-19	-24	-38
36.250	1-Hexanol	3.008 ± 0.016	-10	-18	-28	3.408 ± 0.142	-20	-25	-39
36.883	(Z) 3-Hexen-1-ol	0.085 ± 0.007		-19	-24	0.089 ± 0.006	-18	-23	-37
38.250	(E) 3-Hexen-1-ol	0.064 ± 0.005		-17	-23	0.070 ± 0.006	-15	-17	-32
39.700	(Z) 2-Hexen-1-ol	0.019 ± 0.001			-71	0.022 ± 0.003		-54	
40.308	(E) 2-Hexen-1-ol	0.018 ± 0.003				nd	nd	nd	nd
42.550	1-Octen-3-ol	0.020 ± 0.001		-27	-36	0.040 ± 0.002	-25	-30	-44
42.950	1-Heptanol	0.047 ± 0.006			-31	0.075 ± 0.000	-26	-36	-48
49.392	1-Octanol	0.032 ± 0.001	15		-38	0.070 ± 0.006	-18		-54
67.400	Benzyl alcohol	0.204 ± 0.011		-18		0.193 ± 0.006		-64	-32
69.517	β-Phenylethyl alcohol	58.154 ± 4.077				58.243 ± 2.467			
	TERPENES								
48.633	Linalool	0.020 ± 0.003				0.030 ± 0.001	-20	-19	-34
57.650	α-Terpineol	0.040 ± 0.005				0.033 ± 0.001			-28
61.508	β-Citronellol	0.015 ± 0.002			-29	0.019 ± 0.001	-30	-33	-47
89.875	Geranic acid	0.035 ± 0.004				0.048 ± 0.004			-28
	PHENOLS								
76.800	4-Ethylguaiacol	nd	nd	nd	nd	0.172 ± 0.008	74	138	155
82.092	4-Ethylphenol	0.074 ± 0.000	92	68	82	0.628 ± 0.022	150	294	348
	ACIDS								
65.867	Hexanoic acid	1.425 ± 0.181				1.342 ± 0.035		24	24
76.792	Octanoic Acid	2.659 ± 0.122				2.182 ± 0.048	-15		-14
82.400	Nonanoic acid	0.035 ± 0.007		nd		nd	nd	nd	nd
86.842	Decanoic acid	1.245 ± 0.198		-49	-53	nd	nd	nd	nd
	OTHER COMPOUNDS								
46.850	Benzaldehyde	nf	nf	nf	nf	0.085 ± 0.006	-55	-56	-66
47.425	Vitispirane	0.005 ± 0.001				nd	nd	nd	nd
53.283	γ-Butyrolactone	0.031 ± 0.002				0.044 ± 0.004			

The concentrations of isolated compounds (peak area/IS area) are expressed as the arithmetic average ± SD of three replicates; *nd* not determined since the relative peak contained more than one component as confirmed by mass spectrometry; *nf* not found; dec% = percentage decreases respect to the initial concentration, only significant values (p<0.05) are reported.

Alcohols

Alcohols contribute to wine olfactory properties by giving its base vinous aroma and some herbaceous and floral notes. Quite the same compounds were decreased in the two wines, with few exceptions. The greatest differences were found after -2 dealcoholization, with a higher number of compounds decreased in Wine 2 (-2) in respect to Wine 1 (-2). The losses of isoamyl alcohol found by Diban *et al.* (2008) (13.7-50.9 %) and by Varavuth *et al.* (2009) (23-44 %) were comparable to those found in the present study. The only alcohol not decreased in both wines at all levels of dealcoholization was β-phenylethyl alcohol, responsible for a pleasant olfactory note of rose. For this compound a retention effect by wine matrix, probably due to π-π stacking interactions, was previously found (Rodrìguez-Bencomo *et al.*, 2011). This result is consistent with other studies (Diban *et al.*, 2008; Liguori *et al.*, 2013). Also the losses found after a dealcoholization up to 4% (v/v) of a white model wine by reverse osmosis and of white wines by membrane contactor were little (Labanda *et al.*, 2009; Fedrizzi *et al.*, 2013).

Terpenes

Terpenes, deriving from grape in free or glycoconjugated form, contribute to wine aroma with their floral notes. The concentration of these compounds did not change significantly after the dealcoholization of Wine 1 at all the levels, except for a reduction of β-citronellol (29 %) in Wine 1 (-5). In Wine 2 linalool and β-citronellol were decreased after the three levels of dealcoholization, to a greater extent as the degree of the treatment increased. In Wine 2 (-5) also α-terpineol and geranic acid were decreased, both by 28 %.

Phenols

Two volatile phenols were determined, 4-ethylphenol and 4-ethylguaiacol, both responsible for the off-flavour described as horsy, stable, animal. They are produced in wine by contaminant yeast species (*Brettanomyces*/*Dekkera* genus), (Chatonnet *et al.*, 1992). In both experiments, a great increase of 4-ethylphenol in the dealcoholized wines was found. Concerning 4-ethylguaiacol, it was determined only in Wine 2 and showed an increase after the dealcoholization. Dias *et al.* (2003) found that a greater concentration of ethanol reduced the microbial activity of yeast and made the synthesis of ethylphenols difficult. Probably there was a proliferation of *Brettanomyces* in the bottles, favoured by the low ethanol content in the dealcoholized wines. The fact that the increase of volatile phenols was much higher in Wine 2 (the least alcoholic) corroborates this explanation.

Acids

The fatty acids, formed enzymatically during fermentation, can contribute with fruity, cheese, fatty, and rancid notes to the sensory properties of wine, but seem to give a positive contribution to red wine fruitiness (San-Juan *et al.*, 2011). The concentration of acids was very slightly affected by the treatment. In Wine 1 only decanoic acid, the most hydrophobic one, were decreased after -3 and -5 dealcoholization. In Wine 2 a slight decrease of octanoic acid was found after -2 and -5 dealcoholization. The unexpected increase of hexanoic acid in Wine 2 (-3) and Wine 2 (-5) could be due to the proliferation of *Brettanomyces*, indeed hexanoic acid is among the products of the secondary metabolism of some strains of the yeast (Romano *et al.*, 2008).

Other compounds

Benzaldehyde was found only in Wine 2, in which underwent a decrease after dealcoholization. This compound, principally deriving from grape, can contribute to wine aroma with an almond note. The other two compounds, vitispirane and γ-butyrolactone, were not significantly decreased by the treatment.

3.2.2 Bound volatile fraction

Aglianico is a non-aromatic grape, however the glycosidically bound volatile fraction could play an interesting role in preserving aroma complexity during ageing. In the experimental wines, 17 bound compounds were determined, including alcohols, terpenes and phenols. The effect of the treatment of dealcoholization on the bound volatile fraction was negligible for both wines at the three considered level, except for 1-pentanol and 1-heptanol. Indeed, the former was decreased in Wine 1 by 15 %, 23 %, and 15 % (at -2, -3, and -5 alcoholic degrees of dealcoholization, respectively), and in Wine 2 by 43 % and 38 % (at -3% and -5 % dealcoholization, respectively); the latter was only decreased by 14 % after -5 % dealcoholization of Wine 1.

4. Conclusion

The partial dealcoholization by membrane contactor technique affected the organoleptic properties of red wine, causing a decrease of the intensity of fruity notes and an increase of astringency. Moreover, the process causes a consistent loss of volatile compounds, most of all esters and alcohols. However, a dealcoholization of 2 %, minimally affected sensory properties of wine. The highest considered dealcoholization level (-5 %) caused great modifications of the sensory profiles and very great losses (up to 100 %) of volatile compounds. At the medium level of dealcoholization (-3 %), it was found that the treatment most affected the least alcoholic wine. This result corroborate the recent modification of the UE Regulation, fixing the limit for wine dealcoholisation expressed as a percentage of the initial ethanol content (20 %), rather than the percentage of the wine volume.

5. References

Chatonnet P, Dubourdieu D, Boidron JN & Pons M (1992) The origin of ethylphenols in wines. Journal of the Science of Food and Agriculture, 60, 165-178.

Di Stefano R (1991) Proposal for a method of sample preparation for the determination of free and glycoside terpenes of grapes and wines. Bullettin de l'OIV, 64, 219-223.

Dias L, Pereira-da-Silva S, Tavares M, Malfeito-Ferreira M & Loureiro V (2003) Factors affecting the production of 4-ethylphenol by the yeast Dekkera bruxellensis in enological conditions. Food Microbiology, 20, 377-384.

Diban N, Athes V, Bes M & Souchon I (2008) Ethanol and aroma compounds transfer study for partial dealcoholization of wine using membrane contactor. Journal of membrane Science, 311, 136-146.

Diban N, Arruti A, Barcel´o A, Puxeu M, Urtiaga A, Ortiz I (2013) Membrane dealcoholization of different wine varieties reducing aroma losses. Modelling and experimental validation. Innovative Food Science and Emerging Technologies, DOI 10.1016/j.ifset.2013.05.011

Escudero A, Campo E, Fariña L, Cacho J & Ferreira V (2007) Analytical characterization of the aroma of five premium red wines. Insights into the role of odor families and the concept of fruitiness of wines. Journal of Agricultural and Food Chemistry, 55, 4501-4510.

Fedrizzi B, Nicolis E, Camin F, Bocca E, Carbognin C, Scholz M, Barbieri P, Finato F, Ferrarini R (2013) Stable isotope ratios and aroma profile changes induced due to innovative wine dealcoholisation approaches. Food and Bioprocess Technology DOI 10.1007/s11947-013-1068-x.

Fischer U (1995) Mass balance of aroma compounds during the dealcoholization of wine: correlation of chemical and sensory data. PhD Thesis, Universität Hannover, Hannover, Germany.

Fischer U & Noble AC (1994) The effect of ethanol, catechin, concentration, and pH on sourness and bitterness of wine. American Journal of Enology and Viticulture, 45(1), 6-10.

Fontoin H, Saucier C, Teissedre PL & Glories Y (2008) Effect of pH, ethanol, and acidity on astringency and bitterness of grape seed tannin oligomers in model wine solution. Food Quality and Preference, 19, 286-291.

Gambuti A, Genovese A, Lamorte S, Capuano R, Lisanti MT, Piombino P & Moio L (2007) Study of the influence of the degree of grape ripening on aroma characteristics of Aglianico wines by instrumental and sensory analysis. Acta Horticulturae (ISHS) 754, 533-540.

Gambuti A, Rinaldi A, Lisanti MT, Pessina R & Moio L (2011) Partial dealcoholization of red wines by membrane contactor technique: influence on colour, phenolic compounds and saliva precipitation index. European Food Research and Technology 233(4), 647-655.

Gawel R (1998) Red wine astringency: a review. Australian Journal of Grape and Wine Research 4, 74–95.

Goldner MC, Zamora MC, Di Leo Lira P, Gianninoto H & Bandoni A (2009) Effect of ethanol level in the perception of aroma attributes and the detection of volatile compounds in red wine. Journal of Sensory Studies, 24, 243-257.

Labanda J, Vichi S, Llorens J & Lòpez-Tamames E (2009) Membrane separation technology for the reduction of alcoholic degree of a white model wine. Food Science and Technology, 42, 1390-1395.

Le Berre E, Atanasova B, Langlois D, Etiévant P & Thomas-Danguin T (2007) Impact of ethanol on the perception of wine odorant mixtures. Food Quality and Preference, 18, 901-908.

Liguori L, Russo P, Albanese D, Di Matteo M (2013) Evolution of quality parameters during red wine dealcoholization by osmotic distillation. Food Chemistry, 140, 68-75.

Lisanti MT, Gambuti A, Genovese A, Piombino P & Moio L (2012) Partial Dealcoholization of Red Wines by Membrane Contactor Technique: Effect on Sensory Characteristics and Volatile Composition. Food and Bioprocess Technology, DOI 10.1007/s11947-012-0942-2.

Lisanti MT, Gambuti A, Genovese A, Piombino P & Moio L (2013) Partial Dealcoholization of Red Wines by Membrane Contactor Technique: Effect on Sensory Characteristics and Volatile Composition. Food and Bioprocess Technology, 6, 2289-2305.

Martin S & Pangborn RM (1970) Taste interaction of ethyl alcohol with sweet, salty, sour and bitter compounds. Journal of the Science of Food and Agriculture, 21, 653-655.

Meillon S, Urbano C & Schlich P (2009) Contribution of the Temporal Dominance of Sensation (TDS) method to the sensory description of subtle differences in partially dealcoholized red wines. Food Quality and Preference, 20, 490-499.

Meillon S, Viala D, Medel M, Urbano C, Guillot G & Schlich P (2010) Impact of partial alcohol reduction in Syrah wine on perceived complexity and temporality of sensations and link with preference. Food Quality and Preference, 21, 732-740.

Noble AC (1998) In: Waterhouse & Ebeler (ed), ACS Symposium Series #714, Chemistry of Wine Flavor. Washington, DC.

Nurgel C & Pickering G (2005) Contribution of glycerol, ethanol and sugar to the perception of viscosity and density elicited by model white wines. Journal of Texture Studies 36, 303–323.

O'Mahony M (1986) Sensory evaluation of food. Statistical methods and procedure. Marcel Dekker Inc., New York, USA.

OIV Compendium of International Methods of Wine and Must Analysis (2007) Office International de la Vigne et du Vin, Paris, France.

Pickering GJ, Heatherbell DA, Vanhanen LP, Barnes MF (1998) The effect of ethanol concentration on the temporal perception of viscosity and density in white wine. American Journal of Enology and Viticulture 3:306–318.

Pineau B, Barbe J–C, Van Leeuwen C & Dubourdieu D (2009) Examples of perceptive interactions involved in specific

"Red-" and "Black-berry" aromas in red wines. Journal of Agricultural and Food Chemistry, 57, 3702-3708.

Rinaldi A, Gambuti A & Moio L (2011) Precipitation of salivary proteins after the interaction with wine: the effect of ethanol, pH, fructose and mannoproteins. Journal of Food Science, 77, 485-490.

Robinson AL, Ebeler S, Heymann H, Boss PK, Solomon PS & Trengove R D (2009) Interactions between wine volatile compounds and grape and wine matrix components influence aroma compound headspace partitioning. Journal of Agricultural and Food Chemistry, 57, 10313-10322.

Rodríguez-Bencomo JJ, Muñoz-Gonzàlez C, Andújar-Ortiz I, Martín-Álvarez PJ, Moreno-Arribas MV & Pozo-Bayón MA (2011) Assessment of the effect of the non-volatile wine matrix on the volatility of typical wine aroma compounds by headspace solid phase microextraction/gas chromatography analysis. Journal of the Science of Food and Agriculture, 91, 2484-2494.

Romano A, Perello MC, de Revel G & Lonvaud-Funel A (2008) Growth and volatile compound production by Brettanomyces/Dekkera bruxellensis in red wine. Journal of Applied Microbiology, 104, 1577-1585.

San-Juan F, Ferreira V, Cacho J & Escudero A (2011) Quality and aromatic sensory descriptors (mainly fresh and dry fruit character) of spanish red wines can be predicted from their aroma-active chemical composition. Journal of Agricultural and Food Chemistry, 59, 7916-7924.

Varavuth S, Jiraratananon R & Atchariyawut S (2009) Experimental study on dealcoholization of wine by osmotic distillation process. Separation and Purification Technology, 66, 313-321.

Vidal S, Courcoux P, Francis L, Kwiatkowski M, Gawel R, Williams P, Waters E, Cheynier V (2004) Use of an experimental design approach for evaluation of key wine components on mouth-feel perception. Food Quality and Preference, 15, 209-217.

Whiton RS & Zoecklein BW (2000) Optimization of headspace solid-phase microextraction for analysis of wine aroma compounds. American Journal of Enology and Viticulture, 61(1), 53-60.

Wine of reduced alcohol content: Consumer and society demand vs industry willingness and ability to deliver

Kerry Wilkinson* and Vladimir Jiranck
School of Agriculture, Food and Wine, The University of Adelaide, PMB 1, Glen Osmond SA 5064, Australia

Abstract: Since the 1980's, the alcohol content of Australian wine has steadily increased from 12.5 to 13.0% for white wines and from 12.4 to 14.4% for red wines. This is largely due to winemakers' preferences for riper grapes, which exhibit more intense fruit flavors, but which also contain more sugar and therefore lead to higher alcohol wines. However, given the various health, social and tax implications associated with alcohol production and consumption, it's perhaps not surprising that wine consumers and winemakers alike, are interested in wine of reduced alcohol content. In this paper, the factors influencing consumer demand for low alcohol wine, together with methods for producing wine of reduced alcohol content, are discussed. The aims and objectives of a new Innovative Wine Production research program, underway at the University of Adelaide, Australia, are also outlined, to demonstrate industry's willingness to develop and implement alcohol-reduction strategies.

Keywords: alcohol reduction, consumer preference, industry, low alcohol wine, winemaking

1. Introduction

The alcohol content of table wine typically ranges from nea% for light bodied white or rosé wines, to in excess of 16 % for full bodied red wines. Since the 1980's, the alcohol content of Australian wine has steadily increased from 12.5 to 13.0 % for white wines and from 12.4 to 14.4 % for red wines (Godden and Muhlack, 2010), as shown in Figure 1. These trends are largely attributable to winemakers' preference for riper grapes, which exhibit more intense fruit flavors, but which also contain more sugar and therefore lead to higher alcohol wines.

The issue of high alcohol wines is one of concern for winemakers, given the harmful effects of alcohol on health and behavior (Le Berre *et al.*, 2007) and the potential implications for wine quality. Ethanol makes an important sensory contribution to wine, influencing viscosity and body (Pickering *et al.*, 1998), and therefore wine style, but it can also influence our perceptions of astringency (due to tannin), sourness (due to acid), sweetness, and aroma and flavor (Martin and Pangborn, 1970; Fischer and Noble, 2004; Robinson *et al.*, 2009). Many high alcohol wines also exhibit an unpleasant 'hotness' and can lack fruit flavor and freshness, possibly due to the increased solubility of wine volatiles which affects detection thresholds (Pineau *et al.*, 2007) and headspace partitioning (Robinson *et al.*, 2009). Not surprisingly, winemakers are interested in reducing the concentration of alcohol in wine.

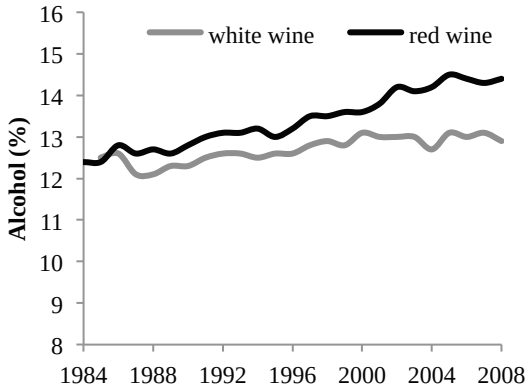


Figure 1. Mean alcohol concentration in white and red Australian wines, 1984–2008. Adapted from Godden and Muhlack (2010).

Wines of reduced alcohol content have been classified as dealcoholized or alcohol free (< 0.5 % v/v), low alcohol (0.5 to 1.2% v/v), reduced alcohol (1.2 to 5.5–6.5 % v/v) and lower alcohol wine (5.5 to 10.5 % v/v), by Pickering (2000) and Saliba *et al.* (2013a); albeit these

Corresponding author : kerry.wilkinson@adelaide.edu.au

ing (2000) and Saliba *et al.* (2013a); albeit these classifications, which are loosely based on labeling and legislative requirements, vary between different countries (Pickering, 2000). Until recently, the Australian New Zealand Foods Standard Code required wine produced in Australia to contain a minimum alcohol content of 8 %, however, Standard 4.5.1: Wine Production Requirements was amended in 2011, and the minimum alcohol requirement is now 4.5 % (Saliba *et al.*, 2013b). As a consequence, some lower alcohol wines containing less than 8 % alcohol that were previously designated as 'wine products', for example Moscato, now meet the requirements to be considered as wine (Rowley, 2013).

In a recent study investigating Australian wine consumers' demand for low alcohol wine, the majority of consumers surveyed, i.e. almost 70%, considered low alcohol wine to contain between 3 and 8 % alcohol (Saliba *et al.*, 2013b); interestingly, of the 851 consumers surveyed, 21 % did not know the alcohol content of the wine they normally consumed. In a similar study involving United Kingdom (UK) wine consumers, the alcohol content of a typical bottle of wine was estimated to be 12.1 %, whereas the alcohol content of low alcohol wine was estimated to be 5.9 % (Bruwer *et al*., 2013). It's perhaps worth noting that the alcohol content reported on wine labels is often understated (Alston *et al.*, 2011); i.e. labeling regulations typically allow alcohol to be reported within a percentage, e.g. 0.5 %, of the actual alcohol content by volume.

Aroma and flavor are undoubtedly critical to the appeal of wine to the consumer, together with color, taste and mouthfeel. The human preference for flavor in fruits and, in turn, the products derived from these is universally self-evident. Such flavor comes from ripening – but ripening is a double-edged sword when making wine, since extended ripening results in the higher accumulation of sugars, which are dutifully converted to alcohol by *Saccharomyces* wine yeast during fermentation. However, winemaker demand for greater flavor is not the only reason for over-ripening of grapes. Global warming, evidenced by the earlier phenology of grapevines, and hot weather events are more common (Webb *et al.*, 2007) and tend to compress vintage, causing transport and scheduling difficulties and fermentation bottlenecks. This inevitably leads to longer ripening. Coupled with the tendency of certain varieties such as Shiraz to dehydrate (Tilbrook and Tyerman, 2008), as well as restrictions on the availability of water for irrigation, the outcome is unintentional over-ripening of grapes and the over-accumulation of sugars; which ultimately results in highly alcoholic wines.

In this paper, the factors influencing consumer and society demand for low alcohol wine are discussed, together with an overview of methods for reducing the concentration of ethanol in wine and thus, the industry's ability to deliver wines of reduced alcohol content. The aims and objectives of a new Innovative Wine Production research program, underway at the University of Adelaide in Australia, are also outlined, to demonstrate industry's willingness to develop and implement alcohol-reduction strategies.

2. Consumer and society demand for wine of reduced alcohol content

Beverage preferences are tending toward lighter styles, i.e. light-bodied white, rosé and Moscato wines. Indeed, Wine Australia recently reported 'substantial growth in the volume of wine exported with 'lower' alcohol content' (Rowley, 2013), which suggests market opportunities analogous to low-alcohol beers.

However, frequent media reports continue to fuel concerns about alcohol abuse and societal effects; while the health benefits previously associated with moderate wine consumption are overshadowed by convincing and probable evidence that a causal relationship between alcohol consumption and some cancers exists (Allen *et al.*, 2009). As a consequence, public health advocates are pushing for increased taxes on wine, as well as greater restrictions on how wine can be marketed (Evans, 2013), so as to reduce alcohol consumption. Whilst it is not clear that wine (vs. other alcohol) is the primary cause of these problems, wine producers strongly advocate responsible consumption of alcohol (Winemakers' Federation of Australia, 2012; Evans, 2013). Thus, the observed changes in beverage preferences are likely to be at least partially attributable to a combination of media scrutiny, consumer education and the introduction of alcohol taxes.

However, these trends may also reflect changes in consumers' attitudes towards low alcohol wines. In 2011, Mueller and colleagues (2011) reported Australian wine consumers'

relative acceptance for low alcohol wine was only 6-8 %. In contrast, a more positive result, i.e. 16 % relative acceptance, was reported in a more recent study (Saliba *et al.*, 2013b); with responsible driving and health cited as the reasons most strongly influencing participants' interest in low alcohol wine.

Bruwer and colleagues (2013) investigated the importance of choice cues on UK wine consumers' wine purchasing decisions and found no significant difference in the importance ratings assigned by buyers and non-buyers of low alcohol wine. Alcohol content was ranked 7th for both groups, with promotional offers (i.e. price discount), grape variety, country of origin and brand familiarity considered to be of more importance. However, the authors still considered alcohol content to be an important consideration, since 43 % of consumers assigned this cue a rating ≥4.0 (on a 5-point Likert scale).

High alcohol wines attract higher import duties and taxes; i.e. costs which must either be absorbed, thereby reducing profitability, or passed on to price-sensitive consumers. A significant proportion of exported Australian wine, i.e. 34 %, is sold in the UK (Wine Australia 2012). At the key price point of £3.99 per bottle, 45 % of the price is represented by typical excise duty of £1.81/bottle. This rises by 33 % to £2.41/bottle (60 % of total price) for wine exceeding 15% alcohol. As such, there are real economic incentives for industry to meet consumer demand for wines with reduced alcohol content, but these wines must also meet consumer expectations of quality.

Wine Intelligence conducted a survey of regular UK wine drinkers' experiences of 5.5 % v/v wines (Halstead, 2013). Some 23 % of UK wine drinkers indicated they had bought and would continue to buy these wines; while another 15 % were open to purchasing 5.5 % v/v wines, but were yet to do so. However almost half the participants indicated they would not buy 5.5 % v/v wines; the majority (80 %) on principle, because 'the product has no relevance or appeal', while the remainder had previously bought 5.5 % v/v wines but would not do so again, presumably due to a poor experience. Indeed, Halstead suggests that the poor quality of many 5.5 % v/v wines may have put some consumers off low alcohol wines for good. In some cases, consumers did not realise they were purchasing low alcohol wines, prompting retailers to flag the 5.5 % alcohol content more prominently.

Attempts have been made to profile consumers who purchase low alcohol wines and several studies suggest a bias towards females, both younger (18-34) and older (45-55), of mid to low income, who drink wine about once a week or who drink wine with food, with a low to medium level of involvement with wine (Saliba *et al.*, 2013b; Halstead, 2013; Bruwer *et al.*, 2013). Consumers' motivations for buying or not buying low alcohol wine have also been studied (Bruwer *et al.*, 2013). Concern for the health, liking of taste, lower price and staying in control were the main motivations for consumers who buy low alcohol wines; whereas the non-availability of low alcohol versions of consumers' favourite brands, the perception of low quality and disliking of taste were the main motivations given by consumers for not buying low alcohol wines. Taste was again considered an important driver of acceptability and preference, in agreement with earlier studies (Saliba *et al.*, 2013b)

The consumer research undertaken to date confirms the opportunity market for low alcohol wine, in both Australia and the UK. It is perhaps worth noting that retailers such as Sainsbury's in the UK have publically set ambitious goals for growing the lowered-alcohol wine segment and reducing the average alcohol content of their wines (Sainsbury, 2011); i.e. actions which further demonstrate confidence in consumer demand for low alcohol wine.

3. Industry's ability to deliver wine of reduced alcohol content

A range of methods are available for achieving wines of reduced alcohol content; most alcohol reduction strategies are based on principles which either reduce the concentration of fermentable sugar present in grapes or juice, or remove alcohol from wine (Pickering, 2000). Several of these strategies are discussed below.

The simplest approach to sugar reduction involves harvesting grapes at an earlier stage of development, i.e. when sugar levels are still low. However, this has implications for grape, and therefore wine, composition and quality, due to the associated reduction in aroma, flavor and color intensity, and increased acid content. Wines made with unripe fruit are typically per-

ceived as thin, 'green', low intensity wines; i.e. wines which exhibit sensory attributes that tend to be less appealing to consumers than those of high alcohol wines.

Glucose oxidase has also been shown to be capable of reducing the glucose content of grape juice. In the presence of oxygen, this dehydrogenase enzyme catalyzes the oxidation of glucose to gluconolactone, which can then be hydrolyzed to gluconic acid (Pickering, 2000). Fermentation of the resulting glucose-depleted juice produces wines with up to 40 % lower ethanol concentrations; albeit wines which require sweetening or deacidification to address acid imbalances due to high levels of gluconic acid (Pickering *et al*., 1999). However, in many countries, glucose oxidase is not a permissible wine additive (Pickering, 2000).

The addition of water to grape must or wine can effectively dilute the concentrations of sugar and alcohol respectively; but the intensity of wine aroma, flavor and color is also diluted, and so quality is diminished. Furthermore, in many countries, Australia and France for example, the addition of water to juice or wine is not legally permitted. Alcohol dilution can instead be achieved via blending (Pickering, 2000); i.e. with either reduced alcohol or partially fermented wine, which are grape-derived and therefore legally permitted. However, there are limitations on the styles of wine that can be produced in this manner: typically sweeter wine styles. When fermentation is arrested early, i.e. before grape sugars are fully converted to ethanol, the resulting wines will have lower alcohol concentrations, but also perceptible levels of sweetness due to high residual sugar levels; being 7.5-20 g/L for semi-sweet wines and 20-150 g/L for sweet wines (Iland and Gago, 1997). Despite their reduced alcohol content, sweet wines are unlikely to appeal to health–conscious consumers, because of their inherent calorie count. Precautions must also be taken to ensure the stability of sweet wine styles; i.e. sterile filtration and/or sulfur dioxide additions.

Various distillation processes have been used to remove alcohol from wine, post-fermentation. Early distillation methods tended to reduce flavor and quality, through the co-evaporation of wine volatiles; but modern distillation processes can remove ethanol at lower temperatures, require much shorter processing times and incorporate aroma recovery techniques (Pickering, 2000). The spinning cone column is a popular example, but the capital investment in infrastructure is significant and while the technology is permitted in the USA it has not yet been approved for use in Europe (Halstead, 2013).

Reverse osmosis is a membrane separation process that has been successfully used to reduce the alcohol content of fermented beverages, including wine, beer and cider (Bui *et al.*, 1986; Lopez *et al*., 2002), via pressure filtration through a membrane permeable to alcohol and water. The loss of flavor typically associated with distillation-based methods for alcohol reduction is largely overcome, because reverse osmosis is performed at low temperature; although some diffusion of aroma compounds, as well as organic acids and potassium has been reported (Pickering, 2000).

Modest reductions in alcohol, i.e. from 16% to below 14%, for example, to avoid import duties without detrimental side-effects on wine sensory properties, are possible if planned in advance, but are more difficult to achieve retrospectively. Industry has the ability to reduce the alcohol content of finished wine by several percent, using distillation or reverse osmosis methods. However, reducing alcohol even further, for example to 9 %, let alone 4.5 % (as in beer), whilst retaining other sensory attributes is simply not possible with any one current technology or approach. Thus, at present, industry cannot produce wines of markedly reduced alcohol content that are indistinguishable from the full alcohol strength equivalent.

Further research is still required to fully understand how alcohol reduction of wine modifies our perceptions of wine aroma, flavor, taste, mouthfeel and quality, from physico-chemical and perceptual perspectives. Research is also needed to determine wine consumers' attitudes towards the different methods used to dealcoholize wine.

4. Industry's willingness to deliver wine of reduced alcohol content: Innovative Wine Production at the University of Adelaide

Making and selling wine in Australia, even in a 'good' year, has become increasingly more challenging. Compounding the environmental stresses in vineyards, are challenges associated with rapidly changing consumer preferences, high labor costs, unfavorable exchange rates, and health and taxation impacts of high alcohol. To overcome these challenges, an unprecedent-

ed combination of researchers and wine industry participants has been assembled to undertake research to address one of the major problems facing industry: that of high sugar accumulation during ripening, resulting in highly alcoholic wines, that are more difficult to achieve flavor and aroma balance in.

In 2013, the Australian Research Council awarded $2.4 million funding to the University of Adelaide to establish an Industrial Transformation Training Centre focused on Innovative Wine Production. This Centre will provide new knowledge, methods and technologies to help the wine industry develop profitable strategies for grape growing and winemaking that achieve the desired balance of taste, mouthfeel, aroma/flavor, color and alcoholic strength.

The specific aims of the project are:

- In the vineyard, to curb sugar accumulation and accelerate the accumulation of aroma and flavor compounds;
- In the winery, to remove sugar prior to fermentation, divert sugar away from alcohol production pathways, improve the reliability and reduce the duration of high sugar fermentations, and enhance the sensory properties of wine;
- Post-fermentation, to selectively remove alcohol and develop additives to adjust levels of sensory compounds in wines from under-ripened grapes or volatiles lost from wines of reduced alcohol content;
- To define current market and consumer perceptions and preferences for wines of reduced alcohol content and, in new markets, use this knowledge to inform the production process.

The Centre brings together researchers from the University of Adelaide, the Australian Wine Research Institute, the Commonwealth Scientific and Industrial Research Organisation and the South Australian Research and Development Institute, and industry partners spanning the entire production chain, i.e. grapegrowers, winemakers, suppliers, downstream/waste processors and retailers. This integrated whole-of-production-chain approach to Innovative Wine Production (Figure 2) is the key to success in achieving incremental alcohol reductions and/or flavor enhancement. In broad terms, the approaches to be used will: (i) reduce sugar or enhance flavor accumulation in the vineyard; (ii) remove sugar or maximize flavor in grape juice; (iii) avoid fermentation of sugar to alcohol; and (iv) remove alcohol post-fermentation.

Research topics fall into three broad areas. Grapegrowing is key to wine composition. Decades of effort honing ways to drive ripeness and sensory intensity need to be revised or reversed to produce the raw material needed for the wine products demanded by the market.

Microbiology remains the vehicle for turning grapes into wine. Understanding microbes, how they interact and how they can be used to improve process efficiency and drive vine behavior, or sensory/alcohol content will be critical for molding grapes into the required wines.

Studies into grape and wine processing and consumer insight will be key where grapes, wines and microbes fall short, and will afford another set of tools for tuning the finished product.

Industry is open to the adoption of innovative wine production practices that deliver economic benefit, without compromising wine quality or consumer acceptability, and the direct involvement of industry partners in the Centre demonstrates industry's willingness to deliver wine of reduced alcohol content.

5. Conclusion

The wine industry is currently facing a number of significant challenges, one of which involves addressing the high alcohol content of wine. For some 30 years, the trend of using riper grapes has contributed to the Australian wine industry's international success, but riper flavor also means grapes rich in sugars, which yeast dutifully convert to alcohol. Thus, red wines with in excess of 16% alcohol are not uncommon. Reducing alcohol content is therefore a priority for many winemakers and so the Innovative Wine Production research outlined in this paper will develop profitable strategies for grape growing and winemaking to achieve reduced alcohol content wines. In this way, the project will deliver both economic and social benefits: industry will be better placed to meet consumers' expectations and demand for wines of reduced alcohol content; excise duty, which is typically applied according to alcohol content, will decrease with reductions in alcohol content; and the adoption of alcohol-reduction strategies will assist the wine industry to achieve its commitment to promote socially responsible alcohol consumption practices.

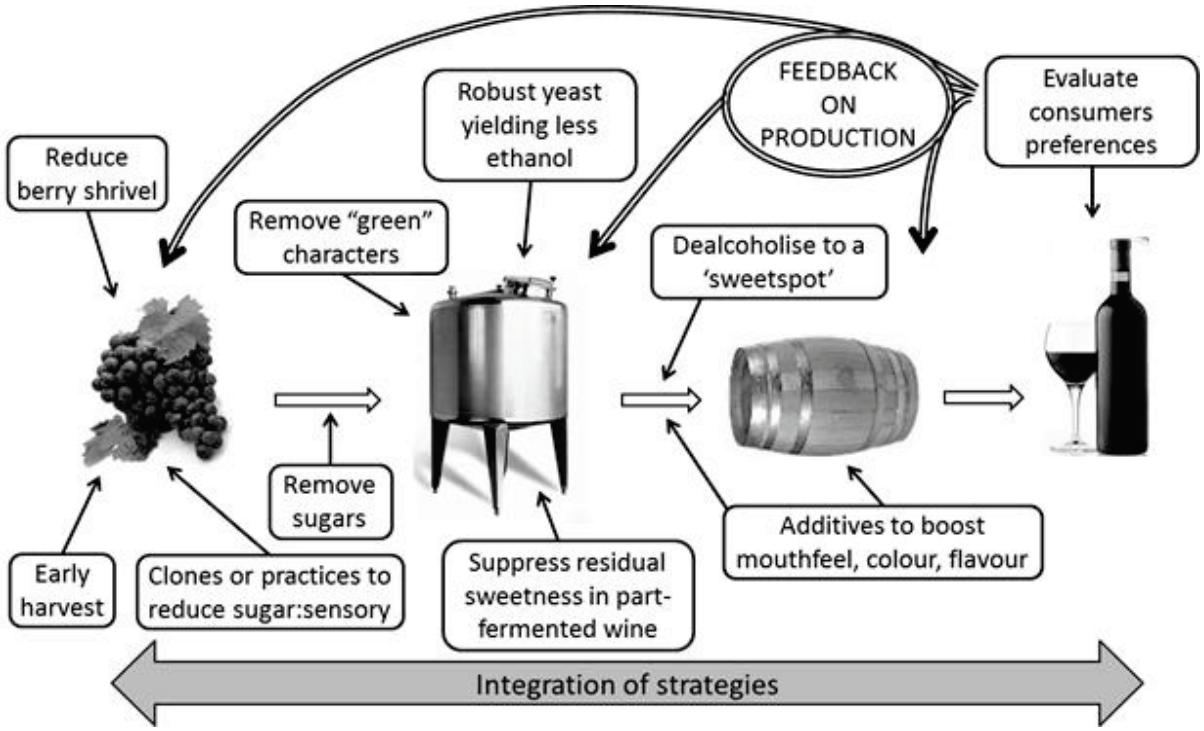


Figure 2. Key targets within the integrated whole-of-production-chain strategy for modulation of wine alcohol content and sensory properties.

6. References

Allen N.E., V. Beral, D. Casabonne, S.W. Kan, G.K. Reeves and A. Brown, 2009. Moderate alcohol intake and cancer incidence in women. *Journal of the National Cancer Institute*, **101**, 296–305.

Alston J.M., K.B. Fuller, J.T. Lapsley, G. Soleas and K.B. Tumber, 2011. Splendide mendax: False label claims about high and rising alcohol content of wine. *AAWE Working Paper No. 82, New York: American Association of Wine Economists.*

Bruwer J., V. Jiranek, L. Halstead and A. Saliba, 2013. Lower alcohol wines in the UK market: some baseline consumer behaviours metrics. *British Food Journal (in press)*.

Bui K., R. Dick, D.G. Moulin and P. Galzy, 1986. A reverse osmosis for the production of low ethanol content wine. *American Journal of Enology and Viticulture*, **37**, 297–300.

Evans P. 2013. Profitability and health our two immediate priorities. *Wine and Viticulture Journal*, **28**, 10.

Fischer U. and A.C. Noble, 2004. The effect of ethanol, catechin concentration, and pH on sourness and bitterness of wine. *American Journal of Enology and Viticulture*, **45**, 6–10.

Godden P. and R. Muhlack, 2010. Trends in the composition of Australian wine, 1984–2008. *Australian and New Zealand Grapegrower and Winemaker*, **558**, 47–61.

Halstead R., 2013. 2013 – the year of the lower alcohol wine? *Wine and Viticulture Journal*, **28**, 62–63.

Iland P. and R. Gawel, 1997. *Australian wine: from the vine to the glass*. Patrick Il and Wine Promotions, Adelaide, South Australia.

Le Berre E., B. Atanasova, D. Langlois, P. Etiévant and T. Thomas-Danguin, 2007. Impact of ethanol on the perception of wine odorant mixtures. *Food Quality and Preference*, **18**, 901–908.

Lopez M., S. Alvarez, F.A. Riera and R. Alvarez, 2002. Production of low alcohol content apple cider by reverse osmosis. *Industrial and Engineering Chemistry Research*, **41**, 6600–6606.

Martin S. and R.M. Pangborn, 1970. Taste interaction of ethyl alcohol with sweet, salty, sour and bitter compounds. *Journal of the Science of Food and Agriculture*, **21**, 653–655.

Mueller S., L. Lockshin and J. Louviere, 2011. Alcohol in moderation: Market potential for low alcohol wine before and after excise tax. *6th AWBR International Conference*. Talence, France: Bordeaux Management School.

Pickering G.J., D.A. Heatherbell, L.P. Vanhanen and M.F. Barnes, 1998. The effect of ethanol concentration on the temporal perception of viscosity and density in white wine. *American Journal of Enology and Viticulture*, **49**, 306–318.

Pickering G.J., D.A. Heatherbell and M.F. Barnes, 1999. The production of reduced alcohol wine using glucose oxidase–treated juice – composition. *American Journal of Enology and Viticulture*, **50**, 299–306.

Pickering G.J., 2000. Low- and reduced-alcohol wine: a review. *Journal of Wine Research*, **11**, 129–144.

Pineau B., J.-C. Barbe, C. van Leeuwen and D. Dubourdieu, 2007. Which impact for β–damascenone on red wines aroma? *Journal of Agricultural and Food Chemistry*, **55**, 4103–4108.

Robinson A.L., S.E. Ebeler, H. Heymann, P.K. Boss, P.S. Solomon and R.D. Trengove, 2009. Interactions between wine volatile compounds and grape and wine matrix components influence aroma compound headspace partitioning. *Journal of Agricultural and Food Chemistry*, **57**, 10313–10322.

Rowley M., 2013. Market analysis for lower alcohol Australian wine. *Wine and Viticulture Journal*, **28**, 63–64.

Saliba A., L. Ovington, C.C. Moran and J. Bruwer, 2013a. Consumer attitudes to low alcohol wine: an Australian sample. *Wine and Viticulture Journal*, **28**, 58–61.

Saliba A., L. Ovington and C.C. Moran, 2013b. Consumer demand for low-alcohol wine in an Australian sample. *International Journal of Wine Research*, **5**, 1–8.

Sainsbury, 2011. www.j-sainsbury.co.uk/responsibility/20x20/alcohol/

Tilbrook J. and S.D. Tyerman, 2008. Cell death in grape berries: varietal differences linked to xylem pressure and berry weight loss. *Functional Plant Biology*, **35**, 173–184.

Webb L.B., P.H. Whetton and E.W.R. Barlow, 2007. Modelled impact of future climate change on the phenology of winegrapes in Australia. *Australian Journal of Grape and Wine Research*, **13**, 165–175.

Wine Australia, 2012. United Kingdom market export snapshot. www.wineaustralia.com/en/winefacts

Winemakers' Federation of Australia, 2012. www.wfa.org.au/health_social_responsibility.aspx

Impact of alcohol reduction on the sensory perception of wine and their acceptability by consumers

Sophie Meillon[1,2,3,4]*, Christine Urbano[1,2,3] and Pascal Schlich[1,2,3]

[1] CNRS UMR 6265 Centre des Sciences du Goût et de l'Alimentation, 21000, Dijon, France
[2] INRA UMR 1324 Centre des Sciences du Goût et de l'Alimentation, 21000, Dijon, France
[3] Université de Bourgogne, UMR Centre des Sciences du Goût et de l'Alimentation, 21000, Dijon, France
[4] Centre de Recherche Pernod Ricard, Créteil, France

Abstract: The significant increase of alcohol content in wines has led to the development of reverse osmosis technology to reduce the alcoholic degree. However little is known about the consequences of this technology on the sensory perception of wines and their appreciation/acceptability by consumers. These studies showed that alcohol reduction by reverse osmosis significantly impacts the sensory properties of wines with a decrease in the perception of hotness, bitterness, aromas and persistency in mouth. An increase in the perception of astringency was also shown in red wines as well as a decrease in the perception of complexity. In blind tasting conditions, the liking of the sensory properties of alcohol-reduced wines was strongly segmented. Wine professionals and consumers with more experience of wine did not like the sensory properties of alcohol-reduced wines, whereas consumers with less experience liked them. In tasting condition with information, the concept of alcohol-reduced wine was rejected even more strongly by consumers with a higher level of expertise. Generally, information about alcohol reduction was less well accepted for red than for white wines. Tastings carried out in real-life settings showed that the sensory dimension is as much important as the cognitive dimension in the acceptability of alcohol-reduced wines by consumers.

Keywords: Reduced-alcohol wine, sensory perception, liking, acceptability, consumers

1. Introduction

Given the significant increase in alcohol content of wines over the last twenty years, wine industry is interested in new technologies such as reverse osmosis to reduce alcohol content of their wines. New partially dealcoholized products are being introduced on the international wine market.

However, little is known about the sensory consequences of alcohol reduction in wine by reverse osmosis. It is also not known about the liking and acceptability of Partially Dealcoholized Wines (PDW).

Do consumers perceive partial alcohol reduction in wine? What are the sensory consequences of alcohol reduction? Do consumers like these sensory modifications? Regardless of the sensory dimension, do consumers accept the concept of alcohol reduction?

The study aims to respond these questions. It is the result of three years of research completed as part of the VDQA project ("Vin de Qualité à teneur réduite en Alcool"). It was carried out with the support of the National Research Agency (ANR) and involved 11 partners of both private and public sectors. The objectives of VDQA project were 1) to optimize the production process of PDW; 2) to investigate the sensory consequences of the alcohol reduction in wine; 3) to emphasize the potential target market for PDW.

2. Materials and method

2.1. Wines

All the wines were dealcoholized by the experimental unit of Pech Rouge (INRA, Narbonne). These wines were initially around 14 % of alcohol (standard). The dealcoholization process by reverse osmosis was completed with pilot equipment in three successive but not continuous steps.

In the first step one hundred and twenty liters of standard wine were treated by reverse osmosis treatment (RO), in a closed circuit, until the required alcohol content. Then the ethanol ex-

*Corresponding author : Sophie.Meillon@dijon.inra.fr

tracted in the permeate was separated from the water either via distillation or via membrane contactors (MC). This step aims to recuperate the water from the standard wine, in order to reincorporate it into the final wine and to obtain a dry extract (mineral content) similar to that of the initial wine. The dealcoholized wine were finally obtained by blending the dealcoholized permeate (<0.2 %) and the osmosed wine (concentrate).

Three different studies were completed over three years. According to wine availability and storage limitations, each study was carried out with a different wine series, made up of standard wines from vintage of the last year. Dealcoholization degree, standard wine type (grape variety) and origin, as well as dealcoholization techniques were readjusted throughout the studies and resulted in the elaboration of three different wine series, which are presented and summarized in table 1.

Table 1. Description of the three series of wine

Series	Grape variety	Origin and vintage	Dealcohol. technique	Dealcohol. level
S1	Chardonnay Sauvignon Merlot Syrah	Languedoc-Roussillon 2005	RO + Distillation	-1.5 %, -3 %
S2	Chardonnay Syrah	Languedoc-Roussillon 2006	RO + MC	-4.5%
S3	Syrah	Australia 2007	RO + MC	-2%, -4 %, -5.5%

2.2. Sensory analysis methods

2.2.1 Sensory perception of alcohol reduction in wine

The aim was to assess whether subjects could perceive a sensory difference between standard wines and the same dealcoholized wines. This question was investigated using sensory discriminative tests that assess the absence or presence of sensory differences between two given products. Triangle tests with forced choice (ISO 4120) were used. Three wines were presented to the participants. Among these wines, two were the same (standard) and one was different (dehalcoholized). Participants had to taste each of the three wines and to identify which one was different from the others.

2.2.2 Description of the sensory differences resulting from the alcohol reduction

The aim was to qualify and quantify the sensory modifications resulting from the dealcoholization process through objective description. Conventional sensory profile method (ISO 13299, 2003) with trained panel subjects was used. To complete the description carried out with the sensory profile, a temporal description methodology was also used: Temporal Dominance of Sensations (TDS) (Pineau *et al.*, 2009).

2.2.3 Measure of perceived Complexity in PDW

The aim was to evaluate if perceived wine complexity is affected by alcohol reduction. Complex wines are often associated with high quality. According to Medel *et al.* (2009), perception of wine complexity is related to eight sensory dimensions such as the perceived number of aromas, familiarity, homogeneity, harmony, balance, facility to describe sensations, strength and persistency. These authors developed an illustrated questionnaire in order to measure perceived wine complexity as well as the eight associated dimensions with non-trained consumers. This questionnaire was used on the wines of the study to assess the impact of alcohol reduction.

2.2.3 Liking and acceptability of PDW by consumers

The aim was to assess whether consumers like and accept PDW. Hedonic tests with and without information (Lange, 2000) were carried out with the same consumers. The blind hedonic test strictly measured sensory liking by isolating the sensory component whereas the hedonic test with information measured the acceptability by determining the impact of information on the overall liking. An innovative methodology was also developed in order to measure the relative impact of each of the component (sensory and information) on the overall liking of wines in real life settings. Three bottled wines were designed by varying the wines into the bottles as well as the information on the labels (figure 1). These three wines were sent to consumers' home and evaluated in real life settings.

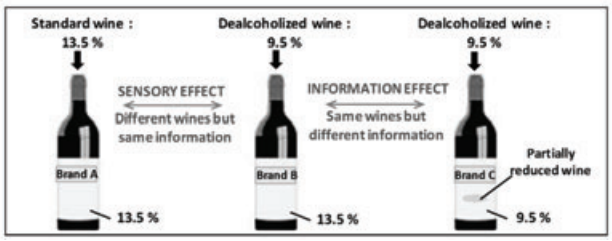


Figure 1. Experimental design of the study

3. Results

3.1. Impact of alcohol reduction by reverse osmosis on the sensory perception of wines

Partial alcohol reduction in wine by reverse osmosis had an impact on the sensory properties of wines since a sensory difference was perceptible between the standard wines and the same wines partially dealcoholized.

The nature and intensity of the sensory differences were variable according to the proportion of alcohol removed as well as the origin and type of the initial wine.

Despite a great variability in the results, common sensory consequences of alcohol reduction appeared across all three series of wine studied. Partial dealcoholization by reverse osmosis induced a decrease in the perception of hotness, bitterness, aroma and persistency. Partial alcohol reduction also resulted in a decrease in sweetness perception in some wines (Syrah and Sauvignon). In the case of red wines, alcohol reduction induced an increase in the perception of astringency to the expense of hotness and bitterness perceptions and a decrease in the perception of complexity.

The sensory consequences of alcohol reduction were not only related to the effect of alcohol content reduction but also to the effect of reverse osmosis treatment. Indeed, independently from the alcohol content of wines, reverse osmosis treatment induced a sensory modification of wines and particularly a decrease in the perception of wine balance.

In order to eventually compensate for the sensory consequences of partial dealcoholization, it would be important to consider sensory compensation strategies. Indeed, adding some grape sugars (7 g/l) to dealcoholized wines significantly reduced the perrception of astringency of the tannins and increased the perception of fruity aroma. However, sugar compensation did not compensate for the decrease in the perception of bitterness and hotness.

3.2. Liking and acceptability of PDW by consumers

In blind conditions, the liking of the sensory properties of PDW by consumers is strongly segmented. This segmentation is mainly driven by the level of expertise and/or exposure in wine. Wine professionals and consumers with high wine experience (frequent wine consumption, high wine knowledge, many bottles in cellar) did not like the sensory properties of PDW whereas less involved consumers liked these wines.

Before even tasting the dealcoholized wines, the consumer expectations were segmented. About 50% of consumers had negative expectations toward PDW and 20% of consumers had positive expectations. Other consumers did not express any expectations.

Many arguments were expressed by the consumers to explain their negative attitude towards dealcoholized wines. First, the dealcoholization process was believed to induce a loss in wine authenticity and tradition. Some consumers had a “tampering with” feeling and worried about dealcoholized wines that are excessively manipulated. Many consumers also worried about the final wine quality and long term storage consequences. On the other hand, consumers that reported to be in favor of dealcoholization believed that the wines are now too strong (alcohol content). They told that reducing alcohol content of wine could help to have a better sobriety, health and slimming diet.

When tasting and assessing the liking for PDW, many consumers were influenced by the information provided on the label. When the information was given, many consumers modified their overall evaluation, either by increasing their liking for PDW, hence expressing acceptance or by decreasing their appreciation, hence expressing a reject of the PDW concept. However the sensory dimension was as much important as the information in the acceptability of PDW.

The concept of PDW was less accepted (expectations and tasting) in red wines than in white wines. Many consumers reported that red wines are more complex than white wines and therefore should be less suitable for dealcoholization. Other consumers reported that dealcoholization is a “tampering process” and given the symbolic, cultural, sacred and traditional status associated with red wines, this “tampering” is psychologically more negatively perceived for these wines.

4. References

ISO 4120, 1983. Analyse sensorielle - Méthodologie - Essai triangulaire (V09-013). *In Analyse Sensorielle*. Paris, AFNOR.

ISO 13299, 2003. Analyse sensorielle - Méthodologie - Directives générales pour l’établissement d’un profil sensoriel. *In Analyse Sensorielle* p 475-506. Paris, AFNOR.

Lange C., 2000. *Étude de l'effet des caractéristiques sensorielles, des attentes induites par l'information et du prix sur l'acceptabilité et le comportement d'achat du consommateur*. Université de Dijon.

Medel M., Viala D., Meillon S., Urbano C., Schlich P., 2009. « A questionnaire for assessing the perceived complexity of wine: Application to the study of the effect of expertise on perception of wine complexity ». *8th Pangborn Sensory science Symposium*, Florence, Italy, 26-30 July, Edited by Elsevier.

Pineau N., Schlich P., Cordelle S., Mathonnière C., Issanchou S., Imbert A., Rogeaux M., Etiévant P., Köster E., 2009. « Temporal Dominance of Sensations: Construction of the TDS curves and comparison with time-intensity ». *Food Quality and Preference,* **20**, 6, 450-455.

Les Partenaires* du Réseau OENOVITI INTERNATIONAL

*The Partners** of OENOVITI INTERNATIONAL Network

Partenaires académiques/*Academic partners*

École de Management de Bordeaux (BEM) - Bordeaux, France
Bordeaux Sciences Agro - Bordeaux, France
École Supérieure d'Agriculture d'Angers - Angers, France
Institut d'éducation Technologique d'Athènes - Athènes, Grèce
Montpellier Supagro - Montpellier, France
Université Bordeaux Segalen - Bordeaux, France
Université Charles Sturt - Wagga Wagga, Australie
Université d'Adélaïde - Adélaïde, Australie
Université de Californie - Davis, États-Unis
Université de Hochschule Geisenheim - Geisenheim, Allemagne
Université de La Rioja - Logroño, Espagne
Université de Padoue - Padoue, Italie
Université de Pécs - Pécs, Hongrie
Université de Saint Catharines de Brock - Ontario, Canada
Université de Stellenbosch - Stellenbosch, Afrique du Sud
Université de Turin - Turin, Italie
Université de Vérone - Vérone, Italie
Université de Zagreb - Zagreb, Croatie
Université des Sciences Agronomiques et de Médecine Vétérinaire de Bucarest - Bucarest, Roumanie
Université d'Udine - Udine, Italie
Université nationale de Cuyo - Mendoza, Argentine
Université Polytechnique de Madrid - Madrid, Espagne
Université Pontificia Catholique du Chili - Santiago, Chili
Université Rovira I Virgili - Tarragone, Espagne
Université technique de Lisbonne - Lisbonne, Portugal

Partenaires industriels/*Industrial partners*

Château Pichon-Longueville - Pauillac, France
Dão Sul - Carregal do Sal, Portugal
JU.CLA.S. SRL (Groupe Vason) - Vérone, Italie
Linde Gas - Arluno, Italie
MBF - Veronella, Italie
Laffort (SARCO) - Floirac, France
Lallemand - Blagnac, France
TORRES S.A - Vilafrance del Penedes, Espagne
VITEC - Falset, Espagne
Winetech - Paarl, Afrique du Sud

*en juin 2013/*as of June 2013*

The OENOVITI INTERNATIONAL Network is the first and unique international network for higher education and research in oenology and viticulture. It aims to promote the exchange of know-how and expertise between the stakeholders of the viticulture and academic communities and the industry. The network enables its members to be influent at the international level by developing the opportunities in terms of joint research and training projects and by encouraging dialogue between the groups involved. The OENOVITI INTERNATIONAL Network comprises more than 30 partners worldwide which form an international consortium of institutions with recognised excellence in the field. In this frame, a joint doctoral programme, OENODOC, was created in order to set up an international doctorate specific to the oenology-viticulture sector. The network, coordinated by the University of Bordeaux Segalen, brings together the academic community and the industry around the many research challenges in oenology and viticulture.

This first symposium of the network will deal with the reduction of alcohol level in wine which is one of the challenges the viticulture and oenology sector will have to face in order to :

- preserve the wine quality,
- bring solutions to viticulturists,
- ensure consumers maximum enjoyment when tasting wines.

Indeed, some climatologists foresee that by 50 years, the sensorial quality of wine will be fundamentally different from the current one because of global warming. Will we be able to : - reduce the sugar level of grapes ? – reduce the alcohol level in wine through technology or biotechnology ? – maintain the acidity levels necessary to the conservation of wine ?

This first international symposium intends to gather knowledge and to suggest actions on this topic, addressing the different possibilities to reduce the alcohol level of wine in terms of :

- viticulture,
- oenology (microorganisms and chemistry),
- potential strategies and rules, technological practices and processes,
- sensorial impact and consumers' preferences regarding wines with a reduced alcohol level.

This international symposium relies on the support of the Château Pichon-Longueville (AXA Millésimes Group), the University of Bordeaux Foundation (original interface between the academic and socio-economic communities), IdEx Bordeaux (investment programme to foster the dynamic of transformation and development of the University of Bordeaux) and of all the academic and industrial partners of the OENOVITI INTERNATIONAL Network as well as on the sponsorship of the OIV. We would like to thank all the people who supported us and our partners for their help in the promotion of the research in oenology and viticulture to come up with the collective answers essential to meet the new challenges in this field.

The 1st OENOVITI INTERNATIONAL Symposium on Alcohol level reduction in wine has been supported by the following instituitions :

fondation Bordeaux Université

UNIVERSITÉ DE BORDEAUX

UNIVERSITÉ BORDEAUX SEGALEN

ŒNOVITI INTERNATIONAL

ISVV INSTITUT DES SCIENCES DE LA VIGNE ET DU VIN BORDEAUX AQUITAINE

Initiative d'Excellence Université de Bordeaux

INVESTISSEMENTS D'AVENIR

OIV

Sous le patronage de l'Organisation Internationale de la Vigne et du Vin