DUBROVNIK
APRIL 28, 2011

PART 1
SENSORY DEVELOPMENT OF HOT-CLIMATE RED VARIETALS DURING FERMENTATION

PART 2
ROSÉ WINE FERMENTATION MANAGEMENT AND THE CURRENT MARKET SITUATION

18

LALLEMAND

DUBROVNIK, APRIL 28, 2011

PART 1
SENSORY DEVELOPMENT OF HOT-CLIMATE RED VARIETALS DURING FERMENTATION

PART 2
ROSÉ WINE FERMENTATION MANAGEMENT AND THE CURRENT MARKET SITUATION

PROCEEDINGS
OF

THE *XXII*^{es} *ENTRETIENS SCIENTIFIQUES LALLEMAND*

LALLEMAND

FOREWORD

This year, the *XXII^es Entretiens Scientifiques Lallemand* were a two-part event focused first on hot-climate red varietals and understanding their sensory development, and, second, on rosé winemaking and the impact of different techniques on the wine style, including a presentation on the rosé wine market.

The meeting gathered some of the top scientists in the field to present these topics to an international crowd, including winemakers from Eastern Europe, and was an opportunity to hand out the Lallemand awards. The student award, the *Prix Michel-Feuillat – Entretiens Scientifiques Lallemand*, was awarded to Dr. Guillaume Antalick from the Université de Bordeaux II for his work on "Biochemical and sensory changes associated with fruity notes in red wines during malolactic fermentation. The importance of esters." The Lallemand – Institute of Masters of Wine bursary was awarded to Sharon Wild, a second-year Master of Wine student from Australia for her essay responding to "Discuss the evolution of rosé wine styles and consumer preferences globally over the past five years." Also on hand were the winners of the ML Wines competition (Madrid 2011), who received their prizes from the president of Lallemand, Mr. Jean Chagnon.

The meeting opened with a presentation by Professor Rémi Guérin-Schneider from IFV Rhône Méditerranée in Montpellier, France, about the impact of yeast on the aromatic potential of grapes during fermentation. His presentation showed that yeast fermentation is a key component in the biotransformation of varietal precursors and is responsible for much more than the production of secondary metabolites from amino acids and sugars.

Professor Fernando Zamora from Universitat Rovira i Virgili in Tarragona, Spain, addressed the impact of climate change on grape ripening and the challenge it presents to the wine industry. Different scenarios were discussed on how to minimize the impact on the wine, such as using lees or inactive yeast to enrich the wine with polysaccharides to increase mouthfeel and reduce bitterness, astringency and herbaceous characters, as well as applying techniques for the partial dealcoholization of wines.

Dr. Eveline Bartowsky from the AWRI, Australia, presented the progress on her work on the influence of malolactic fermentation on the fruity character of red wines. Her results show that significant sensory and compositional differences occur as a result of different malolactic fermentation treatments, including differences in the intensity of perceived fruit flavour. The first part of the meeting concluded with a presentation by Dr. Charles Edwards from Washington State University, United States. The impact of molecular sulphur dioxide (mSO_2) and filtration requirements to control *Brettanomyces bruxellensis* yeast was also presented.

The second part of the *Entretiens Scientifiques Lallemand* focused on rosé wines. Dr. Antonio Palacios presented the results of a joint study by Lallemand, Litmus Wines (United Kingdom), three wineries (in Spain, France and Portugal) and a large U.K. retailer. The study looked at making rosé wines with selected yeasts and a specific protocol.

One key element for rosé wine is fermentation management established with a proper nutrition strategy to avoid struck fermentation and related defects. Baptiste Olivier from the ICV, France, presented Good Nutritional Practices that aim to satisfy the needs of the yeast in order to obtain a viable population large enough to complete alcoholic fermentation. The Centre du Rosé in the Provence region of France was represented by Dr. Laure Cayla, who discussed the numerous tools that have been developed to describe the rosé colour palette and to adapt the selected winemaking process according to the desired colour objective. The management of colour is based on the choice of varietal and the good management of pre-fermentation operations. The winemaker can direct the sensory profile of rosé wines through the choice of techniques, input and equipment, so the sensory quality of the wines meets the needs of different markets. The last presentation of the day was a departure from the scientific ones, as Lucy Clements, from Sainsbury Supermarkets, U.K., presented on the rosé market in the U.K. and the company's approach to consumer preferences.

In both red and rosé wines, the sensory impacts of yeast and bacteria are now better understood, and ongoing research lets us understand the mechanisms behind the processes, so winemakers and, ultimately, wine drinkers can benefit from this information. The primary goal of our investment in research is to translate scientific results into improvements in wine quality.

CONTENTS

IMPACT OF YEAST ON THE AROMATIC POTENTIAL OF GRAPES DURING FERMENTATION

Rémi GUÉRIN-SCHNEIDER[1, 2] and Laurent DAGAN[2]

[1] UMT Qualinnov, INRA-IFV, 2 place Pierre Viala, 34060 Montpellier, France

[2] Analyse et Conseil, bâtiment 28, 2 place Pierre Viala, 34060 Montpellier, France

remi.schneider@supagro.inra.fr

Abstract

Alcoholic fermentation is one of the key steps in the process of winemaking. In addition to the major bioreactions resulting from yeast activity, such as alcohol production, secondary metabolites are produced and some are involved in developing the sensory characteristics of wines. As the major secondary volatile metabolites produced by yeast are generated by non-specific substrates (amino acids, sugars, etc.), until recently yeast was considered unable to interact with qualitative varietal compounds. However, in the past 10 years, research has demonstrated that yeast fermentation is also a key component in the biotransformation of varietal precursors.

Two examples of biotransformation will be developed. The first deals with varietal thiols generated from specific precursors (cysteine or glutathione conjugates) by C-S lyase activity of yeast during fermentation. These aroma compounds are key in several white and rosé wines, and seem to contribute to the blackcurrant olfactory note of several red wines. When the impact of the yeast strain is well documented, the nitrogen composition of the must and its consequences on yeast nutrition also appear to have a major impact on the yield of this transformation.

The second example is the modulation by the yeast of the content of the dimethyl sulphide (DMS) precursor in wine. At low concentrations, this compound contributes to enhancing fruity notes in wines, and is reminiscent of truffle notes at high levels, especially in red wines made from Shiraz grapes. Recent studies have shown that the DMS present in wines is formed mainly during aging from a non-volatile precursor (PDMS) by a pure chemical reaction. That precursor was recently identified as S-methylmethionine. The role of yeast in this mechanism is not yet clear, although no relation was observed between the PDMS levels in must and those found in wines after fermentation. A recent study supported by Lallemand showed that yeast is able to metabolize S-methylmethionine, likely as a sulphur/nitrogen source, and thus lower the precursor level in wines. The strain of yeast and nitrogen nutrition appear to be two elements involved in the modulation of this degradation, and, therefore, in the pilot wine typology.

1. Introduction

The aroma potential of grapes includes several classes of specific non-volatile compounds, the so-called aroma precursors, which have in common being present in grapes at the harvest, and are transformed into volatile compounds only during winemaking. Four categories of aroma precursors are now considered as great contributors to wine aroma (Baumes 2009):

- The carotenoids, accessory pigments of photosynthesis whose degradation leads either to C13-norisoprenoidic glycosides when it occurs *in planta*, or to free norisoprenoid compounds such as β-ionone, which is hypothesized to occur during the maceration phase of red winemaking;
- The aroma glycoconjugates, which include dozens of compounds hydrolyzed mainly during wine storage to form odour compounds responsible for the aromatic complexity of aging wine;

- The S-cysteine and S-glutathione conjugates, precursors of the volatile thiols;
- The precursor of dimethyl sulphide (DMS), i.e., S-methylmethionine.

Yeast during fermentation seems to have no direct impact on carotenoids as their hypothesized degradation is a chemical reaction catalyzed by heat. The production of ethanol by the yeast during the maceration phase could, however, help their dissolution in the must.

On glycoconjugates, the yeast is described as having no impact (Baumes 2009) or a very minor impact (Loscos et al. 2007, and Hernandez-Orte et al. 2008). In the latter case, the compounds released have been proven to have only a small contribution to the aromatic complexity of wine. However, yeast assumes a key role on the release of thiols from their S-conjugates (Dubourdieu et al. 2006) and on the preservation of the DMS potential.

2.]The Formation of Varietal Thiols during Fermentation: The Impact of Yeast

2.1 The nature of the key varietal thiols in wine

Three varietal thiols have been identified as key aroma compounds in Sauvignon Blanc (Darriet 1993, Tominaga et al. 1996, and Tominaga et al. 1998a), and in Colombard (Tominaga et al. 2000), Petite Arvine (Fretz et al. 2005), and red wines from Merlot, Cabernet, Grenache and Shiraz (Blanchard et al. 1999, Murat et al. 2001a, Ferreira et al. 2002, and Masson and Schneider 2009).

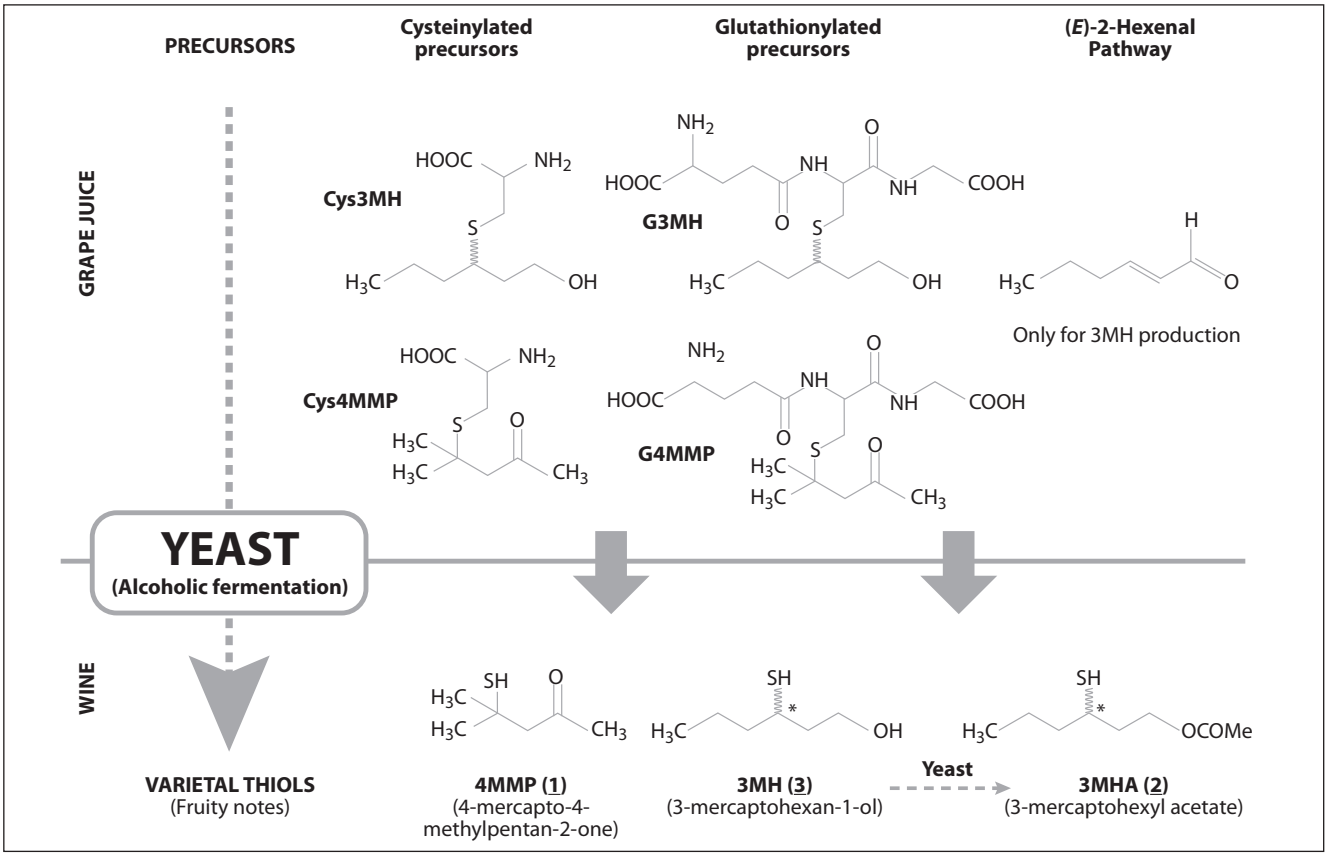
The 4-methyl-4-mercaptopentan-2-one (4MMP) is reminiscent of box-tree or guava (Darriet et al. 1995), whereas 3-mercaptohexanol (3MH) and its acetate (3MHA) exhibit citrus notes (Tominaga et al. 2000) or blackberry scents in red wines (Blanchard et al. 1999). They are present in wines at trace levels (sub ppb), but, as their perception thresholds are very low, they have been shown to be great contributors of the fruity notes in general.

2.2 Biogenesis pathways

Three biogenesis pathways are commonly accepted to explain the release of 4MMP and 3MH in wine. The biogenesis of 3MHA is quite particular because it is produced from 3MH during fermentation, by the action of the yeast ester-forming alcohol acetyltransferase, encoded by the ATF1 gene (Swiegers and Pretorius 2007) (figure 1).

The first pathway involves the cysteinylated precursors, which were initially identified in Sauvignon Blanc grapes (Tominaga et al. 1995, and Tominaga et al. 1998b), then

Figure 4. Biogenesis pathways of varietal thiols.



in Merlot and Cabernet Sauvignon (Murat et al. 2001a), in Semillon (Thibon et al. 2009), in Petit Manseng and Gros Manseng (Lopes et al. 2005), in Riesling, in Melon B. and Gewürztraminer (Roland et al. 2010) and in Koshu (Kobayashi et al. 2010), especially for Cys3MH. These S-cysteine conjugates are cleaved by the yeast, through its β-lyase activity (Tominaga et al. 1998) during alcoholic fermentation (AF). S-3- (hexan-1-ol)-cysteine (Cys3MH) is more ubiquitous and abundant in grapes than S-3- (4-mercapto-4-methylpentan-2-one)-cysteine (Cys4MMP) (Peyrot des Gachons et al. 2000, Murat et al. 2001a, Roland et al. 2010c).

The second pathway concerns the glutathionylated precursors: S-3-(hexan-1-ol)-glutathione (G3MH) identified in Sauvignon Blanc grapes (Peyrot des Gachons et al. 2002), Melon B. (Roland et al. 2010c), Riesling (Roland et al. 2010a), Gewürztraminer (Roland et al. 2010a), Chardonnay (Capone et al. 2010), Pinot Grigio (Capone et al. 2010) and Koshu (Kobayashi et al. 2010), and S-3-(4-mercapto-4-methylpentan-2-one)-glutathione (G4MMP), occurring in Sauvignon Blanc (Fedrizzi et al. 2009), Riesling (Roland et al. 2010c) and Gewürztraminer (Roland et al. 2010c). The mechanism of thiol release from glutathionylated precursors was investigated only for the G3MH. Indeed, the percolation of a Sauvignon Blanc or a Gros Manseng must through an immobilized γ-glutamyltranspeptidase column resulted in the increase of Cys3MH, suggesting that the G3MH could be a pro-precursor (Peyrot des Gachons et al. 2002). However, model (Grant-Preeceet al. 2010, Kobayashi et al. 2010) and Sauvignon Blanc (Roland et al. 2010a) musts were spiked with synthetic G3MH and then fermented with VIN13 or VL3 yeast strains. The release of the 3MH in the resulting wine demonstrated that G3MH constituted another precursor of 3MH. Similar outcomes were observed for G4MMP in experiments on Sauvignon Blanc must (Roland et al. 2010b). Consequently, the G3MH could play two different roles, according to oenological conditions: pro-precursor of Cys3MH (Peyrot des Gachons et al. 2002, Thibon et al. 2011) and precursor of 3MH (Grant-Preece et al. 2010, Kobayashi et al. 2010, and Roland et al. 2010a).

Finally, the third biogenesis pathway involved the C6 unsaturated compounds as (*E*)-2-hexenal which undergo a sulphur addition during AF (Schneider et al. 2006). To date, the sulphur donor has never been identified, but it could be H_2S, cysteine, glutathione or other molecules having an available free thiol function in must.

Because varietal thiols were the result of different biogenesis pathways, the measurement of conversion yields from each precursor can only be based on deuterated markers. This technique presents another advantage as experiments can be performed in real grape must that take into account the impact of must composition on the yeast's ability to convert precursors into thiols.

The first pathway elucidated with this technique was the hexenal pathway, contributing to the production of 3MH, formally proved by adding [^{2}H8]-hexenal to a Melon B. must (Schneider et al. 2006). The release of [^{2}H8]-3MH in the corresponding wine demonstrated that (*E*)-2-hexenal constituted an additional pathway for the 3MH production. This pathway contributed to 10% of the total 3MH released in the Melon B. wine.

Subileau and co-workers (Subileau et al. 2008a) measured the conversion yield of [^{2}H8]-Cys3MH into [^{2}H8]-3MH in a Sauvignon Blanc must from two different origins (Gers and Languedoc) by using two different yeast strains. Whatever the origin of the must or the kind of yeast, molar conversion yield was always below 1%, which explains only 3% to 7% of the total 3MH in the resulting wines.

Using the same strategy, [^{2}H2,3]-G3MH was added to a Sauvignon Blanc must to investigate other biogenesis origins that could explain the total production of 3MH in wine (Roland et al. 2010b). The identification of [^{2}H2,3]-3MH in the resulting Sauvignon Blanc wine showed the direct connection between G3MH and 3MH under oenological conditions. The conversion rate of G3MH into 3MH was estimated to be close to 4.5%, irrespective of the initial amount of [^{2}H2,3]-G3MH spiked in must 1. Similar experiments demonstrated the direct relationship between G4MMP and 4MMP using a Sauvignon Blanc must initially spiked with [^{2}H10]-G4MMP (Roland et al. 2010a). The conversion yield equal to 0.3% justified 20% of the total 4MMP release.

The levels of the three different precursors reported in the literature and the mean conversion yields experimentally determined cannot explain the total amount of thiols present in wines. This observation points out the eventual presence in must of other precursors, especially derivatives of the precursors already identified (aldehyde or cyclic forms). However, modulation of the conversion yield by the nitrogen composition cannot be excluded.

Table 1. Conversion yields under oenological conditions

Conversion yields under oenological conditions (%)					
Yeast type	**Strains**	**Cys4MMP→4MMP**	**Cys3MH→3MH**	**G3MH→3MH**	**G4MMP→4MMP**
Saccharomyces cerevisiae	VL3c	0.06 [a] – 0.8 [b]	0.31 [b]		
	EG8	0.5 [a] – 0.7 [b]	0.41 [b]		
	VL1	0.2 [a]			
	522d	0.06 [a]			
	VIN13	0.6 [b]	0.39 [b]	**4.5**[c]	**0.3**[d]
	VIN7	1.3 [b]	0.30 [b]		
	QA23	1.3 [b]	0.23 [b]		
	NT116	0.5 [b]	0.29 [b]		
	ES1		**0.48 – 0.81**[f]		
S. bayanus	P3				
	TBC28				
Interspecific hybrids ***(S.c. x bayanus)***	H1 to H9	3.5-10.9 [g]			

[a]from Murat et al. 2001, [b]from Subileau 2008, [c]from Roland et al. 2010a, [d]from Roland et al. 2010a, [f]from Subileau et al. 2008a, [g]from Masneuf et al. 2002.
Model or natural medium.

2.3 Oenological conditions that could modulate thiol release by yeast

2.3.1 Fermentation temperature

AF temperature influenced the release of varietal thiols, but reported data appeared quite variable. More specifically, AF conducted at 20°C instead of 13°C resulted in more 4MMP, 3MH and its acetate in model media and wines, despite the yeast strain used (Masneuf-Pomaredeet al. 2006).

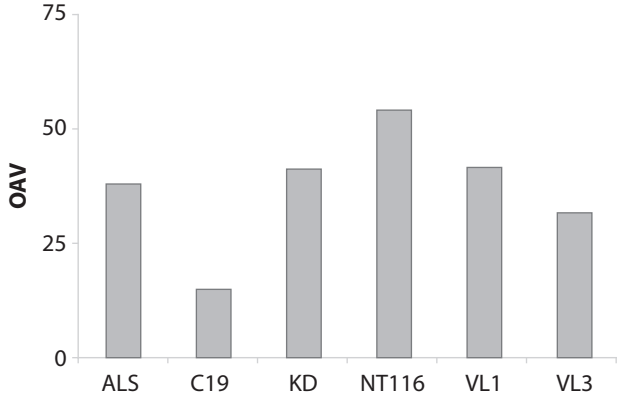
2.3.2 Yeast strain

Some commercial yeast strains, such as VL3c, EG8, VIN13 and VIN7 (Murat, Masneuf et al. 2001, Howell et al. 2004, Dubourdieu et al. 2006, and Swiegers et al. 2006), have demonstrated their ability to release varietal thiols under oenological conditions; 4MMP and 3MH formation in wine can be modulated by yeast strains.

When compared to a single yeast strain, a combination of *Saccharomyces cerevisiae* strains, such as VIN7 and QA23, resulted in an overproduction of 3MH, up to 200 ng/L and of 3MHA, up to 20 ng/L in Sauvignon Blanc (King et al. 2008). Recent investigations have demonstrated that co-fermentation with *Pichia kluyveri* (a non-*S. cerevisiae* yeast) generated more 3MH and 3MHA in Sauvignon Blanc wines (Anfang et al. 2009). In addition, interspecific hybrid *S. cerevisiae* x *S. bayanus* var. *uvarum* yeasts were found to enhance the production of 4MMP from its S-cysteine precursor compared to its parent *S. cerevisiae* (Masneuf et al. 2002, and Dubourdieu et al. 2006).

As shown in figure 2, for a Colombard must, the differences between yeast strains used on an industrial scale could range from one to five.

Figure 2. 3MH release (as Odour Active Value, i.e., the ratio between the 3MH level and its odour threshold) after inoculation with different commercial yeasts on a Colombard must (data from IFV Sud-Ouest)



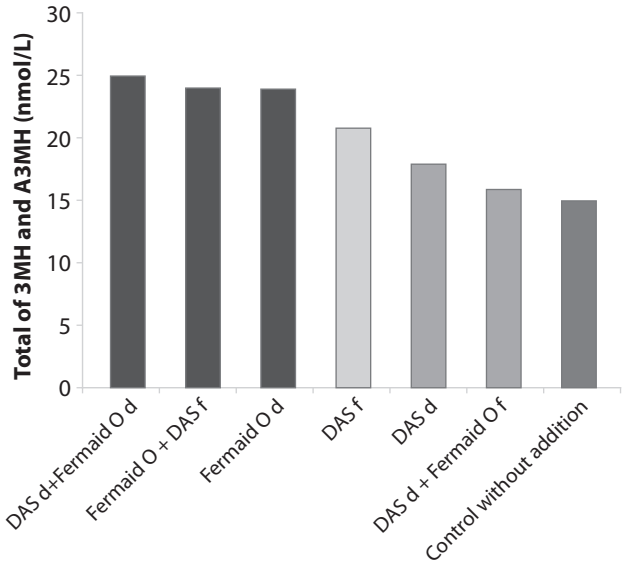
However, studies performed with different musts lead us to moderate this observation, as the must composition, especially in yeast-available nitrogen, could be the first cause of variations in interaction with the yeast's nitrogen needs.

2.3.3 Nitrogen nutrition

Studies were performed on yeast cell precursor transport. In a synthetic medium, Gap1p (general amino-acid permease) constituted at least one transporter of Cys3MH, whose activity regulates thiol production (Subileau et al. 2008b). Thus, the production of varietal thiols by yeast, in such a medium, is modulated via the nitrogen catabolite repression (NCR) mechanism, like the uptake of poor nitrogen sources. Indeed, the substitution of diammonium phosphate (DAP) by urea as the sole source of nitrogen involved an increase of 3MH in a synthetic medium (Subileau et al. 2008b). On grape must, even if Gap1p has not been confirmed as precursor transporter, the addition of DAP, which eventually prolongs NCR, has been shown to decrease thiol release.

This mechanism has crucial technological consequences, since the correction of nitrogen levels in must is widely used. As shown in figure 3, the addition of a complex source of nitrogen (Fermaid O or a mix of Fermaid O and ammonium) gave the best results for 3MH release when the complex nitrogen was added at the beginning of AF. An addition of only ammonium appeared to be less convenient even if added at the end of AF.

Figure 3. Effects of the type of nitrogen source and the timing of its addition on 3MH release during alcoholic fermentation (addition of 50 mg/L yeast-available nitrogen at the end (f) or the beginning (d) of fermentation).



2.4 Effect of yeast on potential dimethyl sulphide

2.4.1 The origin of dimethyl sulphide in wine and its sensory contribution

Dimethylsulphide (DMS) is a light compound in wine highlighted by Du Plessis and Loubser (1974) and has an olfactory perception threshold of about 25 mg/L (Etiévant 1991). Its contents in young wines are often lower than the olfactory perception threshold, but it can reach 900 mg/L in developed wines (Dagan 2006). Recent data show that this compound is more often perceived positively, but its contribution to the aroma of wine is complex. At high concentrations in well-developed wines, mainly those coming from late harvest white grapes, this compound brings truffle notes. At lower concentrations, it contributes to fruity notes in red wine, in particular by a potentialization effect (Anocibar Beloqui 1998, Segurel et al. 2004, and Escudero et al. 2007).

Varietal DMS is produced during AF from S-methylmethionine (SMM) and dimethylsulphoxide (DMSO) of grapes, but only from SMM at pre-fermentation stages (Etiévant 1991, Segurel et al. 2005, Dagan 2006, and Loscos et al. 2008). During AF stages, *S. cerevisiae* yeast is able to reduce DMSO to DMS and some yeast and lactic acid bacteria strains can use SMM as a sulphur source. Nevertheless, because of its high volatility, the varietal DMS produced by yeasts is for the most part removed with the carbon dioxide produced by AF, and in this way DMS concentrations in bottled wines are generally very low.

On the other hand, post-fermentation, varietal DMS contents increase with time and heat during maturing in bottles, to reach levels of about 1 mg/L, but at this stage, DMS is only produced from SMM by a purely chemical reaction (Segurel et al. 2005, and Dagan 2006).

2.4.2 The fate of potential dimethyl sulphide during AF and the role of yeast

The comparison of potential dimethyl sulphide (PDMS) before and after AF on more than 25 experimental vinifications showed that 90% of the PDMS was degraded during AF (Dagan 2006).

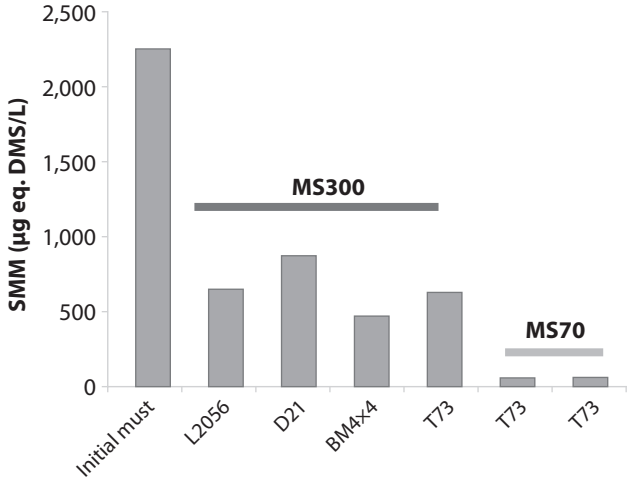
As only the residual PDMS in wine can generate DMS during storage, it could be of interest to pilot PDMS preservation in wine during AF.

Since PDMS was shown to be an amino acid derivate (SMM), the impact of nitrogen nutrition and yeast strains was investigated in a collaborative study performed with Lallemand. The goals of that study were to determine an eventual yeast strain effect, and then to study the effect of nitrogen addition on the preservation of PDMS.

In preliminary work, four yeast strains were tested either on two different synthetic media or on a natural Shiraz must (figure 4), on a laboratory scale (1 L, T= 20°C). The two synthetic media differed only on the level of yeast-available nitrogen (YAN): MS300 presented 420 mg/L of

YAN, whereas MS70, with only 100 mg/L of YAN, exhibited a nitrogen deficiency.

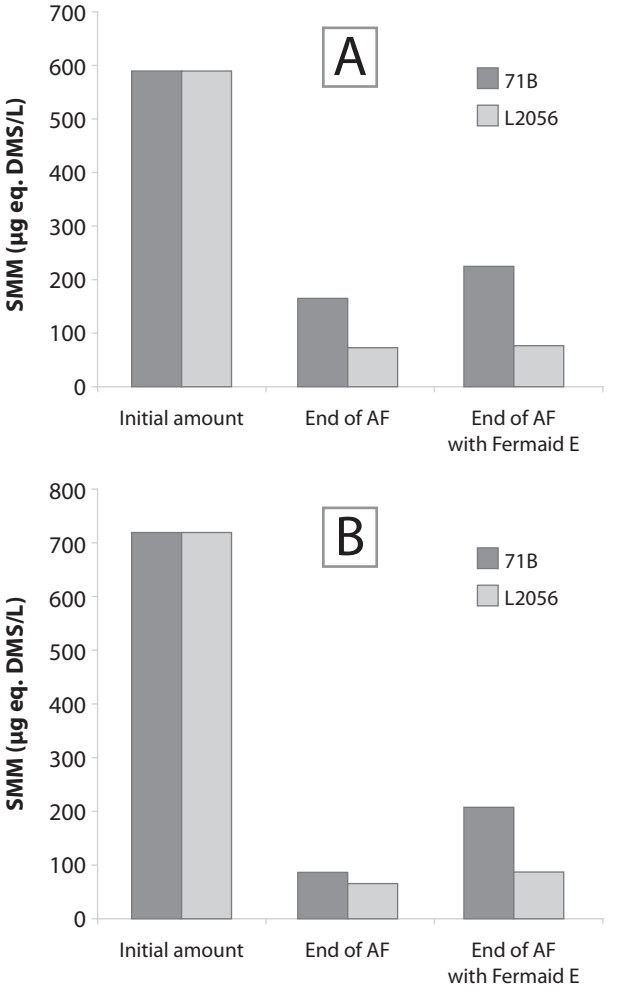
Figure 4. Yeast strain impact on S-methylmethionine degradation during alcoholic fermentation on two different synthetic media (MS300 and MS70)



If the global degradation was about 70% on MS300, the residual SMM varied from 20% to 40% of the initial amount, depending of the yeast strain involved in the AF. In the case of nitrogen deficiency (MS70) this consumption of SMM is greater, resulting in residual SMM at about 3% of the initial amount added. Similar results were observed on a Shiraz must, with YAN at about 350 mg/L (data not shown).

In another experiment, the nitrogen level of the AF was crossed with the nitrogen needs of the yeast, by choosing two yeasts with contrasted needs: L2056, presenting high nitrogen needs, vs. 71B with low nitrogen needs. The AF was performed in a synthetic medium with 130 mg/L of YAN, and in the same Shiraz must that was utilized in the previous experimental set. An addition (300 mg/L) of complex nitrogen (Fermaid E™) was tested. As shown in figure 5, in the context of a nitrogen deficiency, the yeast with the lower nitrogen needs preserved more SMM at the end of AF. The addition of Fermaid E™ enhanced this effect, whereas no effect could be observed on the yeast presenting high nitrogen needs.

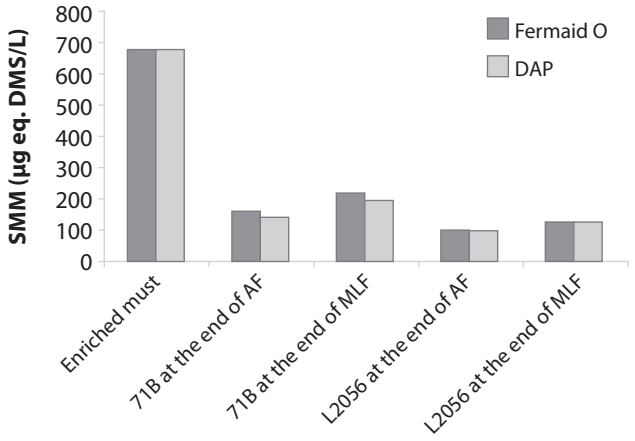
Figure 5. Effect of the interaction between yeast-available nitrogen in a synthetic medium (A) and a natural must (B) and nitrogen needs of yeast on the preservation of S-methylmethionine during alcoholic fermentation



The results obtained on the Shiraz must (figure 5B) were similar even though the differences between the two yeasts were not obvious without nitrogen addition, likely due to the significant initial amount of YAN (350 mg/L). Thus, the addition of a nitrogen source such as Fermaid E™ could be of interest even in a non-deficiency context.

Lastly, the effects of amino acid additions were tested on an industrial scale through the use of Fermaid O™ vs. DAP. The must utilized was a Shiraz presenting 190 mg/L of YAN and was fermented at 24°C with the same two yeasts as the previous experiment.

Figure 6. Effect of DAP or Fermaid O™ addition on the preservation of S-methylmethionine in a Shiraz must fermented with the 71B yeast strain



The results obtained (figure 6) are similar. The 71B strain, with low nitrogen needs, preserved more SMM at the end of AF, and this difference is found also at the end of the malolactic fermentation (MLF). DAP and Fermaid O™ exhibited the same efficiency when used with the low-needs yeast.

In addition, we could observe an increase of SMM during MLF, which is a new result but is consistent with other observations made in our lab, and thus could constitute a new research topic.

3. Conclusions

Yeast is a key element for thiol formation, but yeast nutrition appears to be essential to increase thiol release. As the conversion yields remain very low, it seems more convenient to ensure good fermentation conditions (yeast strain, temperature, nitrogen, etc.) than to try to increase the potential of grapes.

For potential dimethyl sulphide (PDMS), recent results show that yeast is partly responsible for the degradation of this aromatic potential during alcoholic fermentation. The choice of yeast strain and the correction of the nitrogen composition of the must could be tools to modulate PDMS preservation and pilot the wine typology.

References

Anfang, N., et al. 2009. Co-fermentation with *Pichia kluyveri* increases varietal thiol concentrations in Sauvignon Blanc. *Aust. J. Grape Wine R.* 15(1):1-8.

Anocibar Beloqui, A. 1998. *Les composés soufrés volatils des vins rouges*. Victor Segalen Bordeaux II. Ph.D. thesis. 169.

Baumes, R. 2009. Wine aroma precursors. *Wine chemistry and biochemistry*. V. Moreno-Arribas and C. Polo, Springer. 261.

Blanchard, L., et al. 1999. Caractérisation de la fraction volatile de nature soufrée dans les vins de Cabernet et de Merlot. Étude de son évolution au cours de l'élevage en barriques. *Œnologie 99 – 6e Symposium International d'Œnologie*, Bordeaux, Lavoisier Tec & Doc.

Capone, D. L., et al. 2010. Analysis of Precursors to Wine Odorant 3-Mercaptohexan-1-ol Using HPLC-MS/MS: Resolution and Quantitation of Diastereomers of 3-S-Cysteinylhexan-1-ol and 3-S-Glutathionylhexan-1-ol. *J. Agric. Food Chem.* 58(3):1390-1395.

Dagan, L. 2006. Potentiel aromatique des raisins de *Vitis vinifera* L. cv. Petit Manseng et Gros Manseng. Contribution à l'arôme des vins de pays Côtes de Gascogne. *Sciences et Procédés Biologiques et Industriels*, École Nationale Supérieure Agronomique de Montpellier. 238.

Darriet, P. 1993. *Recherches sur l'arôme et les précurseurs d'arôme du Sauvignon*. Université Victor Segalen, Bordeaux II. Ph.D. thesis.

Darriet, P., et al. 1995. Identification of a powerful aromatic component of *Vitis vinifera* L. var. Sauvignon wines: 4-mercapto-4-methylpentan-2-one. *Flavor Fragrance Journal*. 10:385-392.

Dubourdieu, D., et al. 2006. The Role of Yeasts in Grape Flavor Development during Fermentation: The Example of Sauvignon blanc. *American Journal of Enology and Viticulture*. 57(1):81-88.

Du Plessis, C., and G. Loubser. 1974. The bouquet of "late harvest" wine. *Agrochemophysica*. 6:49-52.

Escudero, A., et al. 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(11):4501-4510.

Etiévant, P. X. 1991. Volatile compounds in food and beverages. *Wine*. H. Maarse (Ed.) New York, Basel, Hong Kong, Dekker Inc. 19-33.

Fedrizzi, B., et al. 2009. First Identification of 4-S-Glutathionyl-4-methylpentan-2-one, a Potential Precursor of 4-Mercapto-4-methylpentan-2-one, in Sauvignon Blanc Juice. *J. Agric. Food Chem*. 57(3):991-995.

Ferreira, V., et al. 2002. Chemical Characterization of the Aroma of Grenache Rosé Wines: Aroma Extract Dilution Analysis, Quantitative Determination, and Sensory

Reconstitution Studies. *Journal of Agricultural and Food Chemistry.* 50(14):4048-4054.

Fretz, C. B., et al. 2005. 3-Mercaptohexanol: An Aroma Impact Compound of Petite Arvine Wine. *American Journal of Enology and Viticulture.* 56(4):407-410.

Grant-Preece, P. A., et al. 2010. Synthesis of Wine Thiol Conjugates and Labeled Analogues: Fermentation of the Glutathione Conjugate of 3-Mercaptohexan-1-ol Yields the Corresponding Cysteine Conjugate and Free Thiol. *J. Agric. Food Chem*. 58(3):1383-1389.

Hernandez-Orte, P., et al. 2008. The development of varietal aroma from non-floral grapes by yeasts of different genera. *Food Chemistry.* 107(3):1064-1077.

Howell, K. S., et al. 2004. Variation in 4-mercapto-4-methyl-pentan-2-one release by *Saccharomyces cerevisiae* commercial wine strains. *FEMS Microbiol. Lett.* 240(2):125-9.

King, E. S., et al. 2008. Coinoculated fermentations using *Saccharomyces* yeasts affect the volatile composition and sensory properties of *Vitis vinifera* L. cv. sauvignon blanc wines. *J. Agric. Food Chem.* 56(22):10829-37.

Kobayashi, H., et al. 2010. Analysis of S-3-(hexan-1-ol)-glutathione and S-3-(hexan-1-ol)-L-cysteine in *Vitis vinifera* L. cv. Koshu for aromatic wines. *Am. J. Enol. Vitic*. 61(2):176-185.

Lopes, P., et al. 2005. Nondestructive Colorimetric Method to Determine the Oxygen Diffusion Rate through Closures Used in Winemaking. *J. Agric. Food Chem.* 53(18):6967-6973.

Loscos, N., et al. 2007. Release and formation of varietal aroma compounds during alcoholic fermentation from nonfloral grape odorless flavor precursors fractions. *Journal of Agricultural and Food Chemistry.* 55(16):6674-6684.

Loscos, N., et al. 2008. Identification of S-methylmethionine in Petit Manseng grapes as dimethyl sulphide precursor in wine. *AnalyticaChimicaActa.* 621(1):24-29.

Masneuf-Pomarede, I., et al. 2006. Influence of fermentation temperature on volatile thiol concentrations in Sauvignon blanc wines. *Int. J. Food Microbio.* 108(3):385-90.

Masneuf, I., et al. 2002. Hybrids *Saccharomyces cerevisiae* x *Saccharomyces bayanus* var. *uvarum* having a high liberating ability of some sulfur varietal aromas of *Vitis vinifera* Sauvignon blanc wines. *J. Int. Sci. Vigne Vin.* 36:205-212.

Masson, G., and R. Schneider. 2009. Key Compounds of Provence Rosé Wine Flavor. *American Journal of Enology and Viticulture*. 60(1):116-122.

Murat, M.-L., and I. Masneuf et al. 2001. Effect of *Saccharomyces cerevisiae* Yeast Strains on the Liberation of Volatile Thiols in Sauvignon blanc Wine. *Am. J. Enol. Vitic.* 52(2):136-139.

Murat, M.-L., and T. Tominaga et al. 2001a. Assessing the Aromatic Potential of Cabernet Sauvignon and Merlot Musts Used to Produce Rose Wine by Assaying the Cysteinylated Precursor of 3-Mercaptohexan-1-ol. *J. Agric. Food Chem.* 49:5412-5417.

Murat, M.-L., and T. Tominaga et al. 2001b. Mise en évidence de composés clefs dans l'arôme des vins rosés et clairets de Bordeaux. *J. Int. Sci. Vigne Vin.* 35:99-105.

Peyrot des Gachons, C., et al. 2000. Measuring the Aromatic Potential of *Vitis vinifera* L. cv. Sauvignon Blanc Grapes by Assaying S-Cysteine Conjugates, Precursors of the Volatile Thiols Responsible for Their Varietal Aroma. *J. Agric. Food Chem.* 48:3387-3391.

Peyrot des Gachons, C., et al. 2002. Sulfur Aroma Precursor Present in S-glutathione Conjugate Form: Identification of S-3-(Hexan-1-ol)-glutathione in Must from *Vitis vinifera* L. cv. Sauvignon Blanc. *J. Agric. Food Chem.* 50:4076-4079.

Roland, A., et al. 2010a. Identification and quantification by LC-MS/MS of a new precursor of 3-mercaptohexan-1-ol (3MH) using stable isotope dilution assay: Elements for understanding the 3MH production in wine. *Food Chem.* 121:847-855.

Roland, A., et al. 2010a. Straightforward synthesis of deuterated precursor to demonstrate the biogenesis of aromatic thiols in wine. *J. Agric. Food Chem.* 58:10684-10689.

Roland, A., et al. 2010b. Validation of a nano liquid chromatography-tandem mass spectrometry method for the identification and the accurate quantification by isotopic dilution of glutathionylated and cysteinylated precursors of 3-mercaptohexan-1-ol and 4-mercapto-4-methylpentan-2-one in white grape juices. *J. Chromatogr.* A 1217:1626-1635.

Schneider, R., et al. 2006. Evidence for an alternative biogenetic pathway leading to 3-mercaptohexanol and 4-mercapto-4-methylpentan-2-one in wines. *Anal. Chim. Acta.* 563(1-2):58-64.

Segurel, M. A., et al. 2004. Contribution of Dimethyl Sulfide to the Aroma of Syrah and Grenache Noir Wines and Estimation of Its Potential in Grapes of these Varieties. *J. Agric. Food Chem.* 52(23):7084-7093.

Segurel, M. A., et al. 2005. Ability of Possible DMS Precursors to Release DMS during Wine Aging and in the Conditions of Heat-Alkaline Treatment. *J. Agric. Food Chem.* 53(7):2637-2645.

Subileau, M. 2008. *Paramètres influants sur la libération des thiols variétaux par la levure* Saccharomyces cerevisiae*: d'un milieu synthétique à la complexité d'un moût de Sauvignon blanc*. Montpellier, École Nationale Supérieure d'Agronomie de Montpellier. Ph.D. thesis. 156.

Subileau, M., and R. Schneider et al. 2008a. New Insights on 3-Mercaptohexanol (3MH) Biogenesis in Sauvignon Blanc Wines: Cys-3MH and (E)-Hexen-2-al Are Not the Major Precursors. *J. Agric. Food Chem.* 56(19):9230-9235.

Subileau, M., and R. Schneider et al. 2008b. Nitrogen catabolite repression modulates the production of aromatic thiols characteristic of Sauvignon Blanc at the level of precursor transport. *FEMS Yeast Res.* 8:771-780.

Swiegers, J. H., et al. 2006. Meeting consumer expectations through management in vineyard and winery: the choice of yeast for fermentation offers great potential to adjust the aroma of Sauvignon Blanc wine. *Austral. NZ Wine Ind.* 21:34-42.

Swiegers, J. H., and I. S. Pretorius. 2007. Modulation of volatile sulfur compounds by wine yeast. *Appl. Microbiol. Biotechnol.* 74:954-960.

Thibon, C., et al. 2011. 3-Sulfanylhexanol Precursor Biogenesis in Grapevine Cells: The Stimulating Effect of *Botrytis cinerea*. *J. Agric. Food Chem.* 59(4):1344-1351.

Thibon, C., et al. 2009. Impact of noble rot on the aroma precursor of 3-sulfanylhexanol content in *Vitis vinifera* L. cv Sauvignon blanc and Semillon grape juice. *Food Chem.* 114(4):1359-1364.

Tominaga, T., et al. 2000. Contribution of Volatile Thiols to the Aromas of White Wines Made from Several *Vitis vinifera* Grape Varieties. *American Journal of Enology and Viticulture.* 51(2):178-181.

Tominaga, T., et al. 1996. Identification of 3-mercaptohexanol acetate, compound having a powerful odor reminiscent of box-tree, involved in the aroma of Sauvignon wines. *Vitis.* 35:207-210.

Tominaga, T., et al. 1998a. Identification of new volatile thiols in the aroma of *Vitis vinifera* L. var. Sauvignon blanc wines. *Flavour and Fragrance Journal.* 13(3):159-162.

Tominaga, T., et al. 1995. Mise en évidence d'un S-conjugué de la cystéine, précurseurs d'arôme du Sauvignon. *J. Int. Sci. Vigne Vin*. 29:227-232.

Tominaga, T., et al. 1998b. A New Type of Flavor Precursors in *Vitis vinifera* L. cv. Sauvignon Blanc: S-Cysteine Conjugates. *J. Agric. Food Chem.* 46:5215-5219.

ADAPTING WINEMAKING TO WARM-CLIMATE CONDITIONS

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, España

fernando.zamora@urv.cat

Abstract

The impact of climate change on grape ripening, how it affects wine composition and quality, and how we can mitigate the impact by applying certain technical procedures in the winery are presented. Specifically, global warming provokes a growing imbalance between the primary and secondary metabolisms of the grapevines, causing grapes to quickly reach a very high sugar content, very low acidity and very high pH. This forces the grape harvest before the grapes have reached the correct maturity in the skins and seeds, which seriously affects the wine composition and quality.

This new scenario represents a challenge for wine industry, which must develop new strategies for better winemaking. Different possibilities will be discussed, including:

- Utilizing lees or inactive yeast to enrich the wine with polysaccharides;
- Applying techniques for the partial dealcoholization of wines, even the use of unripe grapes harvested during cluster thinning;
- Applying techniques to increase wine acidity and decrease pH.

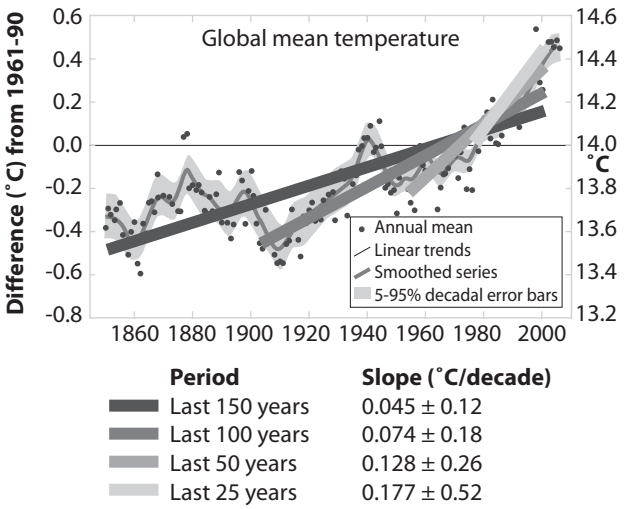
1. Introduction

Is climate change a reality? Is there enough scientific evidence to support this claim? We have all heard these questions more than once and surely we all have friends who are convinced that climate change is an invention of environmentalists and anti-globalization organizations. These arguments remind me of those used by American tobacco companies when they claimed there was insufficient evidence to prove tobacco causes cancer. Today, no one doubts the health problems generated by tobacco use. Similarly, it is impossible to affirm any argument that questions the reality of climate change.

The concept of climate change is nothing new. Many years ago it was described by some scientists who were then dismissed as alarmists. Today, everyone knows the consumption of fossil fuels is causing an increased concentration of carbon dioxide and other gases, which, by reflecting radiation, are causing a greenhouse effect (Crowley 2000, and Zamora 2005) responsible for the warming of the planet. The data are truly frightening. In 1958, the concentration of CO_2 was 315 ppm. Today it is 370 ppm, and in the best case scenario, it will be higher than 500 ppm by the end of the 21st century (Intergovernmental Panel on Climate Change).

Figure 1 shows how the temperature of the Earth has changed during last 150 years. This graph clearly shows that the planet's temperature is increasing, and this process is being accelerated during recent years as the consumption of fossil fuels increases.

Figure 1. Evolution of the average global temperature



2. Climate Change and Viticulture

But how is climate change impacting viticulture? Figure 2 synthesizes the major effects of climate change on the grape-ripening process.

Global warming causes grapes to store sugar and degrade acids faster than in normal conditions. Therefore, the grapes arrive at a very high potential alcoholic degree and pH level sooner than usual, provoking an earlier harvest. However, the grape skins, and especially the seeds, are still unripe. The increasing imbalance between industrial and phenolic maturity is a consequence of climate change. In such conditions, where grapes are not well-ripened, winemakers have a very difficult decision to make. If they carry out a short maceration, the wines will not have enough colour. But, if they carry out a long maceration the risk of extracting astringent, herbaceous and bitter tannins is very high.

3. Facing the Challenge

What should the oenologist do? There are only two possibilities: 1) Harvest when the alcoholic degree and/or pH are at the correct level and then adapt winemaking to the conditions of unripe grapes; or 2) Wait for complete maturity and harvest when grapes are well ripened and then apply techniques for decreasing alcoholic degree and pH.

In the first case, there are different strategies possible for winemaking with unripe grapes, but the most appropriate would be:

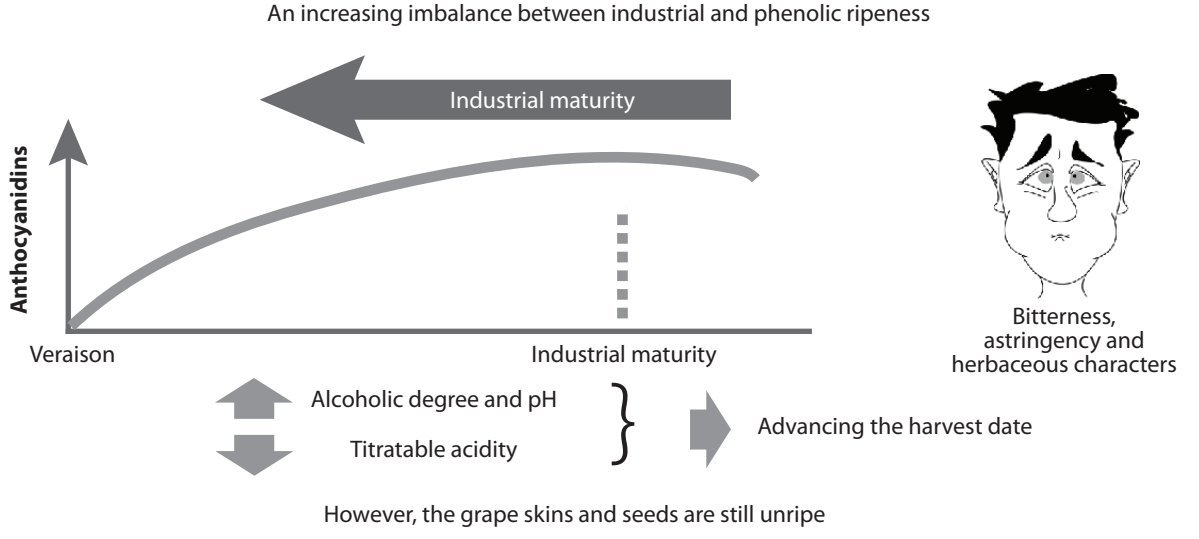
- Shortening the maceration length and simultaneously accelerating the anthocyanin extraction;
- Removing seeds by *délestage*;
- Applying appropriate micro-oxygenation or aging on oak barrels;
- Enriching the wine with polysaccharides.

The first three possibilties were developed in my presentation at the *XIX*^{es} *Entretiens Scientifiques Lallemand* (Margaux, May 9, 2007). For this reason, I will now develop the last point.

3.1 Enrich the wine with polysaccharides

The most usual technique for enriching wine in polysaccharides consists of aging the wine in contact with lees

Figure 2. Impact of global warming on grape ripening



(Zamora 2002). This procedure has several advantages, including:

- Yeast autolysis to increase mouthfeel;
- Oxidation protection;
- Smoothing the astringency;
- Colour stabilization;
- Reduced impact of the wood;
- Appearance of new flavours;
- Increase persistence.

But this technique also presents some serious drawbacks:

- Increased risk of *Brettanomyces*;
- Increased risk of reduction odours;
- Slower evolution of the wine.

Figure 3 illustrates how this technique affects wine composition and quality (Rodríguez et al. 2005).

As this graph shows, the presence of lees during the oak aging of the red wine results in wines with a significant decrease in their astringency and an increase in the mouthfeel perception. These effects are probably due to the enrichment of mannoproteins released by yeasts during autolysis (Klis et al. 2002). Mannoproteins are polymers of mannose β(1➞6) with branches of other monosaccharides and they contain less than 30% peptide fractions (Klis et al. 2002). Therefore, mannoproteins must be considered like polysaccharides.

However, this technique is laborious and, as mentioned, presents the risk of *Brettanomyces* and reduction taints (Pérez-Serradilla and Luque de Castro 2008). In order to avoid these problems, two new strategies have recently appeared. One is the employment of yeast strains with a higher capacity for producing polysaccharides (Gonzalez-Ramos and Gonzalez 2006). The other is the addition of inactive yeast specially grown to favour the release of polysaccharides (Guadalupe et al. 2007, and Rodriguez-Bencomo et al. 2010).

Figure 4 shows the polysaccharide composition of two Cabernet Sauvignon wines, one fermented with the control yeast (EC 1118) and the other with a yeast specially selected for its capacity to release higher amounts of polysaccharides (HPS).

Figure 3. Influence of the presence of lees during oak aging on the colour and phenolic compound composition of red wine

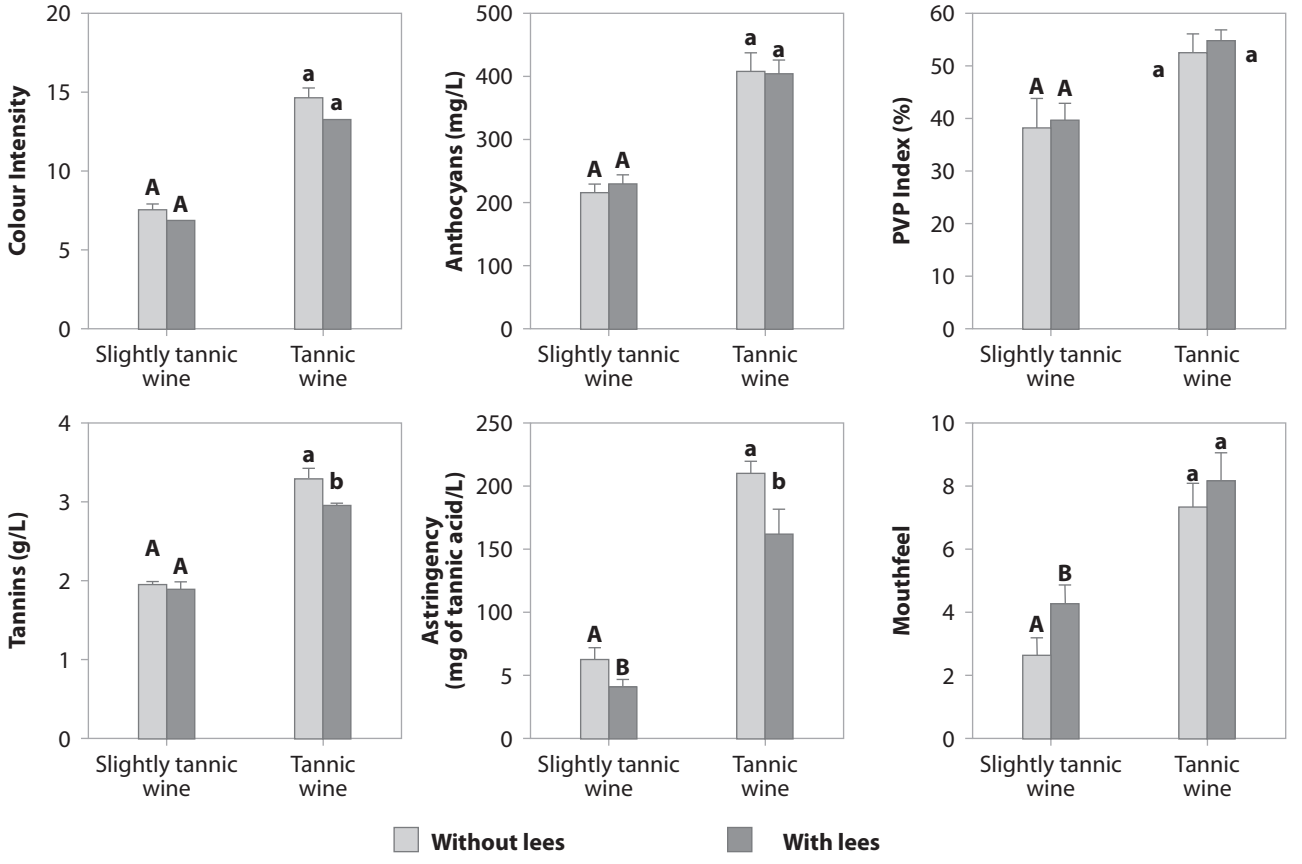
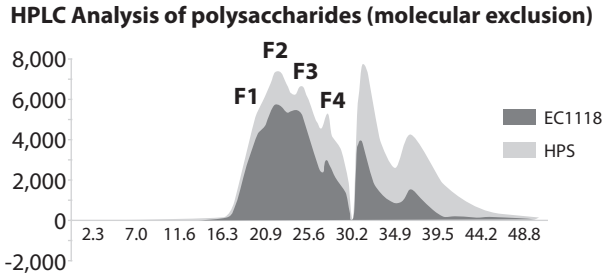


Figure 4. Cabernet Sauvignon wines made with two different yeasts



The results are very clear and indicate that the utilization of the HPS yeast strain generates wines with a 30% higher polysaccharide concentration. These data confirm that the HPS strain may be useful for enriching wine with polysaccharides.

Figure 5 shows the results corresponding to the maceration in a wine model solution of three different commercial inactive yeasts: Optired®, Booster® and Noblesse®.

These data confirm that all these inactive yeast products release polysaccharides at a relatively fast rate. Moreover, it seems that Optired® and Booster® release mainly polysaccharides of high molecular weight (10 to 1100 kDa), whereas Noblesse® releases greater amounts of the lower molecular weight fraction (<10 kDa). These solutions were later dialysed, lyophilized, dissolved in mineral water and tasted informally by a panel that stated the Optired® and Booster® products increased mouthfeel notes, whereas Noblesse® provided sweetness. Further sensory evaluations are needed to confirm these findings.

Simultaneously, different trials were carried out in real winemaking conditions using Cabernet Sauvignon grapes with and without the addition of the three different inactive yeast products. Table 1 shows the results obtained.

The results are also very clear and confirm that the utilization of all inactive yeasts is useful to enrich the wine in polysaccharides.

3.2 Decrease alcoholic degree and pH

As was mentioned, the other possibility is to wait for complete maturity and harvest when the grapes are well ripened and then apply techniques for decreasing alcoholic degree and pH.

The possible strategies to correct high alcoholic degree and high pH include:

- Selecting cultivars and clones which ripen later;

Figure 5. Macerations in model wine solutions

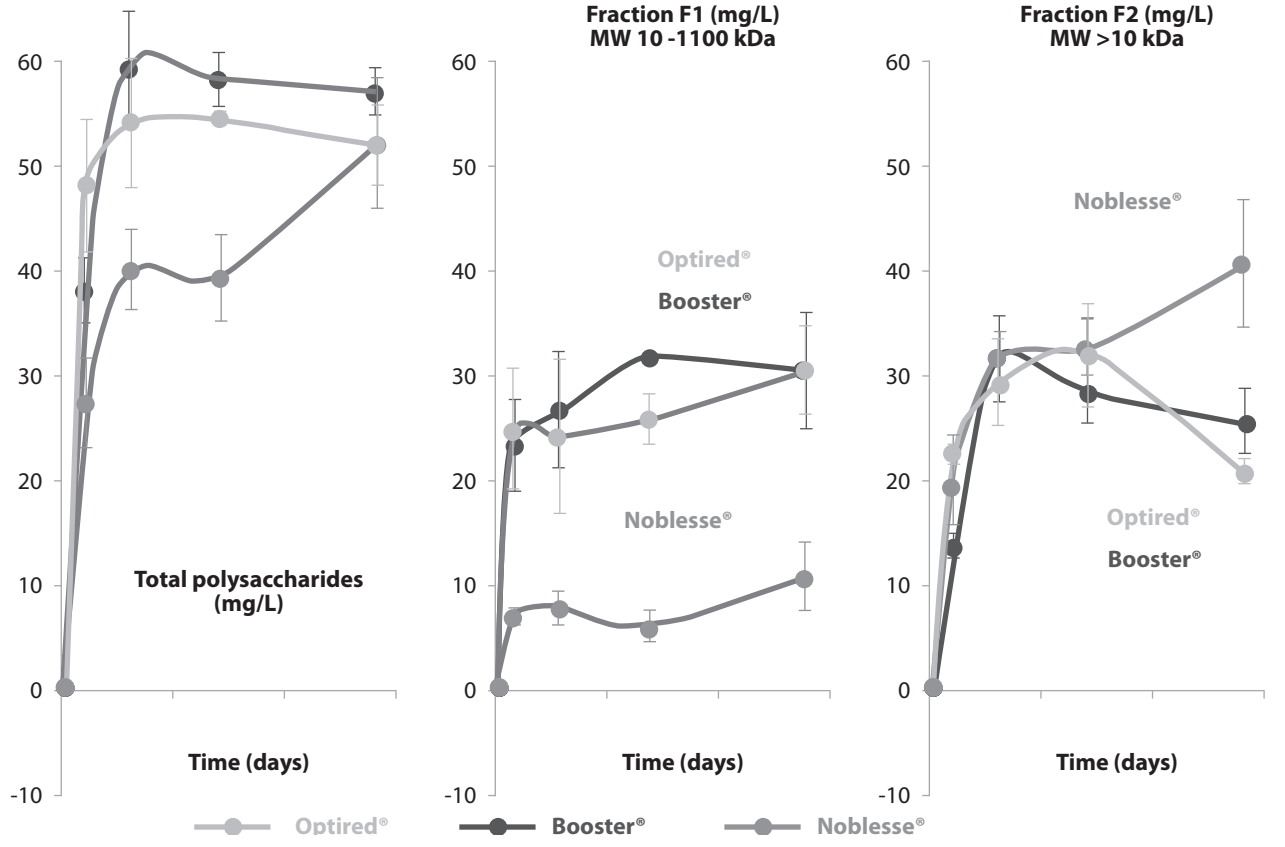


Table 1. Effect of the addition of inactive yeasts on the polysaccharide composition of wines

Fraction	Control	+ Noblesse	Δ (%)	+ Optired	Δ (%)	+ Booster	Δ (%)
F1 (144-1100 kDa)	132.6 ± 5.7	132.5 ± 0.5	-0.1	148.6 ± 4.4	11.9	142.6 ± 3.9	7.5
F2 (40-144 kDa)	187.6 ± 1.3	200.5 ± 20.7	6.9	254.3 ± 3.8	35.5	216.9 ± 3.9	15.6
F3 (6-40 kDa)	258.1 ± 13.9	282.6 ± 20.3	9.5	297.9 ± 6.1	15.4	293.4 ± 23.2	13.7
F4 (1-5 kDa)	95.7 ± 10.7	132.7 ± 18.1	38.7	96.3 ± 96.3	0.7	154.5 ± 27.4	61.5
Total	674.1 ± 46.3	748.3 ± 22.4	11.0	770.3 ± 2.4	14.3	807.5 ± 48.0	19.8

- Adapting culture techniques to this new situation;
- Selecting yeasts with lower yield of transformation sugar/ethanol;
- Decreasing the concentration of ethanol through inverse osmosis;
- Partial dealcoholizing wines with the spinning cone column;
- Lowering pH through cationic interchange or electrodialysis.

Although the first three points are interesting, we are not there yet. Scientific research in these fields is necessary to obtain enough knowledge. The last three points are now better known, but more work is necessary to improve their capacities and applications.

Figure 6 shows how inverse osmosis can be used to decrease the potential alcohol degree in wine.

This technique is now a reality and some businesses even rent the equipment to wineries. We carried out trials with two red wines from the AOC Priorat and Penedès. Table 2 (next page) shows the results obtained.

The results show that the only significant differences were found in alcohol content, while the other laboratory parameters remain unchanged. These wines were tasted by a trained tasting panel using the triangle test. In general, the tasters were able to distinguish between the control and the partially dealcoholized wines, but all tasters said it was much more difficult than they expected at the beginning of the test. Therefore, it seems that reverse osmosis can be a useful procedure to compensate for excess ethanol content.

Another possible strategy is the utilization of unripe grapes harvested during cluster thinning as a method for reducing the alcohol content and pH of wine. Grapes from cluster thinning were used to produce a very acidic

Figure 6. Partial dealcoholization of wine by reverse osmosis

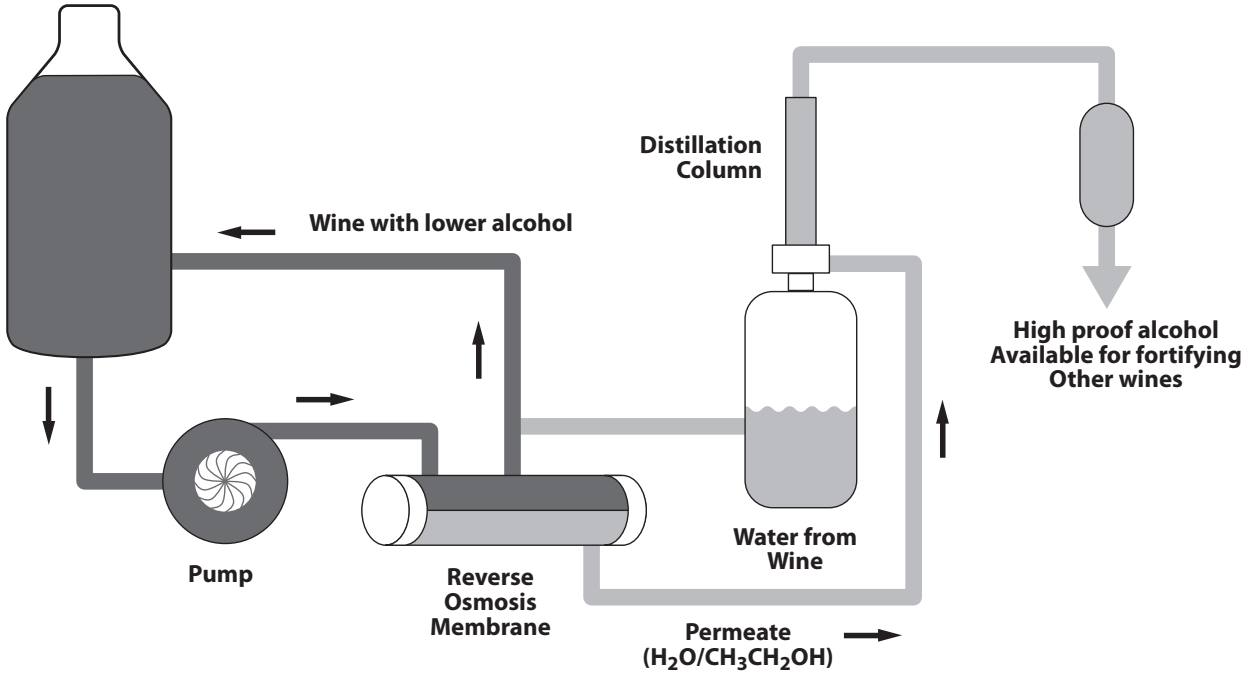


Table 2. Partial dealcoholization of wine by reverse osmosis

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
Titrable acidity (g/L)	4.8 ± 0.1 A	4.8 ± 0.2 A	4.9 ± 0.1 A	5.2 ± 0.1 A	5.2 ± 0.1 A	5.6 ± 0.1 B
Colour intensity	15.3 ± 1.5 A	15.6 ± 0.2 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 ± 0.2 A	68.3 ± 1.5 A	59.3 ± 1.2 A	60.0 ± 0.4 A	59.2 ± 0.5 A
Anthocyanins (mg/L)	567.0 ± 41.0 A	546.0 ± 19.0 A	574.0 ± 14.0 A	200.0 ± 13.0 A	206.0 ± 23.0 A	226.0 ± 11.0 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

low-alcohol wine. This wine was treated with high doses of charcoal and bentonite, and the resulting odourless and colourless wine was used to reduce the pH and ethanol content of wine produced from grapes that had reached complete phenolic maturity. Subsequently, grapes of the cultivar *Vitis vinifera* cv. Cabernet Sauvignon and Merlot grapes from the AOC Penedès, and Bobal from the AOC Utiel-Requeña, 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 used without any low ethanol wine. The other three tanks from the second harvest were used for the alcohol-reduction experiment, where a part of the total volume of the grape juice was removed and replaced with the same volume of low alcohol wine. Figure 7 shows the general parameters of the wines obtained.

As expected, all the wines that had part of their juice replaced by the low-alcohol wine had a lower ethanol content and lower 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 the control wines of the second harvest for the three cultivars.

Figures 8 and 9 show the results regarding the colour and phenolic compounds of the different wines.

Figure 7. Utilization of unripe grapes for decreasing ethanol content and pH: General parameters

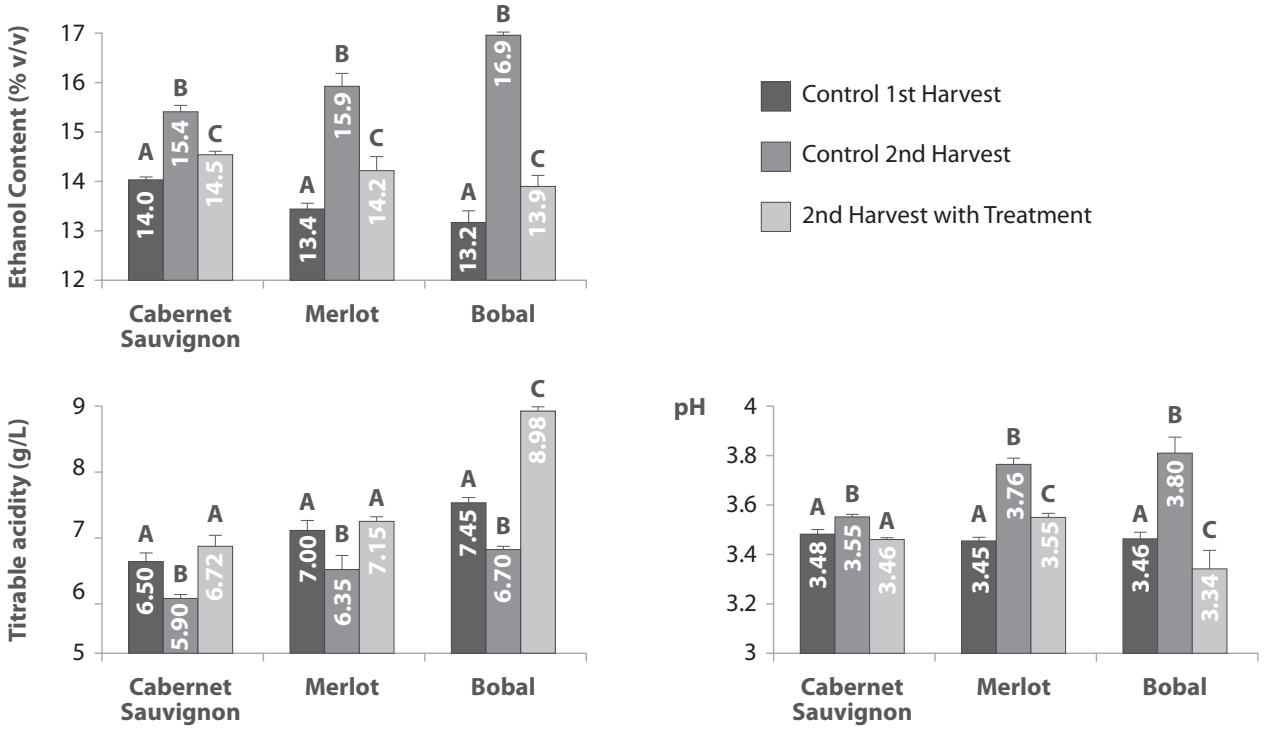


Figure 8. Utilization of unripe grapes for decreasing ethanol content and pH: Colour and phenolic compounds

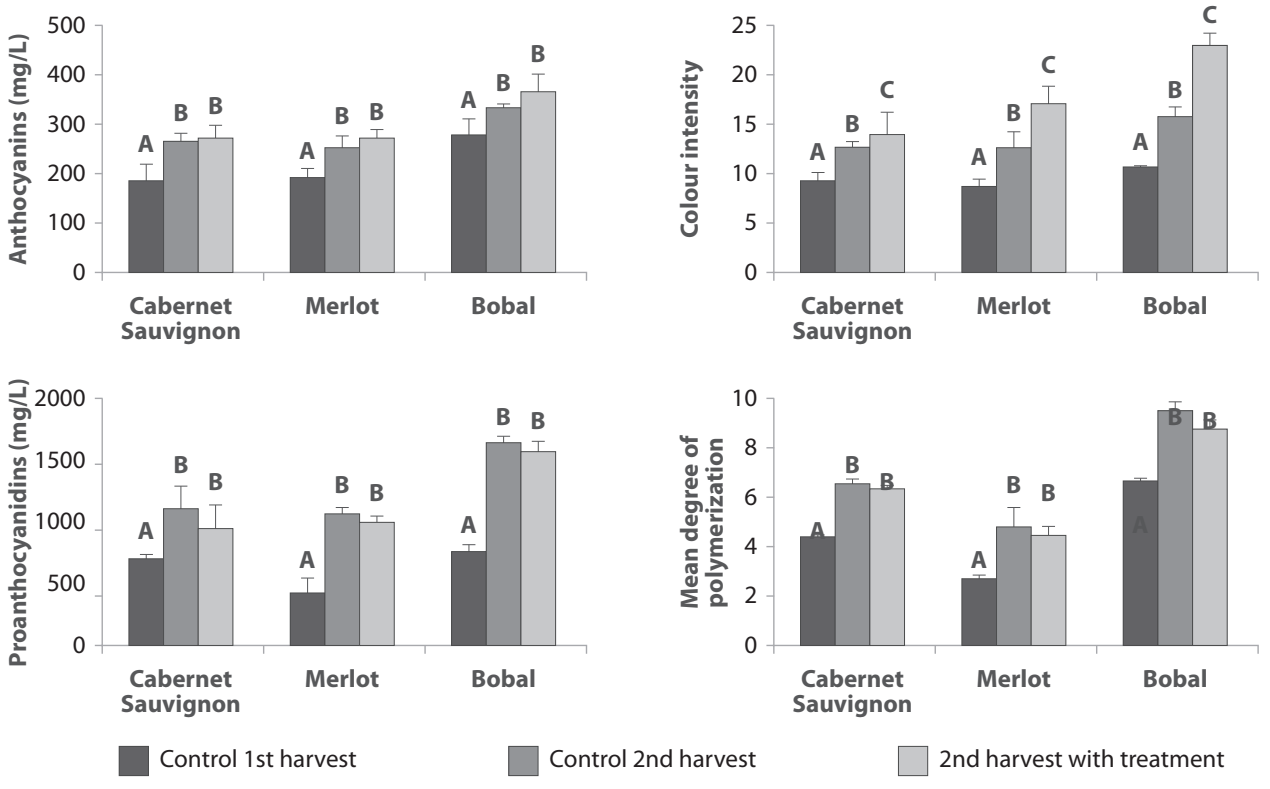
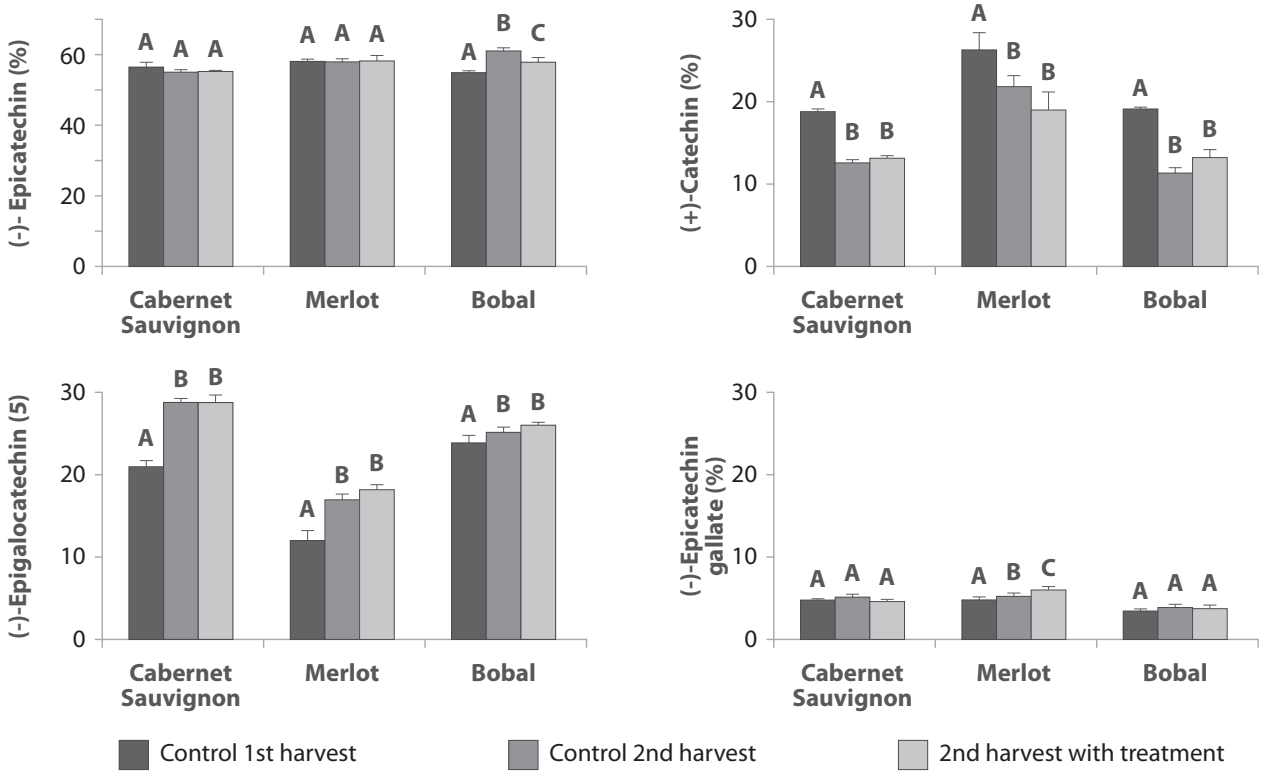


Figure 9. Utilization of unripe grapes for decreasing ethanol content and pH: Percentage of proanthocyanidin monomers



The results are very clear and confirm that all the wines of the second harvest always have higher concentrations of anthocyanins and proanthocyanidins than the wines of the first harvest. This data confirms the great influence of phenolic maturity on these parameters. On the other hand, all the treated wines have concentrations of anthocyanins and proanthocyanins similar to the control wine of the second harvest. Moreover, the structural composition of the treated wine was also identical to the non-treated wine of the second harvest. Furthermore, as the pH levels of the treated wines were significantly lower than those of the non-treated wines, the colour intensity of the treated wines was considerably higher than their controls.

4. Conclusions

It can be concluded that the proposed procedure may be useful for the partial reduction of alcohol content and the simultaneous decrease of the pH level 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.

Climate change is real and inevitable. Winemakers can only try to adapt to it and mitigate its effects. These techniques are available now and they can be very useful to compensate for the impact of global warming in wineries. But the real solution to global warming is on another level.

Fifty years ago, on April 12, 1961, Yuri Gagarin became the first human to travel into space. He was the first to see the Earth from the outside. Before this magnificent landscape, Gagarin said, "Orbiting Earth in the spaceship, I saw how beautiful our planet is. People, let us preserve and increase this beauty, not destroy it!"

Acknowledgements

We thank CDTI (Project CENIT Demeter) and Lallemand for their financial support.

References

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

Gonzalez-Ramos, D., and R. Gonzalez. 2006. Genetic determinants of the release of mannoproteins of enological interest by *Saccharomyces cerevisiae*. *Journal of Agricultural and Food Chemistry*. 54:9411-9416.

Guadalupe, Z., A. Palacios, and B. Ayestaran. 2007. Maceration enzymes and mannoproteins: a possible strategy to increase colloidal stability and color extraction in red wines. *Journal of Agricultural and Food Chemistry*. 55:4854-4862.

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

Klis, F. M., P. Mol, K. Hellingwerf, and S. Brul. 2002. Dynamics of cell wall structure in *Saccharomyces cerevisiae*. *FEMS Microbiological Reviews*. 26:239-256.

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

Pérez-Serradilla, J. A., and M. D. Luque de Castro. 2008. Role of lees in wine production: A review. *Food Chemistry.* 111:447-456.

Rodríguez, M., J. Lezáun, R. Canals, M. C. Llaudy, J. M. Canals, and F. Zamora. 2005. Influence of the presence of the lees during oak ageing on colour and phenolic compounds composition of red wine. *Food Science and Technology International*. 11:289-295.

Rodriguez-Bencomo, J. J., M. Ortega-Heras, and S. Perez-Magarino. 2010. Effect of alternative techniques to ageing on lees and use of non-toasted oak chips in alcoholic fermentation on the aromatic composition of red wine. *European Food Research and Technology.* 230:485-496.

Zamora, F. 2002. La crianza del vino tinto sobre lías; Una nueva tendencia. *Enólogos*. 19:24-29.

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

INFLUENCE OF MALOLACTIC FERMENTATION ON THE FRUITY CHARACTERS OF RED WINE: BRINGING CHEMISTRY AND SENSORY SCIENCE TOGETHER

Eveline BARTOWSKY[1], Peter COSTELLO[1], Sibylle KRIEGER-WEBER[2], Andrew MARKIDES[3], Leigh FRANCIS[1] and Brooke TRAVIS[1]

[1] The Australian Wine Research Institute, P.O. Box 197, Glen Osmond SA 5064, Australia

[2] Lallemand, In den Seiten 53, Korntal-Münchingen D-70825, Germany

[3] Lallemand Australia Pty Ltd, P.O. Box 327, Brooklyn Park SA 5032, Australia

eveline.bartowsky@awri.com.au

Abstract

The effects of malolactic fermentation (MLF) on the wine aroma profile and chemical properties of Australian Cabernet Sauvignon wine over three vintages were explored by inoculation with up to seven different selected malolactic starter culture preparations of *Oenococcus oeni*. The time required for each malolactic culture to complete MLF varied from 12 to 80 days and was dependent on the strain, Cabernet Sauvignon style (lighter to more complex) and wine alcohol concentration (13.5 to15.5% alcohol v/v). The sensory properties of the wines were investigated by descriptive sensory analysis, using a trained panel. In support of the sensory data, chemical analysis of each wine was conducted, including the determination of volatile aroma compounds and organic acids. Significant sensory and compositional differences occurred as a result of the different MLF treatments, including differences in intensity of perceived fruit flavour. Strain-dependent changes in the volatile aroma compounds, including the ester profile, were observed. Increases in total berry fruit esters correlate with increases in fruit-related sensory attributes. These trends were observed over the three vintages in Cabernet Sauvignon fruit sourced from the same vineyard.

1. Introduction

While wine colour is important, the aroma and flavour leave the greatest impression of a glass of wine. Compounds that contribute to the sensory experience of wine originate from the grape and microbial metabolism during winemaking. Yeasts are responsible for the conversion of grape sugars to alcohol and play a major role in the aroma and flavour of wine. However, bacteria are not only responsible for malolactic fermentation (MLF), they contribute to the final sensory experience of wine (Swiegers et al. 2005).

MLF is an important step of the vinification process of red wines, several white wine styles and sparkling base wines. The process of MLF is well understood and its main use is to reduce wine acidity. While numerous species of the lactic acid bacteria family are able to conduct MLF, *Oenococcus oeni* is the preferred species because of its ability to tolerate the high acidity and ethanol concentrations, sulphur dioxide and the low nutrient content of wine. *Lactobacillus* and *Pediococcus* species are able to conduct MLF, but are usually considered undesirable in wine. Recently, *Lb. plantarum* has been reconsidered as an option for MLF (du Toit et al. 2011). In addition to reducing wine acidity, MLF also provides microbial stability and offers the opportunity to modify the sensory properties of the wine.

During MLF, *O. oeni* metabolism modifies the chemical composition of wine and this translates into changes in the appearance, aroma and palate of the wine (Swiegers et al. 2005). *O. oeni* is able to interact with most chemical compounds present in wine, including carbohydrates, polyols, proteins, amino acids, phenolics, organic acids and glycosides (Bartowsky 2005). Many of these alterations in wine composition are strain dependent. However, winemaking techniques can also be used to manipulate the wine composition.

Reduction in wine acidity is the crucial feature of MLF, due to the degradation of malic acid resulting in a slight increase of wine pH by 0.1 to 0.3 pH units. This is sensorially observed as a softening of the wine. Medium- to full-bodied red wines are often described as having blackcurrant, dark cherry, raspberry and plum aromas (Iland et al. 2009). A challenge faced by winemakers is to accentuate the fruitiness of red wine and to keep the green and herbaceous characters at bay.

Our aim in these studies was to increase the understanding of the role of the *O. oeni* and *Lb. plantarum* strains of lactic bacteria to influence the fruity and berry sensory characters of Cabernet Sauvignon wines through changes in the volatile fermentation-derived compound composition.

2. Materials and Methods

2.1 Winemaking (overview)

Cabernet Sauvignon grapes (sourced from different South Australian viticultural regions) were either handpicked or machine harvested at commercial maturity, and fermented with *Saccharomyces cerevisiae* yeast (L2056 [2006] or DV10 [2008-2010]). Malolactic fermentation was conducted in replicate at 18 to 25 L or 1 L volumes (stainless steel kegs or glass vessels) with different malolactic (ML) strains (seven *O. oeni* strains and one *Lb. plantarum* strain). Following MLF, the wines were stabilized, sulphur dioxide added at a rate of 30 to 35 mg/L, filtered and bottled (375 mL glass bottles) with screw-cap closures. All bottled wines were stored at 15°C until analysis.

2.2 Wine analysis

Malic acid concentrations were determined enzymatically (Roche Boehringer Mannheim enzyme kit obtained from Arrow Scientific). MLF was deemed complete at <0.1 g/L malic acid. Volatile fermentation-derived compounds were determined by gas chromatography-mass spectrometry (GC/MS) at Metabolomics Australia (Siebert et al. 2005).

2.3 Wine sensory analysis (overview)

Wines were initially assessed in a bench tasting with six to eight experienced tasters, to ensure that the wines were fault-free. Descriptive sensory analysis was undertaken with 10 assessors, all of whom had extensive experience in sensory descriptive analysis. Aroma and flavour attributes to be rated in the wines were generated by the panel using a consensus approach. Wine samples were presented in a randomized design, in coded glasses, and assessed three times. Data was collected using Fizz 2.46A (Biosystems, France) and statistical analysis was carried out using JMP 5.0.1a (SAS Institute, Cary, NC).

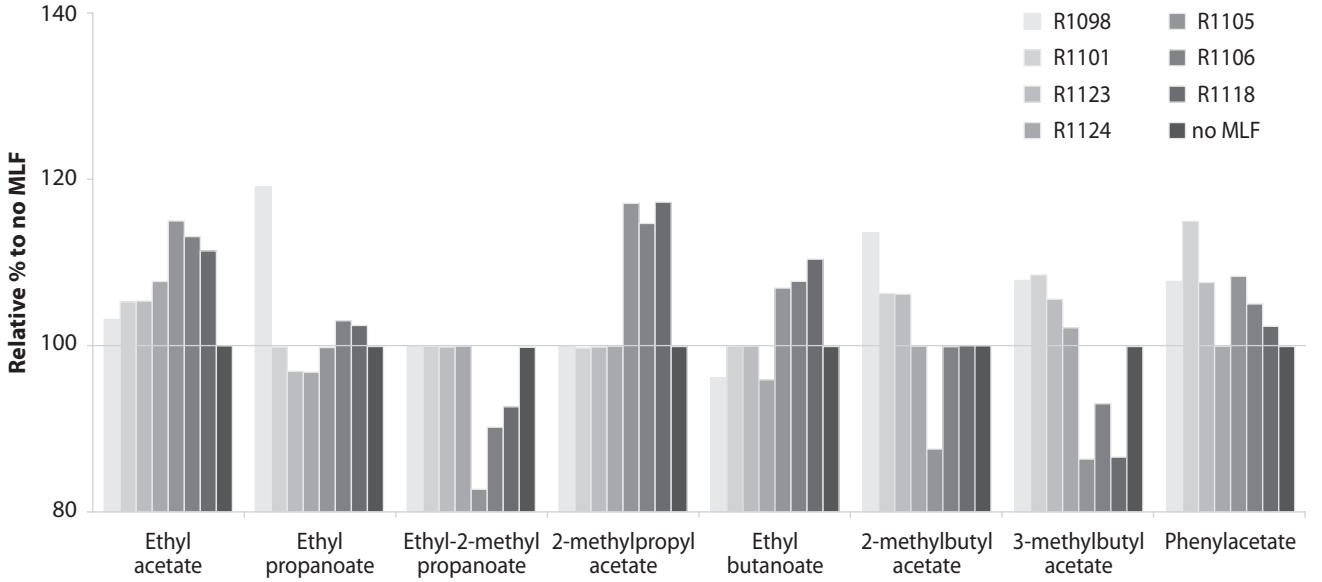
3. Results and Discussion

Wine chemical composition plays an important role in the growth and metabolism of *O. oeni* during malolactic fermentation. Recent studies have highlighted the ability of *O. oeni* to influence various groups of volatile fermentation-derived compounds (esters, acetates, acids and higher alcohols), organic acids and amino acids (Terrade and de Orduña 2009a, and Terrade and de Orduña 2009b). Ethyl esters can impart various fruity characters (berry, pineapple and banana) to wine (Siebert et al. 2005). Several of our studies on Chardonnay and Cabernet Sauvignon wines have shown that ethyl esters tend to increase, and there is a general decrease of acetate esters following MLF (Abrahamse and Bartowsky 2011, and Bartowsky et al. 2008). The variability of *O. oeni* strains to influence wine ester concentrations during MLF are shown in figure 1. As expected, ethyl acetate concentrations increase regardless of the *O. oeni* strain, but to a different extent. Several compounds show increases or decreases dependent upon the *O. oeni* strain (e.g., 2-methylpropyl acetate, ethyl butanoate and ethyl proponate). Differences in *O. oeni* metabolism will be a reflection of expression variations within the *O. oeni* genome.

Recent work has suggested that red wine berry fruit aroma is a complex interaction between fruity esters, norisoprenoids, dimethyl sulphide, ethanol and other components (Escudero et al. 2007, and Pineau et al. 2009). Pineau and co-workers have proposed a group of esters that specifically contributes to red berry aroma and a separate group that gives rise to blackberry aroma, and they suggest that these esters could be used to gauge the type of berry fruit aroma in a red wine.

Studies in Australian Cabernet Sauvignon were undertaken to determine *O. oeni* strain variation in synthesis of esters that contribute to fruity berry aromas, as well as the importance of pre-MLF wine composition and viticultural region (i.e., the source of Cabernet Sauvignon grapes).

Figure 1. Relative changes in various fruity esters by seven selected *Oenococcus oeni* strains to no malolactic fermentation. MLF was performed in Cabernet Sauvignon (Adelaide Hills, 2008)



The group of esters proposed by Pineau and colleagues was used as a chemical parameter to indicate the fruity sensory characters of the wines.

3.1 Effect of wine pH on malolactic fermentation

To study the role that *O. oeni* strains play in enhancing the fruity characters in red wine, we investigated the influence of wine pH on the ability of *O. oeni* to produce volatile fermentation-derived compounds during MLF. Wine pH is one of the three essential wine chemical parameters (i.e., pH, alcohol and SO_2) which greatly influence the growth and subsequent MLF rate of *O. oeni* (Henick-Kling 1993). To examine the effect of wine pH on the kinetics of MLF, chemical composition and sensory attributes, Cabernet Sauvignon wine was divided into two lots, where one lot was adjusted to pH 3.3 and the second to pH 3.7. These wines were inoculated in triplicate with three *O. oeni* strains; malic acid metabolism and cell viability is shown in figure 2. As would be predicted, wines at pH 3.7 supported the growth of *O. oeni* and a rapid degradation of malic acid, which completed within three weeks, whereas the wines at pH 3.3 took approximately 12 weeks to complete MLF. The *O. oeni* population viability was closely linked to the rate of MLF. In the wines with pre MLF pH 3.3, the *O. oeni* population was maintained at ~5 x 10^5 CFU/mL, which was sufficient to sustain a slow malic acid degradation rate. This confirmed that pH was a crucial factor in slowing *O. oeni* growth and MLF rate in this Cabernet Sauvignon wine.

Figure 2. Malic acid metabolism and cell viability of three *Oenococcus oeni* strains during malolactic fermentation of Cabernet Sauvignon (Clare Valley, South Australia 2006; 14.7% alcohol v/v) conducted at either wine pH 3.3 or 3.7

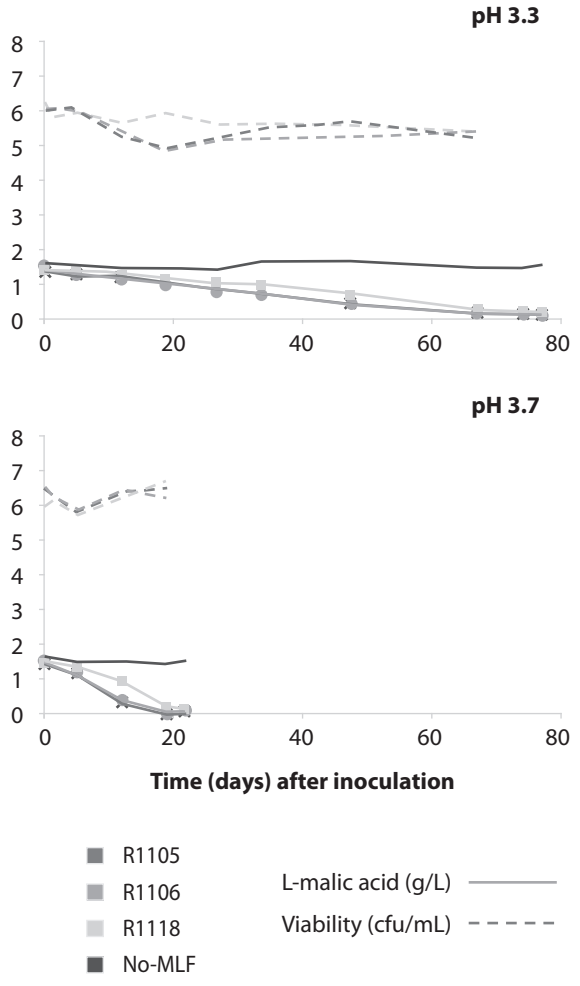
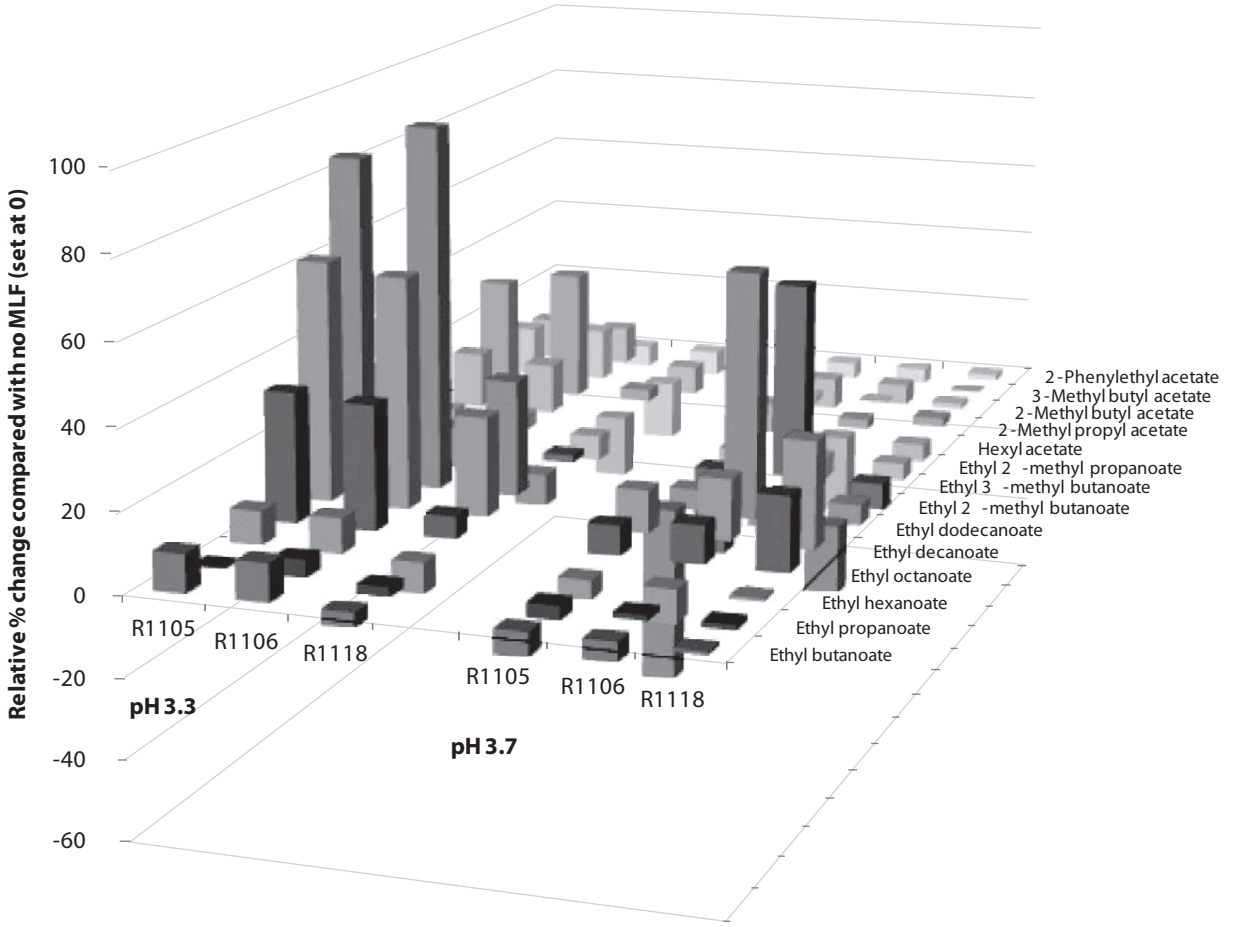


Figure 3. Volatile fermentation-derived compounds of Cabernet Sauvignon wines (Clare Valley, South Australia, 2006; pre-MLF wine pH 3.3 or 3.7) after malolactic fermentation by three *Oenococcus oeni* strains. Changes in concentration are shown as relative percentage change to no MLF (set at 0)



The volatile fermentation-derived compounds of these eight wines were determined and changes relative to wines that did not go through MLF are shown in figure 3. Pre-MLF wine pH influenced the concentration of the fermentation-derived compounds; the lower pre-MLF wine pH (3.3) tended to have higher concentration of these compounds. Ethyl esters (C4-C8) had the highest concentrations after MLF in wines conducted at pH 3.3. *O. oeni* strains varied in the production of the different compounds, with O. *oeni* strain R1118 exhibiting the lowest concentration in this Cabernet Sauvignon wine, irrespective of pre-MLF wine pH.

A formal sensory descriptive analysis of these nine Cabernet Sauvignon wines was undertaken. The pre-MLF wine pH was an important factor in sensory differences between the wines, particularly fruit-related descriptors (figure 4). Wines which went through MLF at pH 3.3 were described has having more fruity characters and higher raspberry aroma, especially with *O. oeni* strains R1105 and R1106.

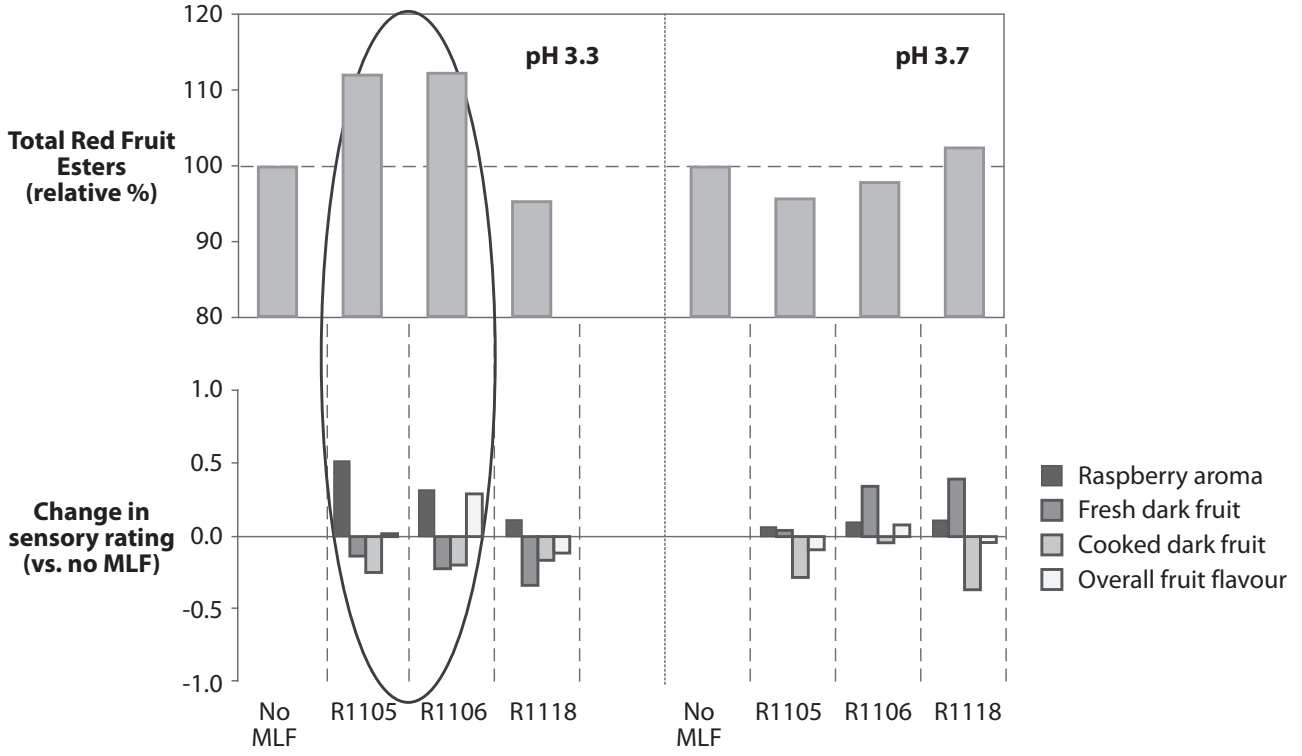
3.2 Linking volatile fermentation-derived compounds with sensory attributes: red fruity characters

The metabolism of *O. oeni* strains at different wine pH levels clearly influences their ability to produce the ethyl esters which contribute to red fruit berry aromas in Cabernet Sauvignon wines (figure 4). The total red fruit ester concentration was much higher for the pre-MLF wine pH 3.3 compared to the non-MLF wine. Moreover, at pre-MLF wine pH 3.7, the concentration of total red fruit esters decreased relative to the no-MLF wines. *O. oeni* strains R1105 and R1106 both had elevated concentrations of volatile fermentation-derived compounds, particularly those which have been reported to contribute to red fruit character (Pineau et al. 2009). This clearly demonstrates the link between increases of specific ethyl esters and consequent increase in sensory perception of red fruit in these Cabernet Sauvignon wines following MLF with specific *O. oeni* strains.

3.3 *Oenococcus oeni* strain performance in Cabernet Sauvignon wine from one vineyard over several vintages and vineyards from different viticultural regions

We were interested in investigating whether *O. oeni* strains behave similarly during MLF in Cabernet Sauvi-

Figure 4. Total red fruit esters and sensory attribute rating scores expressed as relative percentage change from no MLF for Cabernet Sauvignon wines (Clare Valley, South Australia, 2006) after malolactic fermentation induced with three *Oenococcus oeni* strains



gnon wine: 1) from fruit sourced from the same vineyard over several vintages, and 2) from fruit sourced from different viticultural regions.

3.4 Cabernet Sauvignon from one vineyard over several vintages

To investigate vintage variations on MLF, we used Cabernet Sauvignon grapes from a vineyard in Clare Valley, South Australia, over three vintages (2006, 2008 and 2009). The 2006 wine had higher alcohol content compared to the 2008 and 2009 wines (14.7%, 13.9% and 14.1%, respectively). The MLF trial in 2006 was conducted at pH 3.3, whereas MLF in wines from 2008 and 2009 was conducted at pH 3.45. MLF was induced with three *O. oeni* strains (R1105, R1106 and R1118), and completed within 80 days (2006), 25 days (2008) and 25 to 45 days (2009).

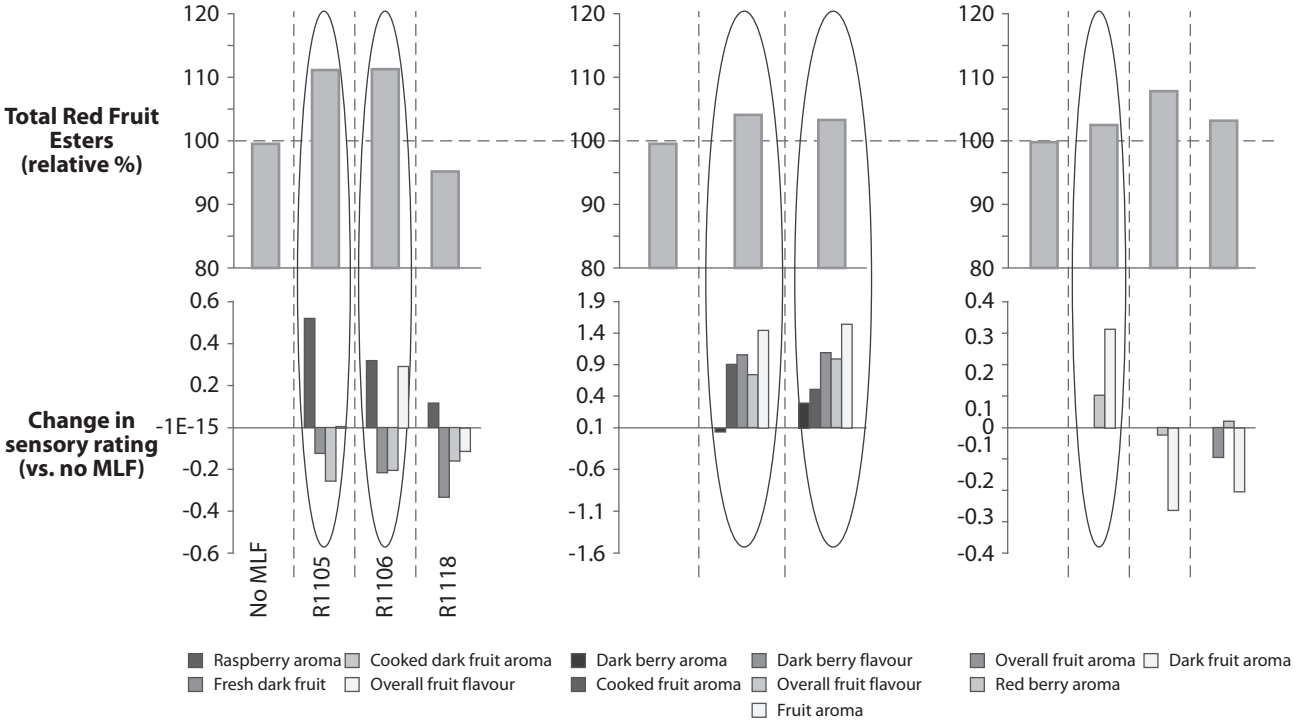
Over the three vintages, *O. oeni* strains R1105 and R1106 consistently produced wines with increased concentrations of volatile fermentation-derived compounds which relate to the red fruit aromas of Cabernet Sauvignon wines (figure 5, next page). These Cabernet Sauvignon wines were also described as having higher dark fruit and red berry aromas, increased overall fruit flavour and fruit aftertaste. From this study and other studies (Krieger-Weber personal communication, Bartowsky et al. 2008, and Schmid et al. 2007), *O. oeni* strain R1105 consistently produces red wines with enhanced red fruity sensory characters. Total red fruit esters also were produced at higher concentrations in Cabernet Sauvignon wines undergoing MLF with *O. oeni* strains R1105 and R1106. The sum of esters proposed by Pineau (2009) which contribute to red berry aroma appears to be a good gauge of fruit aroma in Cabernet Sauvignon wines.

3.5 Cabernet Sauvignon sourced from different viticultural regions

To investigate the viticultural region's influence on MLF performance and *O. oeni* ester production, Cabernet Sauvignon fruit was sourced from four different viticultural regions in South Australia in 2008 (Clare Valley, Langhorne Creek, Padthaway and Adelaide Hills). Wines produced had similar alcohol content (13.9%, 14.7%, 14.4% and 14.0%, respectively) and were adjusted to pH 3.45. MLF was induced with *O. oeni* strains R1105, R1106 and R1118. Strains R1105 and R1106 completely metabolized malic acid within 20 to 25 days, whereas strain R1118 needed slightly longer to complete MLF (25 to 37 days); it did not complete MLF in the Clare Valley wine.

The three *O. oeni* strains produced fruit-related esters in a similar mode in all four Cabernet Sauvignon wines (figure 6). Red berry, blackberry and total fruity esters had the

Figure 5. Comparison of Cabernet Sauvignon wines after malolactic fermentation with three *Oenococcus oeni* strains; total red fruit esters and sensory descriptors. Fruit was sourced from the same vineyard over the three vintages (Clare Valley, South Australia)



lowest concentrations in the Langhorne Creek Cabernet Sauvignon wines. There were variations in the concentrations of the esters produced, which most likely reflect differences in precursor concentrations dependent on viticultural region differences.

3.6 *Oenococcus oeni* and *Lactobacillus plantarum* strains and fruity ester production

Previous studies focused on the performance of three *O. oeni* strains and their ability to modulate red fruit characters in Cabernet Sauvignon wines. In the 2010 vintage we expanded the *O. oeni* strains to investigate three other strains and one *Lb. plantarum* strain using Cabernet Sauvignon fruit sourced from two South Australian viticultural regions, Clare Valley and Coonawarra.

The two Cabernet Sauvignon wines (Clare Valley, 14.1% alcohol v/v and Coonawarra, 13.7% alcohol v/v) were adjusted to pH 3.4 and pH 3.5 for inoculation with *O. oeni* and *Lb. plantarum* strains, respectively. All bacterial strains completed MLF within 20 days (Coonawarra) or 35 to 40 days (Clare Valley). The total fruity esters for each of the Cabernet Sauvignon wines are shown in figure 7. The *Lb. plantarum* strain produced sound wines with increased fruity aroma and flavour characters, which correlated well with the production of fruit-related esters. Strain-dependent variation in ester production was observed with the *O. oeni* strains, and some differences were observed in the fruit aroma and flavour descriptors.

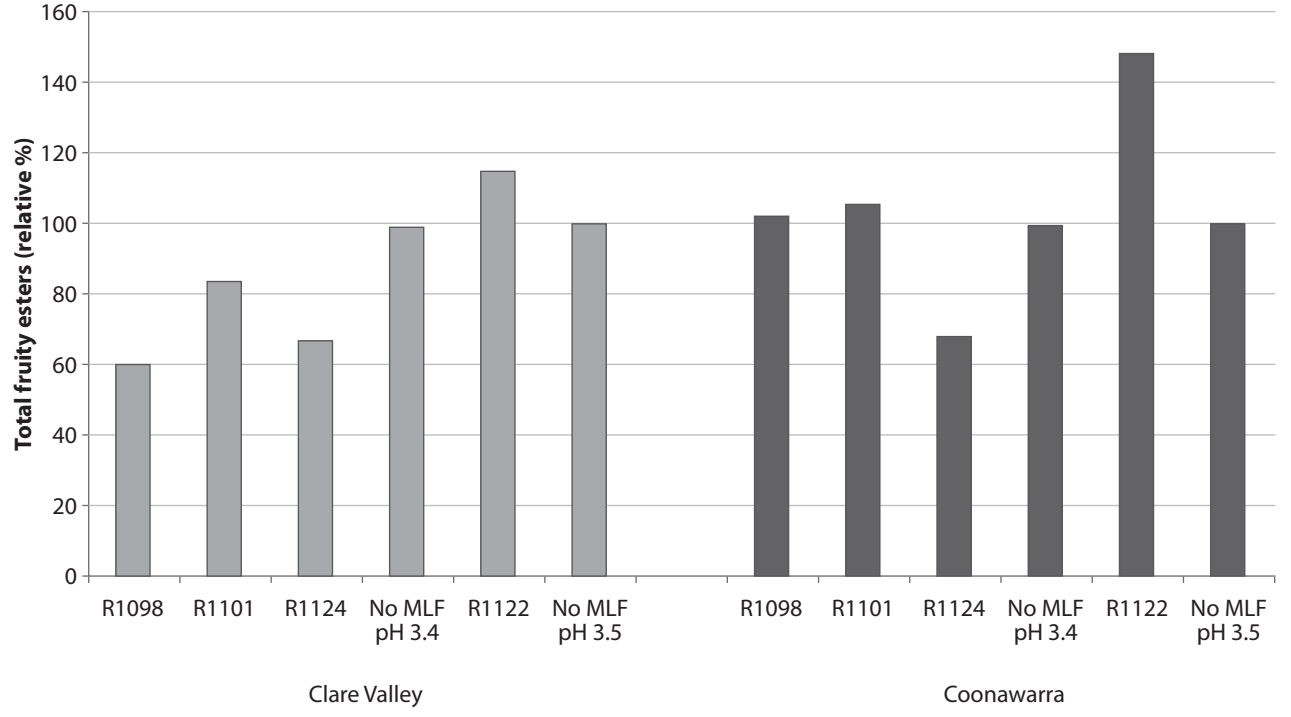
4. Conclusions

In these series of studies on Cabernet Sauvignon wines that underwent malolactic fermentation with different *Oenococcus oeni* and *Lactobacillus plantarum* strains, there was generally an increase in total fruity berry esters, and where this occurred an increase in sensory ratings of the fruity- and berry-related terms was found, thus confirming that ester concentrations are a good indicator of potential fruity and berry aromas in Cabernet Sauvignon wines, and that different strains can have a marked effect on these compounds.

Wine chemical composition plays an important role in the metabolism of *O. oeni* during MLF. Preference by *O. oeni* to metabolize either organic acid or sugars has been shown to be wine pH dependent; at lower wine pH (below 3.5), organic acids are metabolized in preference to sugars, and conversely, at higher wine pH (over 3.7), *O. oeni* will preferentially metabolize sugars, which may lead to an increase in volatile acidity (Bartowsky 2005, Krieger et al. 2000, and Ribéreau-Gayon et al. 2006). Other changes in wine composition at different wine pH values (3.3 compared with 3.7) were also demonstrated, specifically the volatile fermentation-derived compounds, including ethyl esters. In Cabernet Sauvignon, at lower wine pH there were greater increases in total fruity esters compared with MLF conducted at wine pH 3.7. These dif-

Figure 6. Sum of esters contributing to fruity characters (red berry, blackberry and total fruity esters), expressed as relative percentage to no MLF (100%), in Cabernet Sauvignon wines produced from four South Australian viticultural regions (vintage 2008) after malolactic fermentation induced by three *Oenococcus oeni* strains

Figure 7. Total fruity esters, relative to no MLF, in Cabernet Sauvignon wines after malolactic fermentation conducted by three *Oenococcus oeni* strains (R1098, R1101, R1124) and *Lactobacillus plantarum* strain (R1122). Fruit was sourced from two South Australian viticultural regions (Clare Valley and Coonawarra, 2010)



ferences in total fruity esters were reflected in higher sensory ratings for fruity and berry descriptors.

MLF conducted in Cabernet Sauvignon fruit sourced from the same vineyard over three vintages (2006, 2008 and 2009) with three *O. oeni* strains, showed that the ester production and fruity sensory attribute rating differences for the three strains behaved similarly over the vintages. This was confirmed in the 2010 vintage, where all malolactic bacteria strains modulated the fruity characters in the wine, and the *Lb. plantarum* strain exhibited an increase in fruity esters and increased sensory rating of dark fruit, confirming that utilizing selected *Lb. plantarum* strains can produce fault-free wines.

These comprehensive studies in Cabernet Sauvignon wines have together shown that changes in ester concentration following MLF are consistently observed and are dependent upon numerous factors, including the *O. oeni* strain, wine composition, vintage and viticultural region. Increases in ester concentration are reflected in enhanced fruity and berry aroma, and in the flavour descriptors applied to the Cabernet Sauvignon wines.

The opportunity to use MLF to alter the sensory properties of wine is increasingly becoming a pertinent component influencing the winemaker's decision to conduct MLF in red and white wines. Bacterial metabolism during MLF in wine influences a vast pool of secondary metabolites, including organic acids, diacetyl, fermentation-derived volatile compounds (esters, acetates, acids and higher alcohols) and oak compounds (when wood is used). These studies highlight the importance of *O. oeni* strain selection and the wine conditions under which MLF is conducted to enhance the fruity and berry characters of red wine. Thus, MLF can be a powerful winemaking tool, not only to reduce wine acidity, but to positively influence the aroma and flavour profile of the wine as well.

References

Abrahamse, C. E., and E. J. Bartowsky. 2011. Timing of malolactic fermentation inoculation in Shiraz grape must and wine: Influence on chemical composition. *World Journal of Microbiology & Biotechnology*. DOI:10.1007/s11274-011-0814-3.

Bartowsky, E., P. Costello, and J. McCarthy. 2008. MLF – adding an 'extra dimension' to wine flavour and quality. *Australian & New Zealand Grapegrower& Winemaker*. 533a:60-65.

Bartowsky, E. J. 2005. *Oenococcus oeni* and malolactic fermentation – moving into the molecular arena. *Australian Journal of Grape and Wine Research*. 11:174-187.

duToit, M., L. Engelbrecht, E. Lerm, and S. Krieger-Weber. 2011. *Lactobacillus*: the next generation of malolactic fermentation starter cultures – an overview. *Food Bioprocess Technology*. 4,6:876-906(31).

Escudero, A., E. Campo, L. Farina, J. Cacho, and V. Ferreira. 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.

Henick-Kling, T. 1993. Malolactic fermentation. In: G. H. Fleet (Ed.), *Wine Microbiology and Biotechnology*. Harwood Academic Publisher, Amsterdam, 289-326.

Iland, P., P. Gago, A. Caillard, and P. Dry. 2009. *A Taste of the World of Wine*. Patrick Iland Wine Promotions Pty Ltd, Campbelltown, South Australia.

Krieger, S. A., E. Lemperle, and M. Ernst. 2000. Management of malolactic fermentation with regard to flavor modification in wine. *Proceedings of the 5th International Symposium on Cool Climate Viticulture and Oenology*, Melbourne, Australia.

Pineau, B., J. C. Barbe, C. Van Leeuwen, and D. Dubourdieu. 2009. Examples of Perceptive Interactions Involved in Specific "Red-" and "Blackberry" Aromas in Red Wines. *Journal of Agricultural and Food Chemistry*. 57:3702-3708.

Ribéreau-Gayon, J., D. Dubourdieu, B. Donèche, and A. Lonvaud. 2006. Lactic Acid Bacteria. In: J. Ribéreau-Gayon, D. Dubourdieu, B. Donèche and A. Lonvaud (Eds.), *Handbook of Enology: The microbiology of wine and vinifications*. Second ed. John Wiley & Sons, Ltd. 115-181.

Schmid, F., Y. Li, B. Liebich, J. Culbert, C. Day, and V. Jiranek. 2007. Evaluation of red wine made on a small scale utilizing frozen must. *Journal of Agricultural and Food Chemistry*. 55:7156-7161.

Siebert, T. E., H. E. Smythe, D. L. Capone, C. Neuwöhner, K. H. Pardon, G. K. Skouroumounis, M. J. Herderich, M. A. Sefton, and A. P. Pollnitz. 2005. Stable isotope dilution analysis of wine fermentation products by HS=SPME-GC-MS. *Analytical and Bioanalytical Chemistry*. 381:937-947.

Swiegers, J. H., E. J. Bartowsky, P. A. Henschke, and I. S. Pretorius. 2005. Yeast and bacterial modulation of wine aroma and flavour. *Australian Journal of Grape and Wine Research*. 11:139-173.

Terrade, N., and R. M. de Orduña. 2009a. Arginine and citrulline do not stimulate growth of two *Oenococcus oeni* strains in wine. *FEMS Microbiology Letters*. 290:98-104.

Terrade, N., and R. M. de Orduña. 2009b. Determination of the essential nutrient requirements of wine-related bacteria from the genera *Oenococcus* and *Lactobacillus*. *International Journal of Food Microbiology*. 133:8-13.

CONTROLLING *BRETTANOMYCES*

Charles G. EDWARDS

School of Food Science, Washington State University, Pullman, WA 99163-6376, U.S.A.

edwardsc@wsu.edu

ABSTRACT

Brettanomyces bruxellensis yeast is a major threat to red wine quality, causing such off-odours as Band-Aid®, barnyard or even sewage during aging. However, the use of sulphites (SO_2) as a means for adequate microbial control has been questioned given the high concentrations sometimes required. Addition of SO_2 to red wines inoculated with *Brettanomyces* at $\approx 10^5$ cells/mL resulted in a rapid decline of culturability, as determined by standard plate counts on non-selective media (<300 CFU/mL). However, fluorescence microscopy using the carboxyfluorescein diacetate succinimidyl ester (CFDA-SE) and propidium iodide (PI) stains revealed the presence of high populations of metabolically active cells (>10^4 cells/mL) in these wines, even in the absence of culturable cells. Scorpion™ assays, based on specific genetic markers that detect *Brettanomyces*, confirmed these findings. While *Brettanomyces* could be injured due to the presence of sulphites, these yeasts may also have entered a physiological state known as "viable-but-not-culturable." Yeast cells from SO_2-treated wines were observed to be physically smaller than non-treated wines, potentially changing the required pore sizes of filtration systems used by wineries to remove the yeast. While strain B1b inoculated into a red wine was retained by 1.2 µm absolute membranes, strain F3 passed through and eventually grew to populations that led to wine spoilage. However, filtration of wine inoculated with F3 through a 0.8 µm membrane resulted in removal of the yeast (by evaluation of wines) up to 250 days after filtration. Application of hurdle technology towards minimizing *Brettanomyces* infections will be discussed.

1. Introduction

Brettanomyces/Dekkera yeasts are well known wine spoilage microorganisms whose growth can result in haziness or the production of off-odours sometimes described as medicinal, mousy, Band-Aid®, barnyard or other odours (Gilliland 1961, Heresztyn 1986, Fugelsang et al. 1993 and Sponholz 1993). Previously described species of *Brettanomyces* isolated from wines have been reclassified several times, with *Dekkera bruxellensis* and *D. anomala* now believed to be the microorganisms associated with wine spoilage (Grbin and Henschke 2000).

While many wine microorganisms, including *Acetobacter*, *Oenoccocus oeni*, *Lactobacillus hilgardii*, *L. plantarum*, *L. brevis*, *Pediococcus pentosaceus*, *P. damnosus* and *Saccharomyces*, can synthesize 4-vinyl guaiacol or 4-vinyl phenol from ferulic and p-coumaric acids, respectively, most are not able to reduce the vinyl intermediates to 4-ethyl guaiacol or 4-ethyl phenol (Chatonnet et al. 1992, 1995; and Shinohara et al. 2000). Because of this observation, analysis of 4-ethyl phenol has been used as an indicator of *Brettanomyces* infections. However, some microorganisms, most notably *L. plantarum* (Chatonnet et al. 1992, 1995; and Cavin et al. 1993) and *Pichia guilliermondii* (Dias et al. 2003a), are reported to produce either very small amounts of these ethyl phenols or do not survive in wine.

Controlling the growth of the spoilage yeast in a winery is not an easy task, as *Brettanomyces* can be relatively tolerant to sulphites. However, little information is available regarding the toxicity of sulphites towards this spoilage

yeast. Interestingly, current research in Australia suggests a much wider genetic diversity of *Brettanomyces* than previously thought (Anonymous, 2003), a finding that could imply a range of tolerances to SO_2. In addition, some wine microorganisms may enter a controversial phase known as "viable-but-not-culturable" (VBNC) after exposure to SO_2 (Millet and Lonvaud-Funel 2000), a status that may result in physically smaller cells (Oliver 2005). Thus, physical removal by filtration may require smaller pore size membranes than previously thought, a finding in agreement with the findings of Millet and Lonvaud-Funel (2000).

2. Materials and Methods

Red wines, standardized to 12.5% ethanol, were inoculated with 10^5 CFU/mL of *Brettanomyces bruxellensis*. After an incubation of approximately 14 days, enough potassium metabisulphite was added to yield various concentrations of molecular SO_2 (0.3, 0.5 and 0.8 mg/L as calculated using free SO_2 and wine pH). Yeast viability and 4-ethyl phenol was determined using: a) plate counts with non-selective media and b) epi-fluorescence microscopy (with the direct epi-fluorescent filter techniqueor DEFT) using the fluorescence stains carboxyfluorescein diacetate succinimidyl ester (CFDA-SE) and propidium iodide (PI).

B. bruxellensis strains B1b and F3 were inoculated at 10^5 CFU/mL into sterile-filtered commercial red wines. Once populations reached 10^6 CFU/mL, half the wine received enough potassium metabisulphite to reach 0.5 mg/L of molecular SO_2 (mSO_2). After six days, each wine was passed through a 1.2 or 0.8 µm absolute membrane and 4.5 L was aseptically transferred into each of three fermentation vessels. For each treatment replicate (a single filter cartridge), three filtered wines were prepared (a total of nine wines). Bubble point tests were performed on each filter prior to use to ensure membrane integrity. Culturability (plate counts using a non-selective medium), viability (epi-fluorescence microscopy), and genetic detection (Scorpion™ probes analysis) were performed on the wines before and after filtration.

3. Results/Conclusions

A staining method using a direct epi-fluorescent filter technique (DEFT) was developed and applied to a wine fermentation involving *Brettanomyces bruxellensis* strain B1b. Unlike other research performed elsewhere, the newly developed DEFT method utilizes two stains: carboxyfluorescein diacetate succinimidyl ester (CFDA-SE) and propidium iodide (PI). Here, CFDA-SE passively diffused through cell membranes and is acted upon by internal esterases to yield the carboxyfluorescein succinimidyl ester (CF-SE), a compound that fluoresces green. Because other yeasts like *Saccharomyces* can quickly export the ester, retention of CF-SE within a cell has been improved by the addition of the succinimidyl ester group (which forms conjugates with intracellular amines). Unlike CFDA-SE, PI enters cells through aberrations or "holes" within the cell membrane and so indicates an injured or dead cell by staining red. Using the combination of stains therefore allows simultaneous viewing of cells with metabolic activity (green) or those which are dying or dead (red).

B. bruxellensis strain B1b grew well in the red wine, achieving populations in excess of 10^6 CFU/mL (plating) or cells/mL (epi-fluorescence microscopy). However, the addition of 0.30 mg/L of molecular SO_2 resulted in an initial decline in populations of approximately 3 logs (>10^6 reduced to 10^3 CFU/mL). A more dramatic decrease was noted after the addition of 0.53 or 0.89 mg/L of mSO_2. After a time, populations again increased and eventually reached >10^5 CFU/mL by day 21.

As viewed using epi-fluorescence microscopy, three distinct cell populations were observed in the wine fermentations before and after addition of SO_2: a) CF-SE fluorescence (epi-CF-SE) where cells of *Brettanomyces* were green, b) PI fluorescence (epi-PI) where cells were red/orange, or c) both CF-SE and PI fluorescence (epi-CF-SE/PI) with distinctive green and red/orange areas within a given cell.

For all fermentations, epi-CF-SE cells were in excess of 10^6 cells/mL prior to adding SO_2 on day 12, in agreement with the populations determined using standard plating methods. After the addition of SO_2, however, the number of green cells decreased by approximately one log, inversely proportional to the increase in the concentration of red cells between days 14 and 22. This observation indicated a shift from viable cells (green) to injured/dead cells (red) due to the addition of SO_2. However, viable cells (green) remained in the wines even though the strain was not detected using direct plating methods. Because of this observation, it is probable that these green cells represent those in an injured or perhaps "viable-but-not-culturable" (VBNC) state.

In all fermentations with SO_2, there were a high number of epi-CF-SE/PI cells, cells that contained both green- and red-stained areas. For all fermentations, the populations of epi-CF-SE/PI cells increased between day 12 and 14 by approximately 0.5 to 1 log and eventually achieved populations of almost $9x10^7$ cells/mL by day 18. Having

evidence of both metabolic activity (green) and membrane aberrations (red) suggests that these cells are either potentially injured or slowly dying. From a winemaking point of view, it remains unknown whether these cells can resuscitate and once again become viable cells or will just eventually die off.

Image analysis of the size and morphology of *Brettanomyces* after exposure to SO_2 revealed possible decreases in the sizes of cells. Here, all cells dramatically decreased in physical size upon exposure to SO_2, sometimes as much as 50%. This observation was in agreement with the occasional observation of other researchers that cells in the VBNC state tend to be far smaller. In agreement, Millet and Lonvaud-Funel (2000) noted that the size of *Acetobacter* cells in the VBNC state decrease during lengthy residency in wine, allowing the bacterium to potentially pass through 0.45 µm membranes (unconfirmed results). Given that the wine industry prefers to minimize filtration, especially for red wines, a series of experiments are planned to determine the impact of cell-size reduction on the filterability of *Brettanomyces*.

In the first filtration experiment, strain B1b was not detected post-filtration through absolute membranes of 1.2 µm >100 days, either by direct plating or by Scorpion™ methods in wines without or with 0.5 mg/L mSO_2. This finding indicates that *Dekkera* could be removed from a wine using absolute membranes of 1.2 µm pore size or less, even from wines with excessive populations (>10^6 CFU/mL). However, results from the second filtration with strain F3 indicated that some cells in the wine without SO_2 were small enough to pass through this membrane. While cells were initially detected by Scorpion™ immediately after filtration, several weeks were needed to observe colony growth on plates. Cells were not detected in wines with 0.5 mg/L of mSO_2. Given these results, filtrations of another red wine inoculated with the same strain of *Brettanomyces* F3 were conducted using 0.8 µm membranes. Unlike the observation with the 1.2 µm filter, strain F3 was removed by the 0.8 µm membranes, as the yeast was not detected based on culturability (plate counts) or Scorpion™. Based on experiments with these two strains, it appears that a 0.8 µm membrane could be sufficient to remove *Brettanomyces* from wines.

Acknowledgements

This research was performed at Washington State University in support of ARC project WNP00846, and is part of the dissertation of N. L. Umiker. Appreciation is given to the Washington Wine Advisory Committee and Washington State University for financial support. Lastly, sincere thanks are extended to G. Specht/N. Sieczkowski (Lallemand Montréal, Québec, Canada), R. Swanson (Scott Laboratories, Petaluma, CA), and G. Burns/R. DeScenzo (ETS Laboratories, St. Helena, CA) for analyses, supplies and additional support.

References

Anonymous. 2003. Investigations into the relationship between *Dekkera/Brettanomyces* yeasts and red wine in Australia. *Australian Wine Research Institute Annual Report*. 11-12.

Cavin, J. F., V. Andioc, P. X. Etievant, and C. Divies. 1993. Ability of wine lactic acid bacteria to metabolize phenol carboxylic acids. *Am. J. Enol. Vitic*. 44:76-80.

Chassagne, D., J. Guilloux-Benatier, H. Alexandre, and A. Voilley. 2005. Sorption of wine volatile phenols by yeast lees. *Food Chem.* 91:39-44.

Chatonnet, P., D. Dubourdieu, and J.-N. Boidron. 1995. The influence of *Brettanomyces/Dekkera* sp. yeasts and lactic acid bacteria on the ethylphenol content of red wines. *Am. J. Enol. Vitic*. 46:463-468.

Chatonnet, P., D. Dubourdieu, J.-N. Boidron, and M. Pons. 1992. The origin of ethylphenols in wines. *J. Sci. Food Agric.* 60:165-178.

Dias, L., S. Dias, T. Sancho, H. Stender, A. Querol, M. Malfeito-Ferreira, and V. Loureiro. 2003a. Identification of yeasts isolated from wine-related environments and capable of producing 4-ethylphenol. *Food Microbiol.* 20:567-574.

Dias, L., S. Pereira-da-Silva, M. Tavaares, M. Malfeito-Ferreira, and V. Loureiro. 2003b. Factors affecting the production of 4-ethylphenol by the yeast *Dekkera bruxellensis* in enological conditions. *Food Microbiol.* 20:377-384.

Edwards, C. G., R. B. Beelman, C. E. Bartley, and A. L. McConnell. 1990. Production of decanoic acid and other volatile compounds and the growth of yeast and malolactic bacteria during vinification. *Am. J. Enol. Vitic.* 41:48–56.

Fugelsang, K. C., M. M. Osborn, and C. J. Muller. 1993. *Brettanomyces* and *Dekkera*: Implications in wine making. *American Chemical Society Symposium Series.* 536:110-129.

Gilliland, R. B. 1961. *Brettanomyces*. I. Occurrence, characteristics, and effects on beer flavour. *J. Inst. Brew.* 67:257-261.

Grbin, P. R., and P. A. Henschke. 2000. Mousy off-flavor production in grape juice and wine by *Dekkera* and *Brettanomyces* yeasts. *Aust. J. Grape Wine Res*. 6:255-262.

Heresztyn, T. 1986. Formation of substituted tetrahydropyridines by species of *Brettanomyces* and *Lactobacillus* isolated from mousy wines. *Am. J. Enol. Vitic.* 37:127-132.

Oliver, J. D. 2005. The viable but nonculturable state in bacteria. *J. Microbiol.* 43:93-100.

Rodrigues, N., G. Goncalves, S. Pereira-da-Silva, M. Malfeito-Ferreira, and V. Loureiro. 2001. Development and use of a new medium to detect yeasts of the genera *Dekkera/Brettanomyces*. *J. Appl. Microbiol.* 90:588-599.

Shinohara, T., S. Kubodera, and F. Yanagida. 2000. Distribution of phenolic yeasts and production of phenolic off-flavors in wine fermentation. *J. Biosci. Bioeng.* 90:90-97.

Sponholz, W. R. 1993. Wine spoilage by microorganisms. *Wine Microbiology and Biotechnology*. G. H. Fleet (Ed.). Harwood Academic Publishers, Chur, Switzerland. 395-420.

Uscanga, M. G. A., M. L. Delia, and P. Strehaiano. 2000. Nutritional requirements of *Brettanomyces bruxellensis*: growth and physiology in batch and chemostat cultures. *Can. J. Microbiol*. 46:1046-1050.

BIOLOGICAL MANAGEMENT FOR THE PRESERVATION OF THE VARIETAL AND FRUITY CHARACTERS IN ROSÉ WINE FOR THE INTERNATIONAL MARKET

Ann DUMONT[1], José Maria HERAS[2], Anthony SILVANO[3], Sam HARROP[4], and Antonio PALACIOS[5]

[1] Lallemand Canada, 1620, rue Préfontaine, Montréal, QC Canada H1W 2N8

[2] Lallemand Ibérica, C/Zurbano 71, Oficina 6, 28010 Madrid, España

[3] Lallemand France, 19, rue des Briquetiers, 31702 Blagnac CEDEX France

[4] Litmus Wines Ltd, 344-354 Grays Inn Road, London, WC1X 8BP England

[5] Laboratorios Excell Ibérica S.L., Polígono la Portalada II, Logrono, España

adumont@lallemand.com

Abstract

The commercialization of rosé wines is undergoing very positive change around the world, with demand growing – particularly in the English market. From a technical point of view, because it is halfway between white wine (avoiding extractions of phenol compounds at the tannin level) and red wine (with potential problems in colour extraction and structure), producing rosé wine involves certain interesting difficulties that need investigation and resolution. The result of applying short maceration times to high quality rosé wine production is fragile wines that could develop rapidly. One of the most frequent developments on the aromatic level is the appearance of premature lactic aromas that can override the fruity aromas on the nose. This work shows the results of research into the making of rosé wines in different countries with selected yeasts in a specific protocol. The wines were then submitted to thorough sensory analysis by an international tasting panel to evaluate whether the biological strategy preserved the primary and fruity character of the rosé wines produced and, thus, their suitability in the current international market.

1. Introduction: The Evolution of Rosé Wine

Historically, rosé wines have been characterized as dry and delicate, exemplified by rosés from France's Anjou and Loire regions. In fact, today's rosé wines were probably originally claret wines, typically produced in the Bordeaux region. Semi-sweet rosé wines became popular after World War II when Mateus rosé and American blush – classic 1970s wines – appeared on the mass market. But today the pendulum appears to be swinging towards the dry, full-bodied rosé wines, produced with grapes from the Rhône region (Syrah, Grenache and Carignan) in warmer regions like Provence, Languedoc and Australia. In France, rosé wines are selling better than white wines. In 2005 the United States had a record harvest of monovarietal grapes with an increase in production in California, and a wide variety of red grapes used in rosé wines, even though red wine sales were not as high.

In English-speaking countries, rosé wine sales have increased 30% and show no sign of slowing down. Today, nearly nine bottles of wine out of 100 sold are rosés – 8.7% of total sales. Just three years ago they were closer to 5%. While this increase is due in large part to the production of the big American brands, sales statistics for rosé wines from all wine-producing regions in the United Kingdom are also showing a solid increase. With consumers trying rosé wines of all styles, from all regions and in all price ranges, this trend is unlikely to change anytime soon.

The rosé wine market has developed considerably around the world due to the growing demand, particularly in English-speaking countries. From a technical point of view, rosé wine production presents certain challenges for researchers. The production system is somewhere between that of white wine (where winemakers avoid maceration to extract phenolic materials from the tannins or other oxidizable compounds) and that of red wine (involving the extraction of colour and phenolic structure at various levels). Using short maceration periods to produce quality rosé wines can result in delicate wines that evolve more quickly. One of the most observed changes that these wines undergo is the premature appearance of dairy, lactic and creamy aromas, which cancel out both the fruity nose and the refreshing sensation in retronasal perception.

The analytical and sensory results presented in this study are taken from a project involving the collaboration of three key players in wine production and marketing: 1) a fine wine distribution expert in England who keeps up to date on direct-to-consumer sales, 2) a business specializing in oenological biotechnology, and 3) three wineries in three different countries operating on an international scale. The participating wineries produced rosé wines according to standardized methods and protocols using three selected natural yeasts. A panel of international wine tasters conducted an exhaustive sensory analysis of the wines to determine their fruitiness and suitability for today's successful international rosé wine market.

2. Project Objective

The goal of the project was to produce rosé wines on an industrial scale and ensure their longevity by enhancing certain sensory compounds associated with varietal aromas, primary fruit and fresh aromas, thereby reducing the negative compounds and sensory descriptors (dairy, creamy, reduction and vegetal) that mask or decrease the impact of the desired compounds.

3. Methodology

3.1 Phase 1: The chemical and sensory characteristics of commercial rosé wines

The objective of the first phase was to obtain a chemical and sensory analysis of a large number of rosé wines available in the market in the United Kingdom to identify the correlations between positive and negative chemical compounds. This process would later prove useful in determining the best oenological practices for producing rosé wines that will be successful on the international market.

Most of the wines analyzed were at the end of their useful commercial lives, having been bottled for a long time. At this stage we were able to assess the overall quality and look for sensory trends that would identify which compounds to avoid in future research. For this very reason, the aromatic and chemical fractions of the commercial wines tasted were also thoroughly analyzed through routine analysis and gas chromatography-mass spectrometry.

Two expert wine tasters tasted 24 of the rosé wine samples. In the end, seven of the wines had a successful international sales profile, whereas six had a negative profile due to the presence of dairy aromas. These preliminary conclusions were determined through chemical analysis of seven positive and six negative wines.

To this end, we conducted a statistical study, comparing the analytical data of volatile compounds with wines analyzed at the sensory level through principal component analysis (PCA).

According to these analyses and their statistical interpretation, a higher concentration of a number of positive chemical compounds was found in those wines which the tasters identified as being more fruity and fresh. These compounds consisted of medium-chain fatty acids, and mainly ethyl esters, isoamyl alcohol and isoamyl acetate, such as diacetyl, damascenone and higher alcohols (2-phenylethanol), and terpenoids. It is worth noting that some of these compounds, like diacetyl and isoamyl alcohol, were initially considered negative due to their supposed contribution of heavy dairy aromas. On the other hand, high levels of negative chemical compound concentrations (including butyric and isobutyric acids, acetoin, ethyl lactate, isobutanol, succinic acid esters and vinylphenols) were found in the wines described as already evolved and as having heavy dairy aromas.

A preliminary sensory evaluation and volatile compound analysis of the rosé wines produced the following conclusions:

- Wines with dairy, butter and confectionery characteristics had higher levels of compounds like acetoin, which are often associated with oxidative metabolism in yeast;
- Wines mainly described as fruity and having fresh fruit aromas had higher levels of hexyl acetate, isobutyl acetate, and ethyl butyrate – all associated with fresh aromas.

Techniques that could help stimulate the compounds that create aromatic stability and increase the longevity of rosé wines include:

- Managing malolactic fermentation or a certain bacterial metabolism;
- Using certain yeasts and enzymes to enhance the aromatic potential; or
- Using inactive yeasts to stabilize aromas once achieved.

3.2 Phase 2: Industrial production of rosé wines

In Phase 2, three wineries (in France, Spain and Portugal) all applied the same production protocol and conducted sensory and chemical analyses through both routine and aromatic analyses. The wines were analyzed and tasted three different times: right after the end of fermentation (November 2009), six months later (May 2010), and one year later (December 2010).

The following production protocol was applied to the 2009 grape harvest.

- Receiving the grape harvest: Add 3 g/hL of SO_2 to grapes, add macerating enzymes (Lallzyme® Ex at a dosage of 2 g/hL), and correct pH level with tartaric acid to a maximum pH of 3.5.
- Crushing and destemming, pressing: When necessary, cool the must at 12°C to 14°C. Three to six hours of pre-fermentation maceration, depending on the colour obtained and the must tasting.
- Bleeding the tanks: Protected under a CO_2 atmosphere, gradually add 2 g/hL of SO_2 when the must is extracted, plus 5 g/hL of ascorbic acid to obtain 40 mg/L of SO_2.
- Clarifying the must: With must cooled to 10°C or 12°C and protected under CO_2, allow the precipitation of gross lees for 24 to 48 hours at 10°C. Meanwhile, accelerate the process by adding pectolytic enzymes (1 g/hL of Lallzyme® C-Max) and keep the must at 70 to 90 NTU.
- Alcoholic fermentation: Add 25 g/hL of selected yeasts (ICV-GRE, ICV Opale and Rhône 4600), add 20 g/hL of Optiwhite® inactive yeasts, 15 g/hL of nutrients (Fermaid® E White) to the must, and 15 g/hL of 1070 density. Control fermentation at 15°C to 18°C.
- Post-fermentation treatments: When necessary, treat with polyvinylpolypyrolidone (PVPP) during fermentation. Remove lees once a day during the last part of fermentation (during the last 40 g/L of sugar). When fermentation is complete (dry wine stage), add 4 g/hL of SO_2 if the pH level is below 3.4, and 5 g/hL if it is equal to or above 3.4. Add 5 g/hL of ascorbic acid. The following day, decant in a CO_2 atmosphere. Keep the wine below 14°C. One week later, repeat decanting in a CO_2 environment. Maintain 25 ppm of free SO_2 when the pH level is below 3.5, and 30 ppm when the pH level is above 3.5. Clarify and stabilize wine with bentonite and isinglass, and stabilize at a cool temperature for 10 days.

3.3 Results of standard parameters analysis

Analysis of the standard chemical parameters was conducted according to official OIV methods. As illustrated in Table 1 (next page), there were no major differences between the wines. The main difference was the deeper colour in the wines (from all three countries) that were fermented with ICV-GRE yeast.

3.4 Results of sensory analysis

Sensory analysis was conducted according to the ISO 11035 standard using previously defined and quantifiable descriptors by 10 international expert tasters who were already familiar with the wines and the tasting method being used. Figure 1 (page 41) presents the tasting sheet of descriptors chosen by tasters by consensus. The intensity of each descriptor was rated on a scale of 0 to 5, with 0 being the lowest intensity of the given descriptor.

The results obtained from the general wine tasting were analyzed with XLSTAT, a statistical software program used for principal component analysis (PCA). The data from the different tasting phases was analyzed separately, except for the visual evaluation, which presented no substantial differences between the wines from different countries.

The wines were tested in three separate tastings. The first tasting was done upon completion of the processes referred to in the protocol by the three wineries. The tasting took place in La Rioja (at Laboratorios Excell Ibérica) in November 2009. The second tasting took place six months later (June 2010) in London. The third and final tasting took place close to a year later (December 2010) at London's Litmus Wines. The goal was to taste wines throughout their commercial life, while also conducting aromatic and chemical analyses to identify the correlations between composition and sensory impact.

The first tasting, held when the wines were just finished, showed that the Opale yeast (in mainly French and Portuguese wines) and the Rh 4600 yeast (France and Portugal) had a greater sensory impact due to their metabolism during the transformation of must into wine. However, the technologies utilized in the winery and the strength of the varietal potential strongly affect the final sensory impact of the wine. Spanish wines, for example, are mostly related to such descriptors as fresh fruit, vanilla, ripe fruit and aromatic intensity. In the gustatory phase, wines are grouped mostly according to their country of origin. The Rh 4600 yeast is mainly associated with such aromatic

Table 1. Results of standard analyses of the wines obtained from the three wineries, and fermented with the three yeasts

	Analysis (03/11/2010)		
	PT (GRE) OS	**PT (4400) OS**	**PT (OPALE) OS**
Alcohol level (vol.)	12.1	12.1	12
Colour intensity (DO 420 nm + 520 nm + 620 nm)	1.33	1.05	0,89
Total acidity (g tartaric acid/L)	5	5.5	5.3
Volatile acidity (g acetic acid/L)	0.3	0.37	0.27
pH (20°C ± 0.02)	3.16	3.22	3.23
L-lactic acid (g/L ± 0.1)	0.1	0.1	0.1
L-malic acid (g/L ± 0.1)	0.9	1.1	1
Glucose + fructose (g/L ± 0.1)	0.7	0.6	0.7
Glycerol (g/L ± 0.1)	6	6.2	6.3
Hue	1.01	0.85	0.77
Total polyphenols index (DO 280 nm ± 0.3)	9	12.4	11.6

	Analysis (03/11/2010)		
	GRE SP	**4600 SP**	**OPALE SP**
Alcohol level (vol.)	12.7	12.9	12.9
Colour intensity (DO 420 nm + 520 nm + 620 nm)	1.56	1.1	1,61
Total acidity (g tartaric acid/L)	5	6.6	5.1
Volatile acidity (g acetic acid/L)	0.42	0.57	0.48
pH (20°C ±0.02)	3.27	3.3	3.28
L-lactic acid (g/L ± 0.1)	0.1	0.1	0.1
L-malic acid (g/L ± 0.1)	2.4	2.1	2.2
Glucose + fructose (g/L ± 0.1)	4.1	3.6	0.9
Glycerol (g/L ± 0.1)	5.6	6.1	5.4
Hue	0.57	0.96	1.03
Total polyphenols index (DO 280 nm ± 0.3)	9.8	15.4	10.2

	Analysis (03/11/2010)		
	GRE FR	**4600 FR**	**OPALE FR**
Alcohol level (vol.)	12.4	12.35	12.4
Colour intensity (DO 420 nm + 520 nm + 620 nm)	0.97	0.49	0,4
Total acidity (g tartaric acid/L)	4.7	5.5	5.4
Volatile acidity (g acetic acid/L)	0.3	0.3	0.32
pH (20°C ± 0.02)	1.24	1.31	1.45
L-lactic acid (g/L ± 0.1)	0.1	0.1	0.1
L-malic acid (g/L ± 0.1)	1.4	1.4	1.5
Glucose + fructose (g/L ± 0.1)	1.1	0.7	0.4
Glycerol (g/L ± 0.1)	5	5.3	5.1
Hue	1.12	1.04	1.14
Total polyphenols index (DO 280 nm ± 0.3)	6.9	11.1	11.1

Figure 1. Tasting sheet used by the international jury

DESCRIPTIVE ANALYSIS ISO11035 METHOD

Date	
Name of Taster	

LALLEMAND

WINE
Rosé Wine

SCORING
Mark with an X the perceived value:
0 equals absent
5 equals a very high intensity

DESCRIPTOR	DEFINITION	Ref:					
APPEARANCE OR VISUAL EVALUATION							
Tone	From onion peel to violet	0	1	2	3	4	5
Intensity	Colour quantity	0	1	2	3	4	5
Limpidity	Clarity	0	1	2	3	4	5
Brilliance	Colour vibrancy	0	1	2	3	4	5
AROMAS OR OLFACTIVE EVALUATION							
Aromatic intensity	Aromatic intensity of first nose (before agitation)	0	1	2	3	4	5
Herbaceous	Vegetal, asparagus, moss	0	1	2	3	4	5
Floral	Aromatic flowers	0	1	2	3	4	5
Aromatic plants	Tea, thyme, eucalyptus, mint	0	1	2	3	4	5
Fresh Fruit	Strawberry, prune, peach, raspberry, blackcurrant	0	1	2	3	4	5
Ripe Fruit	Black fruit, jam, compote, gummy candy	0	1	2	3	4	5
Dried fruit	Raisins, dried figs	0	1	2	3	4	5
Confectionery	Cream, whipped cream, custard, pastries	0	1	2	3	4	5
Butter	Margarine	0	1	2	3	4	5
Dairy	Yogurt, fresh cheese, milk	0	1	2	3	4	5
Vanilla	Cinnamon, coconut	0	1	2	3	4	5
Nut	Hazelnut, almond	0	1	2	3	4	5
Spice	Clove, black pepper, cedar	0	1	2	3	4	5
Oak	Oaky, smoky, toasted	0	1	2	3	4	5
Balsamic	Eucalyptus, menthol	0	1	2	3	4	5
Yeast	Bread crust, freshly baked bread, hot bread	0	1	2	3	4	5
Reduction	Closed, aroma linked with the presence of sulphur compounds	0	1	2	3	4	5
Oxidation	Apple, acetaldehyde, brandy, sherry	0	1	2	3	4	5
Alcohol	Burning nasal sensation	0	1	2	3	4	5
TASTE AND MOUTHFEEL							
Sweet	Sweet first mouthfeel	0	1	2	3	4	5
Thick	Glycerol, smooth, velvety, silky, thin, round, full	0	1	2	3	4	5
Fresh	Pleasant mid-palate acidity	0	1	2	3	4	5
Acid	Too much acidity	0	1	2	3	4	5
Bitter	Bitter and rough finish	0	1	2	3	4	5
Vegetal	Herbaceous character, grass	0	1	2	3	4	5
Chemical	Chemical sensations in mouth	0	1	2	3	4	5
Length	Taste sensation duration	0	1	2	3	4	5
Burning	Hot mouthfeel, sense alcohol	0	1	2	3	4	5
RETRO NASAL							
Fruity	Fruity all types, fruit	0	1	2	3	4	5
Dairy	Dairy, milk, yogurt, fresh cheese	0	1	2	3	4	5
Oak	Oak, wood, barrique aging	0	1	2	3	4	5
Reduction	Sulphuric retro nasal aromas	0	1	2	3	4	5
Heat	Alcohol, heat perception	0	1	2	3	4	5
Complexity	Offers many different perceptions	0	1	2	3	4	5
Persistence	Retro nasal perception duration	0	1	2	3	4	5

Signature of Taster

descriptors as dried fruit, balsamic, butter, dairy and reduction. The Opale yeast is mainly associated with aromatic plants, floral, herbaceous, spice, yeast and oak. The GRE yeast is mostly associated with fresh fruit, ripe fruit, nut and oxidation descriptors. In the taste and mouthfeel phase, the taste descriptors that stand out most are the Opale yeast's positive descriptors, i.e., fresh and length in mouth. In the retronasal phase, the complexity descriptor is mainly associated with the Opale yeast.

The second tasting, held in May 2010, yielded the following results. The yeast that had the greatest sensory impact was the GRE yeast. This was observed in the statistical analysis of all three of the tasting phases. However, the technology employed in the winery and the strength of the varietal potential had a particularly pronounced effect on the wine's final sensory impact. The Spanish wines, for example, appeared to be the most heavily favoured according to the tasters' assessments, because they possessed more fresh fruit flavours and greater aromatic intensity, as well as being associated with the most sweet, fresh and length descriptors, with fruitier, more complex and persistent retronasal attributes. When the common descriptors were reported according to the yeasts, the Rh 4600 yeast was more strongly correlated with such aromatic descriptors as vanilla and reduction, as well as being more vegetal and acidic in the mouth. The Opale yeast was more closely correlated with descriptors of the milky, dried fruit, marmalade and ripe fruit areas, so that this yeast appears to be the one that develops most rapidly, although in a positive direction. The wines with the GRE yeast are the ones most closely correlated with the aromatic intensity, fresh fruit and vanilla descriptors, and with a very fresh mouthfeel and a great deal of body, so that this yeast is one of the most interesting of the three.

3.5 Analysis of the wines after one year – final analysis

3.5.1 Aroma or olfactive phase

Figure 2 depicts the variables (previously described descriptors) and findings (wine samples) in the olfactive phase. The principal axes explain 56.72% of the variance.

Compared with results from earlier tastings (tasting 1, wines after fermentation; and tasting 2, wines after six months), the wine clusters reflected the country of origin more than the selected yeasts used, and were more intense than the first two tastings. The Spanish wines formed a well-defined cluster, as in the previous tasting, which included a single Portuguese sample (Opale Portugal). This group was situated in the lower portion of the graph and, for the most part, in the negative portion of the F1 axis. It was mostly associated with aromatic plants, aromatic intensity, ripe fruit and fresh fruit descriptors (especially for Rh 4600 yeast), as was the case in previous tastings. It appeared that, in this case, the winemaking technology or the strength of the grape's varietal potential developed in the given environments had a greater impact on the sensory profiles of the wines.

The Portuguese wines (GRE Portugal and Rh 4600 Portugal) were mostly associated with dairy, butter, marmalade and floral aromas, particularly those fermented with GRE yeast. All the French wines were in the upper right quad-

Figure 2. Principal component analysis of the wines produced in the three wineries with the three yeasts, according to the aromatic descriptors one year after fermentation

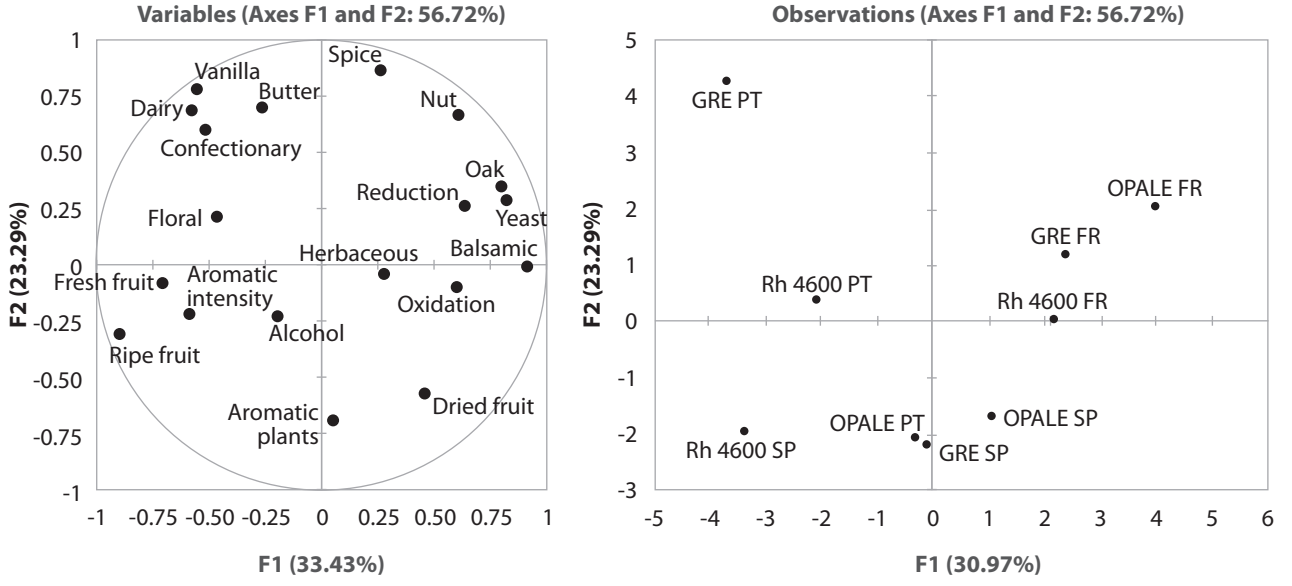
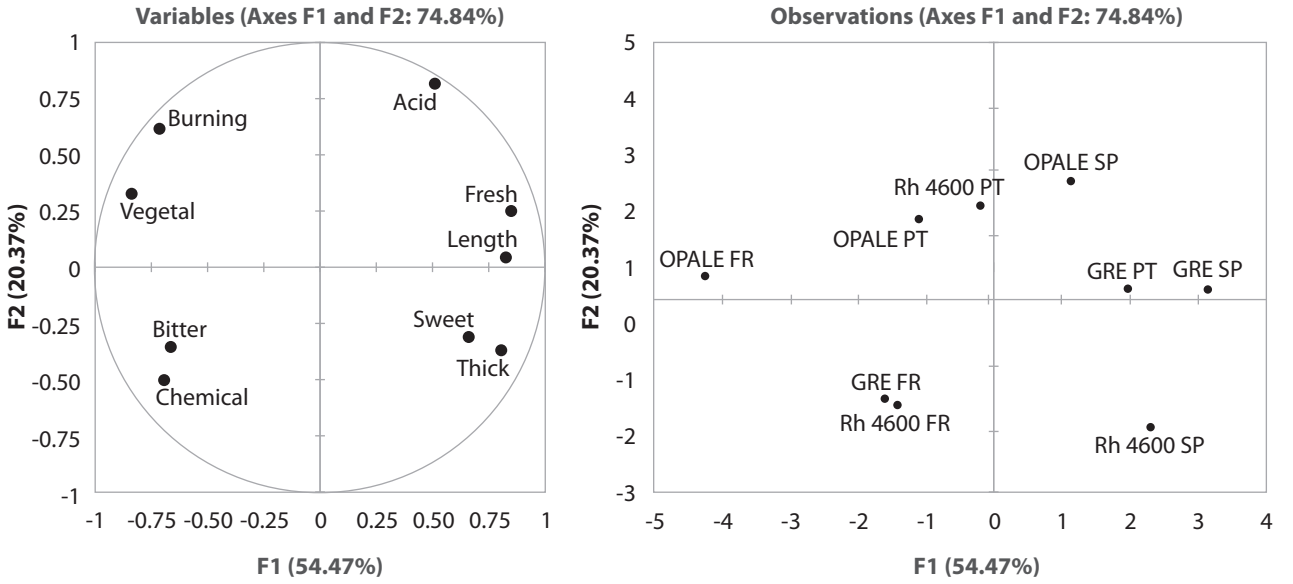


Figure 3. Principal component analysis of the wines produced in the three wineries with the three yeasts, according to the gustatory descriptors one year after fermentation



rant of the graph and were noted for spice, yeast, vegetal and reduction aromatic descriptors.

3.5.2 Taste and mouthfeel or gustatory phase

Figure 3 depicts the variables (previously defined descriptors) and findings (wine samples) in the gustatory phase. The axes explain 74.84% of the variance.

In the gustatory phase, the GRE (GRE Spain and GRE Portugal) samples had the most similarities, as in the previous two tastings. In this phase of the tasting, as in the aromatic phase, the samples were mostly clustered by country of origin. The French wines were associated with chemical, vegetal and bitter descriptors, while the Spanish wines were associated with sweet, fresh and length gustatory descriptors, particularly those produced with GRE and Opale yeasts.

The wines from Portugal were more tightly clustered than in the previous tasting and were situated in an area less defined by the tasting descriptors used (in the centre of the graph), except for the GRE Portugal sample, which was in an area dominated by Spanish wines in the gustatory phase of the tasting. The samples fermented with GRE yeast, regardless of the country, seemed to have the most similar mouthfeel. GRE yeast was regarded as having the most positive sensory characteristics based on the project objectives.

3.5.3 Retronasal phase

Figure 4 (next page) depicts the variables (previously defined descriptors) and findings (wine samples) in the retronasal phase. The factorial axes explain 78.77% of the variance. Given the complexity and number of descriptors being used, this explanation of the variance is considered very acceptable. The PCA representation in the retronasal phase was similar to that in the gustatory phase. The variance in the aromatic phase was lower, meaning that the wines were more distinguishable in the gustatory tasting.

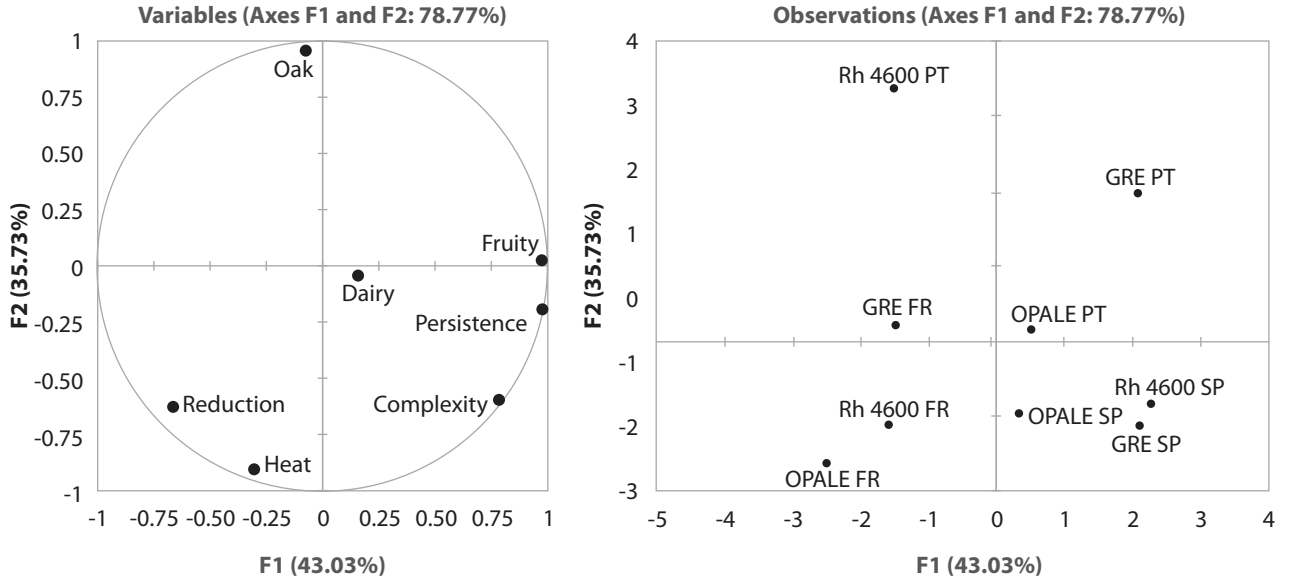
In the retronasal phase of the tasting, the wine samples were once again clustered together by country, although more so than in the previous two tastings. All the Spanish wines were associated with complexity and persistence descriptors, placing them in the lower right quadrant of the graph. The French Opale and Rh 4600 wines were still strongly associated with reduction in the retronasal phase. The Portuguese wines were still situated in an area less defined by the tasting descriptors being used, except for the GRE Portugal sample, which was described as fruity in this phase.

3.5.4 Hedonistic preference test

The tasters were asked to rank the wines by preference with the highest scores representing the lowest rankings. The results are shown in figure 5 (next page). The most preferred wines were ICV-GRE from Spain, Opale from Portugal and ICV-GRE from France, while those least preferred were Opale from France, and Rhône 4600 from Portugal.

Due to its metabolism throughout the transformation of must into wine, the GRE yeast had the greatest sensory impact. These characteristics were determined through statistical analysis of the three tasting phases. The technology employed in the wineries and varietal potential also contributed to the final sensory impact of the wines from the three countries.

Figure 4. Principal component analysis of the wines produced in the three wineries with the three yeasts, according to the retronasal descriptors one year after fermentation



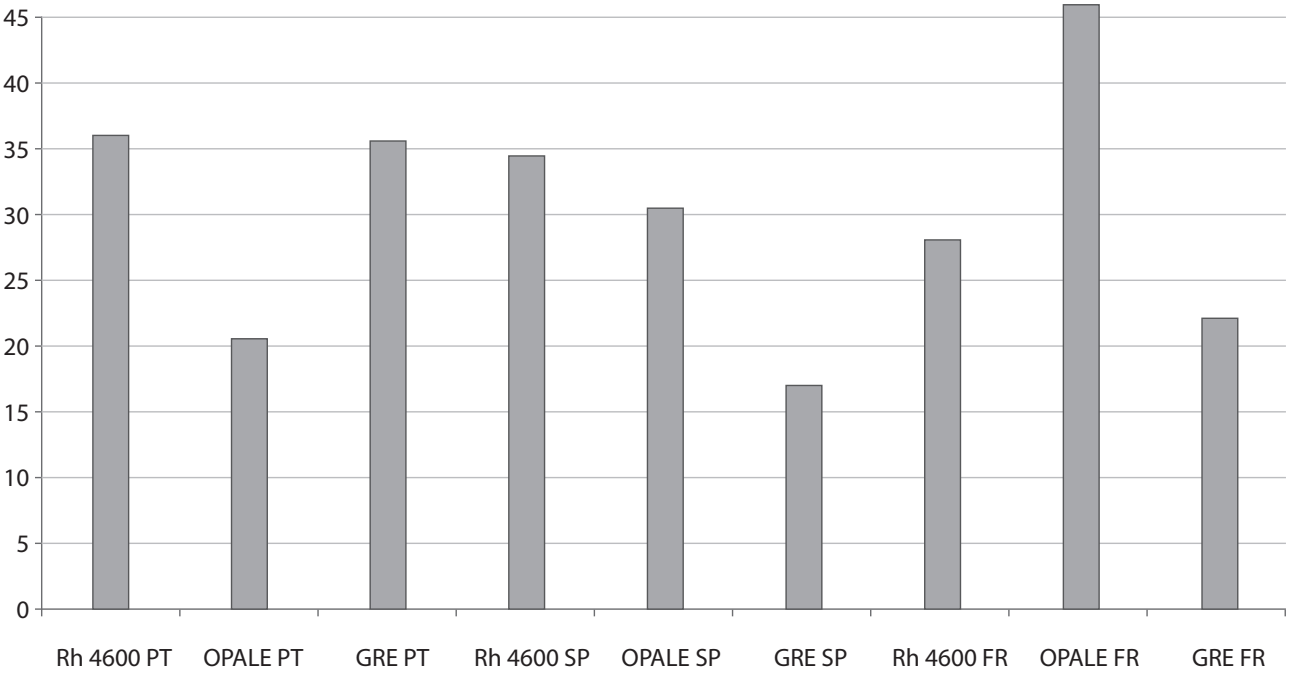
The tasters seemed to prefer the Spanish wine samples since they had the most pronounced fresh fruit aromas and the strongest aromatic intensity, all while having the most sweetness, freshness and mouthfeel, and being mostly associated with the fruity, complexity and persistence retronasal descriptors. It is worth noting that the aromatic profile of the samples produced with this yeast were also associated with hints of dairy, particularly in the retronasal phase, and yet without detracting from the tasters' overall appreciation.

With regard to commonalities among yeasts, the Rh 4600 yeast was mostly associated with floral aromatic descriptors, while being appreciated for its fresh and acidic mouthfeel and complexity retronasal descriptors.

The Opale yeast was mostly described as having an herbaceous nose, and being more vegetal and burning in the mouth.

Lastly, the samples produced with the GRE yeast were mostly associated with vanilla, spice and dried fruit aromatic descriptors, as in earlier tastings. The findings were

Figure 5. Representation of hedonistic preferences for the nine wines – the higher the number, the lower the ranking



similar with regard to gustatory descriptors – the samples were found to be sweet, with volume and length – demonstrating that this yeast is the best of the three studied for producing quality rosé wines using this protocol and under these conditions.

4. Aromatic Analysis of the Wines

Further analysis was carried out by gas chromatography and mass spectrometry of the aromatic fractions in relation to major and minor volatile compounds. The wines were analyzed on two separate occasions (March 2010 and November 2010) in order to observe the chemical evolution after being bottled for almost a year. This was fundamental in identifying the correlations between time and the volatile composition of the wines.

The detailed composition of the nine wines (November 2010) is shown in table 2 (major aromas) and table 3 (minor aromas) (on next page). Based on this data, an additional statistical analysis was conducted by principal components analysis (PCA) and using the chemical parameters as statistical variables and the wines as observations. This made it easier to determine which wines were best in terms of taste and chemical composition. Figure 6 (page 47) provides an example of such representations.

The gray areas in figure 6 represent the wine samples preferred by the tasters according to the desired profiles. In terms of major volatile components, diacetyl and ethyl propanoate were found in one area of preference, and the fatty acids, ethyl esters and γ-butyrolactone in the other.

In figure 7 (page 47), the minor volatile components with positive characteristics (shown in the gray areas) were isobutyl and butyl acetates, with whiskey lactone and β-ionone isomers in one area and compounds such as α-terpineol, vanillin ethyl, δ-nonalactone, phenylacetaldehyde and acetovanillone in another.

5. Conclusions

When the differences between the wines and the final sensory impact were determined, it was found that the

Table 2. Major volatile composition of the nine wines produced

Client Ref.	GRE Pt	4600 Pt	OPALE Pt	GRE Sp	4600 Sp	OPALE Sp	GRE FR	4600 Fr	OPALE Fr
Acetaldehyde	1.59	2.74	2.86	5.51	5.52	5.48	2.91	4.24	4.45
Ethyl acetate	20.36	17.05	20.32	44.16	33.47	32.14	25.44	26.18	23.26
Ethyl propionate	0.10	0.15	0.13	0.09	0.14	0.08	0.10	0.16	0.13
Diacetyl	6.69	2.99	2.76	1.60	1.27	2.09	0.75	0.83	1.57
Ethyl butyrate	0.20	0.18	0.14	0.25	0.26	0.16	0.18	0.22	0.18
Isobutanol	18.57	16.42	20.77	12.64	10.05	12.26	15.89	10.96	14.36
Isoamyl acetate	0.53	0.40	0.42	1.01	1.02	0.89	0.55	0.79	0.24
1-butanol	0.61	0.43	0.33	0.90	0.48	0.32	0.52	0.39	0.24
Isoamyl alcohol	107.69	129.77	175.57	125.29	117.80	133.71	105.62	116.56	143.37
Ethyl hexanoate	0.40	0.33	0.24	0.56	0.47	0.31	0.39	0.41	0.29
Hexyl acetate	0.02	0.01	0.01	0.07	0.08	0.05	0.03	0.05	0.03
Acetoin	3.85	2.86	3.66	14.10	5.43	4.48	3.67	3.49	3.61
Ethyl lactate	13.19	13.78	11.63	9.50	6.52	5.88	11.84	10.86	8.90
1-hexanol	0.60	0.63	0.71	1.15	1.25	1.09	0.82	1.10	1.04
Cis-3-hexenol	0.04	0.63	0.04	0.33	0.34	0.33	0.20	0.19	0.19
Ethyl octanoate	0.28	0.04	0.17	0.57	0.39	0.27	0.35	0.44	0.28
Acetic acid	61.11	0.27	56.25	223.79	136.47	149.15	105.14	89.13	82.18
Isobutyric acid	0.65	49.76	1.31	0.58	0.51	0.84	1.17	0.84	1.29
γ-butyrolactone	2.93	0.83	2.45	3.32	3.91	1.84	3.55	3.74	1.72
Butyric acid	1.44	3.17	1.12	1.55	1.27	1.31	1.20	1.40	1.00
Ethyl decanoate	N.A.	1.45	N.A.	0.10	0.10	0.06	0.06	0.08	0.05
Isovaleric acid	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.
Diethyl succinate	1.78	2.28	2.69	1.67	1.31	1.64	1.69	1.57	2.06
Methionol	0.61	0.74	0.87	0.67	0.52	0.54	1.03	0.64	0.77
Phenylethyl acetate	0.54	0.53	0.48	0.73	0.49	0.33	0.29	0.46	0.39
Hexanoic acid	4.86	4.45	2.98	5.89	5.53	4.64	5.64	5.15	4.19
Benzilic alcohol	0.10	0.08	0.09	0.03	0.03	0.03	0.09	0.07	0.07
β-phenylethanol	15.14	21.52	22.51	27.12	13.99	13.28	12.70	17.46	18.98
Octanoic acid	2.98	2.93	1.92	5.14	4.69	2.94	3.70	4.30	3.21
Decanoic acid	0.09	0.10	0.07	0.51	0.44	0.26	0.17	0.25	0.18

Table 3. Minor volatile composition of the nine wines produced

Client Ref.	GRE Pt	4600 Pt	OPALE Pt	GRE Sp	4600 Sp	OPALE Sp	GRE FR	4600 Fr	OPALE Fr
Ethyl isobutyrate	138.66	167.85	245.13	104.37	76.44	138.25	186.89	136.62	222.52
Isobutyl acetate	71.72	43.11	48.36	67.03	58.83	62.47	70.77	66.51	58.33
Butyl acetate	5.99	4.58	3.82	8.81	6.77	6.08	5.53	8.80	6.13
Ethyl	10.06	12.94	19.91	9.93	6.36	13.18	13.22	10.98	19.63
Ethyl isovalerate	22.25	27.92	38.16	18.87	13.50	26.01	28.21	27.70	38.23
Benzaldehyde	7.37	10.12	4.89	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.
Linalool	7.48	6.37	6.59	3.06	2.87	2.31	5.04	5.26	4.87
Linalool acetate	N.A.	N.A.	N.A.	N.A.	N.A.	N.D.	N.A.	N.A.	N.A.
Ethyl furoate	24.23	19.19	18.15	37.76	33.43	31.08	28.41	33.88	24.11
Phenylacetaldehyde	120.71	126.20	122.18	120.19	114.01	105.05	114.25	101.97	103.57
α-terpenol	12.00	12.59	11.84	3.61	3.24	2.39	4.98	4.82	5.72
β-citronellol	0.97	1.30	2.32	1.19	1.64	3.19	0.61	1.95	3.48
α-ionone	3.25	3.34	3.17	4.74	5.76	5.87	4.30	4.76	4.65
Geraniol	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.
Guaiacol	2.18	2.74	1.88	N.A.	1.47	0.76	1.59	1.51	2.07
Ethyl	4.30	4.25	4.22	1.63	1.64	1.79	5.82	6.70	6.61
(Z)-whiskey lactone	0.20	0.60	0.65	1.27	0.55	0.64	0.68	0.71	0.77
β-ionone	1.81	1.10	1.05	4.46	4.96	2.27	1.67	2.75	1.54
(E)-whiskey lactone	0.15	0.17	0.15	0.27	0.27	0.29	0.16	0.19	0.20
δ-octalactone	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.
o-cresol	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.
4-ethylguaiacol	1.18	1.23	1.32	0.89	0.87	0.95	1.33	1.35	1.49
δ-nonalactone	4.80	5.24	5.40	4.85	4.23	4.23	3.24	3.48	3.52
m-cresol	0.60	0.58	0.58	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.
4-propylguaiacol	0.52	0.47	0.47	0.15	N.A.	0.19	0.26	0.30	0.31
Ethyl cinnamate	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.
γ-decalactone	N.A.	N.A.	N.A.	0.29	N.A.	N.A.	N.A.	N.A.	N.A.
Eugenol	1.23	0.84	1.46	0.80	1.98	0.78	1.47	1.18	1.40
4-ethylphenol	1.48	1.39	1.45	1.49	1.43	1.38	1.24	1.41	1.39
δ-decalactone	2.89	2.60	2.64	0.16	0.20	0.16	0.20	0.19	0.22
4-vinylguaiacol	32.76	29.80	25.21	37.59	44.33	34.41	36.66	50.84	39.34
(E) isoeugenol	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.
2,6 dimethoxyphenol	3.86	4.41	4.25	4.19	4.07	4.36	2.81	2.90	3.02
4-vinylphenol	15.27	17.70	16.90	14.76	12.11	13.29	23.11	20.71	25.90
Dimethoxyphenol	2.02	2.27	2.16	1.89	1.56	1.57	3.23	3.36	3.63
Vanillin	5.47	2.75	2.73	N.A.	N.A.	1.71	N.D.	3.77	3.24
Methyl vanillate	40.16	39.68	39.90	2.94	3.90	4.84	46.85	51.03	53.07
Ethyl vanillate	284.10	218.30	213.15	15.18	27.62	19.45	65.34	66.31	64.51
Acetovanillone	80.77	80.11	82.00	74.56	71.13	72.53	68.14	69.81	72.27
Syringaldehyde	24.74	11.47	19.28	7.67	15.05	33.29	3.89	3.30	2.42

factors with the greatest impact on the sensory quality of the wines were the winemaking technology utilized in the winery and the strength of the grapes' varietal potential.

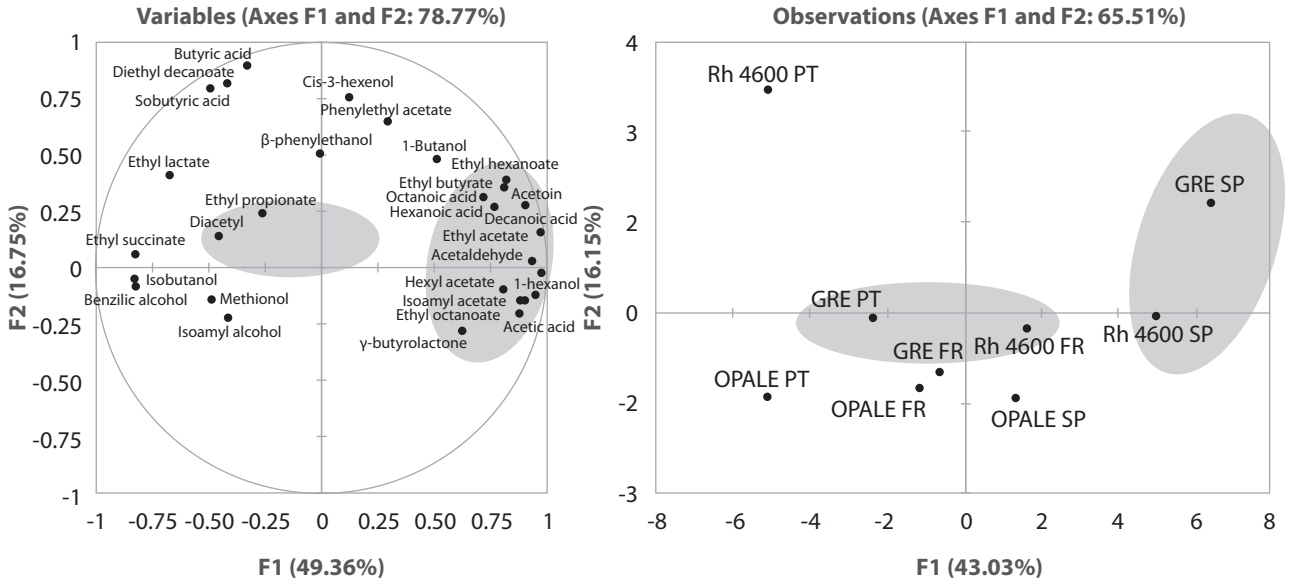
Terpenoids, fatty acids, ethyl ester (isoamyl acetate), diacetyl, damascenone and octalactone are considered positive molecular compositions for attaining the right rosé wine aromas when employed in a balanced concentration.

In low concentrations, diacetyl may enhance red fruit aromas (especially the aroma of strawberries), which are quite appropriate for fresh, international rosé wines.

Sulphur compounds and molecules responsible for the vegetal characteristic in wines radically shorten the useful commercial life of rosé wines, acting in synergy when they occur simultaneously.

Wines produced with the Rhône 4600 yeast were most often associated with dried fruit, balsamic, butter and dairy descriptors. These wines were mainly appreciated for their volume and acidic mouthfeel, and fresh retronasal sensations.

Figure 6. Principal components analysis representation of the wines produced and their correlations with major aromatic composition



The wines made with the ICV Opale yeast were associated mainly with aromatic plant, floral, herbaceous, spice and yeast descriptors.

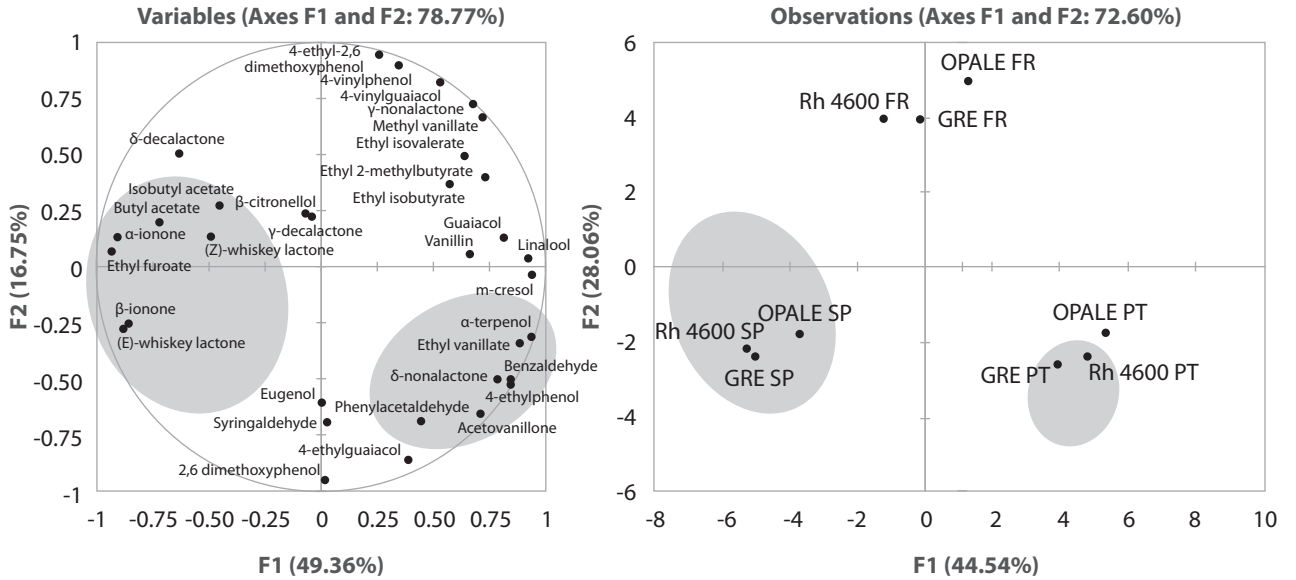
The wines made with the ICV-GRE yeast were frequently associated with fresh fruit, ripe fruit and nut aromas, and had excellent mouthfeel. This yeast was presented as the optimal biological tool for producing rosé wines with longevity and that best correspond to the profile defined in the project's main objectives.

In terms of production, the intense clarification of the must before alcoholic fermentation can generate more ethyl esters from fatty acids, which will later increase fruitiness as the wine ages in the bottle. This intense clarification is not likely to compromise the security and kinetics of the actual fermentation process. Yeast protection using protectors in the rehydration water may help with this.

The use of free cinnamyl-esterase enzymes prevents the appearance of vinylphenol-type compounds. These compounds are considered very negative in terms of nose and mouthfeel freshness, and can shorten the useful life of rosé wines.

The yeasts selected with significant amyl characteristics could be very useful in such practices as blending, in order to create a fruity profile in the final wine.

Figure 7. Principal components analysis representation of the nine wines produced and their correlations with minor aromas



To prevent the accumulation of succinic acid in rosé wines, it is best not to ferment the wine in overly reductive conditions. Using open decanters in the first third of AF or using micro-oxygenation can help with this.

Malolactic fermentation in rosé wines from cold climates or made from grapes with high levels of acidity should be researched as an interesting practice for moderating the production of compounds, like diacetyl and ethyl lactate, which are capable of enhancing the fruity characteristics. Co-inoculation and bulk aging to promote the production of butanediols are recommended techniques given the project's objectives.

Using wood derivatives during fermentation helps prevent the appearance of sulphur compounds, brings new aromas that appeal to international styles, and increases antioxidant characteristics and, therefore, the wine's longevity.

Bibliography

Aznar, M., R. López, J. Cacho, and V. Ferreira. 2001. Identification and quantification of impact odorants of aged red wines from Rioja. GC-Olfactometry, quantitative GC-MS, and odor evaluation of HPLC fractions. *J. Agric. Food Chem*. 49:2924-2929.

Blouin, J. 2003. Analyse et composition des vins. *Comprendre le vin*. Ed. La Vigne.

Carro, D., and B. Piña. 2007. Identificación de cepas de levadura de interés enológico. *ACE Revista de Enología*. 84.

Escudero, A., E. Campo, A. Fariña, L. J. Cacho, and V. Ferreira. 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 Agriculture and Food Chemistry.* 55,11:4501-4510.

Ferreira, V., M. Aznar, R. López, and P. Cacho. 2001. Quantitative gas chromatography-olfactometry carried out at different dilutions of an extract. Key differences in the odor profiles of four high-quality Spanish aged red wines. *American Chemical Society*. 49,10:4818-4824.

Flanzy, C. 2003. *Enología, fundamentos científicos y tecnológicos*. AMV Ediciones. Mundiprensa, 2nd edition.

Geiger, E., and A. Piendl. 1975. Technological factors in the formation of acetolactate and acetohydroxybutyrate during fermentation. *Brew Dig*. 67:50-63.

Hashizume, K., and T. Samuta. 1997. Green Odorants of Grape Cluster Stem and Their Ability to Cause a Wine Stemmy Flavor. *J. Agric. Food Chem*. 45:1333-1337.

Jackson, R. 2002. *Wine Tasting: A Professional Handbook*. Food Science and Technology, International series. Academic Press.

Júdez, L. 1989. *Técnicas de análisis de datos multidimensionales*. Ed. Centro de publicaciones del Ministerio de Agricultura, Pesca y Alimentación. Madrid.

Lafon-Lafourcade, S., and P. Ribéreau-Gayon. 1984. Les altérations des vins par les bactéries acétiques et les bactéries lactiques. *Connaissance de la Vigne et du Vin*. 18:67-82.

Martineau, B., and T. Henick-Kling. 1995. Performance and diacetyl production of commercial strains of malolactic bacteria in wine. *J. of Appl. Bacteriology*. 78:526-536.

Peynaud, É. 2000. *El gusto del vino*. Ed. Mundi Prensa.

Radler, F., and H. Schütz. 1982. Glycerol production of various strains of *Saccharomyces*. *American Journal of Enology and Viticulture*. 33,1:36-40.

Ribéreau-Gayon, J., E. Peynaud, P. Ribéreau-Gayon, and P. Sudraud. 1975. Sciences et Techniques du vin. *Traité d'œnologie*, Vol. 2. Dunod, Paris.

Ribéreau-Gayon, P., and E. Stonestreet. 1965. Le dosage des anthocyanes dans le vin rouge. *Bull. Soc. Chim.* 9:2649-2652.

Ribéreau-Gayon, P., and E. Stonestreet. 1966. Dosage des tanins du vin rouge et détermination de leur structure. *Chim. Anal*. 48:188-196.

Ruiz Hernández, M. 2010. Viña, productividad y defectos: Evaluación sensorial de vinos. *La Semana Vitivinícola*. 296:166-168.

Wainwright, T. 1970. Hydrogen sulphide production by yeast under conditions of methionine, pantothenate or vitamin B6 deficiency. *J. Gen. Microbiol.* 61:107-119.

Y Vinos (blog). 2010. La rueda de los aromas. http://www.yvinos.com/rueda-de-aromas-del-vino.

YEAST NUTRITION AND THE ALCOHOLIC FERMENTATION OF ROSÉ WINES

Baptiste OLIVIER[1] and Daniel GRANÈS[2]

[1] Consultant, Groupe ICV, Vallée du Rhône – Beaumes de Venise, France

[2] Scientific Director, Groupe ICV, Lattes, France

dgranes@icv.fr

bolivier@icv.fr

Abstract

Successful winemaking depends, in part, on the management of alcoholic fermentation (AF), which must be regular, complete and produce a minimum of negative sulphur odours. Among the numerous factors becoming better understood, yeast nutrition remains key to this technique. With rosé wines, it would be almost inexcusable to not take the necessary actions, because AF occurs most often during the liquid phase, which means it is particularly easy to intervene. Nutritional Good Practices must aim to satisfy the needs of the yeast in order to obtain a viable population large enough to complete AF within a reasonable time, without generating any notable sensory defects. Based on any deficiencies measured initially and the specific needs of the yeast chosen, the quantitative aspect is the easiest to manage. In parallel, the quality of supplementation plays a major role. When it is necessary, as is most often the case in the Mediterranean zone, the adding of nitrogen depends on the nitrogen's origin (organic, mineral or a mix of the two) and the timing of additions. Beyond the rigorous management of nitrogen, on musts that are often very clarified and with fermentation temperatures generally low, enrichment with micronutrients and sterols is a further guarantee of fermentation safety.

1. Introduction

Beyond their colour, rosé wines present three main style families in the international market: technological fruity (banana and red fruit), exotic fruity (thiol type), and ripe fruit and richness. But whatever the sought-after style, it is essential to develop clean and stable aromas, volume and freshness with no dryness in the mouth.

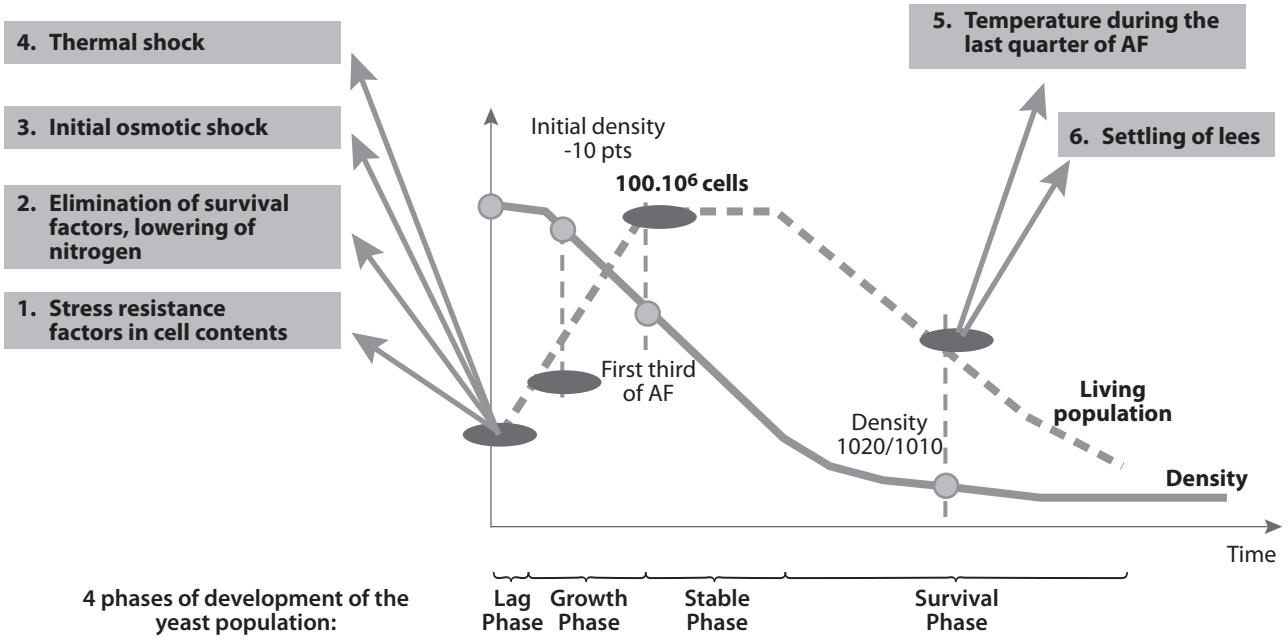
2. Fermentation Goals and Specificities Related to Rosé Wines

The objective is to obtain complete fermentation of the sugars while managing the aimed-for aromatic and gustatory profile. Two aspects are fundamental: the regularity of the alcoholic fermentation (AF) and its completion.

Regular fermentation demonstrates the good physiological health of the yeast population. Regular fermentation limits the risk of producing volatile acidity during the first third of AF and of producing sulphur odours during fermentation.

The **completion** of AF with the absence of residual sugars is an additional guarantee for the microbiological stability of the wine. The sulphiting, maturing and blending operations are further facilitated when the wines have completed AF.

Figure 1. Summary of fermentation conditions related to the production of rosé wines, and the theoretical curves for density and yeast population during alcoholic fermentation



It is important to briefly recall the nutritional needs of *Saccharomyces cerevisiae* yeast:

- Minerals and vitamins;
- Fatty acids and sterols;
- Yeast-available nitrogen (YAN) in mineral or organic form.

The fermentation conditions encountered during the vinification of rosé wines are often difficult. After racking, the turbidity of the juice is low and may lack sufficient growth and survival factors. In addition, the temperature of the must is often low at the time the yeast is added and during AF.

Initial osmotic shock

Six stress factors are shown in figure 1.

1. The content of yeast cells in terms of stress factors, which are related to the quality of production of the yeast strain itself, and to the yeast's potential.

2. The elimination of survival factors and the level of YAN. A consequence of racking – an essential step to attain the style objectives – is the elimination of survival factors and the lowering of the concentration of YAN (measured at up to 30%).

3. The initial osmotic shock, which risks producing glycerol and acetic acid by the yeast. Irreparable damage to the membrane may occur at this stage.

4. Thermal shock. Related to the temperature difference between the rehydrated yeast starter and the juice, thermal shock risks creating stress for the yeast, with a possibility of sensory deviation – even yeast mutation – leading to lower resistance to ethanol.

5. Low temperature during the survival phase, which amplifies stress on the yeast – which is already under difficult conditions (alcohol, fermentation waste, etc.) – with the risk of sluggish or stuck fermentation.

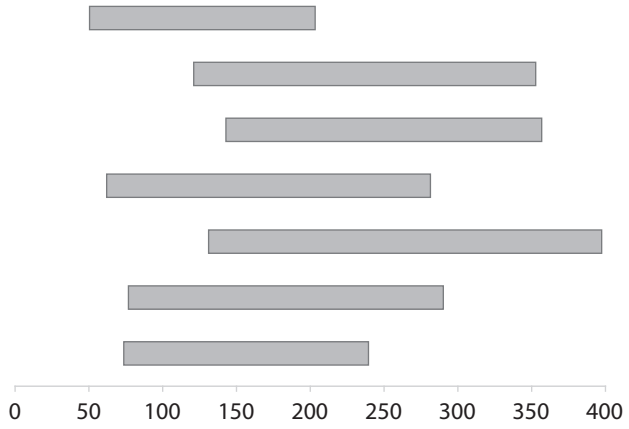
6. Lees settling. Viable yeasts, imprisoned in the lees as it settles and compacts, will die because they are no longer in contact with the still sweet juice, leading to a loss of yeast population and the risk of sluggish or stuck fermentation. In addition, lees settling can produce a sulphur odour with a risk of not achieving the wine style desired.

2.1 Nitrogen Deficiencies

Evaluate the deficiency

Nitrogen deficiencies are characteristic of harvests in the Mediterranean region. Figure 2 presents the maximum and minimum concentrations of YAN over seven vintages. These measurements were taken annually on 30 parcels of land.

Figure 2. Maximum and minimum concentrations of yeast-available nitrogen over seven vintages



Out of the seven vintages, it was demonstrated that more than 50% of the grapes present a YAN level under 150 mg/L, i.e., a nitrogen deficiency.

Beyond the inter-annual variations, the differences come from:

• The varietal – Mourvèdre, Grenache Noir, Syrah and Vranec very often have a YAN deficiency;

• The *terroir* – hydric stress situations accentuate the deficiencies, and variations may come from the quality of the soil and the viticulture techniques.

Analysis of the grape before fermentation is very instructive, and lets the winemaker anticipate the effects of the vintage or qualify vineyard parcels. However, there is no direct link between the value obtained by analyzing the grapes and the value obtained from the must after pressing. Such an analysis of the grapes is therefore of no interest unless it is carried out every year on the same parcels of land. Thus, the results obtained on the grapes and on the must in the preceding years will let the winemaker anticipate future results for the current vintage's must, based on the grape results.

A nitrogen deficiency is a combination of several factors, and is, notably, linked to the yeast, whose needs can vary on a scale of 1 to 2 according to the strain and the potential alcohol in the must, as shown in table 1.

Correcting the deficiency

The quantitative aspects are easier to correct. The necessary additions of YAN are calculated according to the yeast's needs based on the measured concentration in the must. The different products available on the market present known concentrations of YAN, which let the winemaker know how much to add, based on the calculated deficiency. The winemaker must avoid exceeding the calculated dosage, as that could induce deficiencies after mid-fermentation.

The qualitative aspects are more complex. The timing of the nitrogen addition affects the yeast's multiplication and cellular activity: the earlier the addition is made, the greater the impact, but the higher the risk of encouraging an excessive yeast population, which will then put the must in a deficiency situation. The more AF progresses, the less effective nitrogen is on the yeast population and its fermentation. The type of nitrogen (mineral or organic or a mix of the two) impacts the yeast metabolism and aromatic compounds (whether positive or negative) that it prefers to produce.

If the winemaker does not know the initial nitrogen level, the best moment to add nitrogen is at the end of the first third of AF or until mid-AF. In practice, in order to avoid encouraging an excessive yeast population, when the addition is large it is recommended to divide the addition into two increments, as shown in table 2.

The mineral form of nitrogen is rapidly assimilated, but when added at the beginning of AF it can generate very high yeast population levels and induce deficiencies during fermentation. Additions made after mid-AF can generate sulphur odours as well.

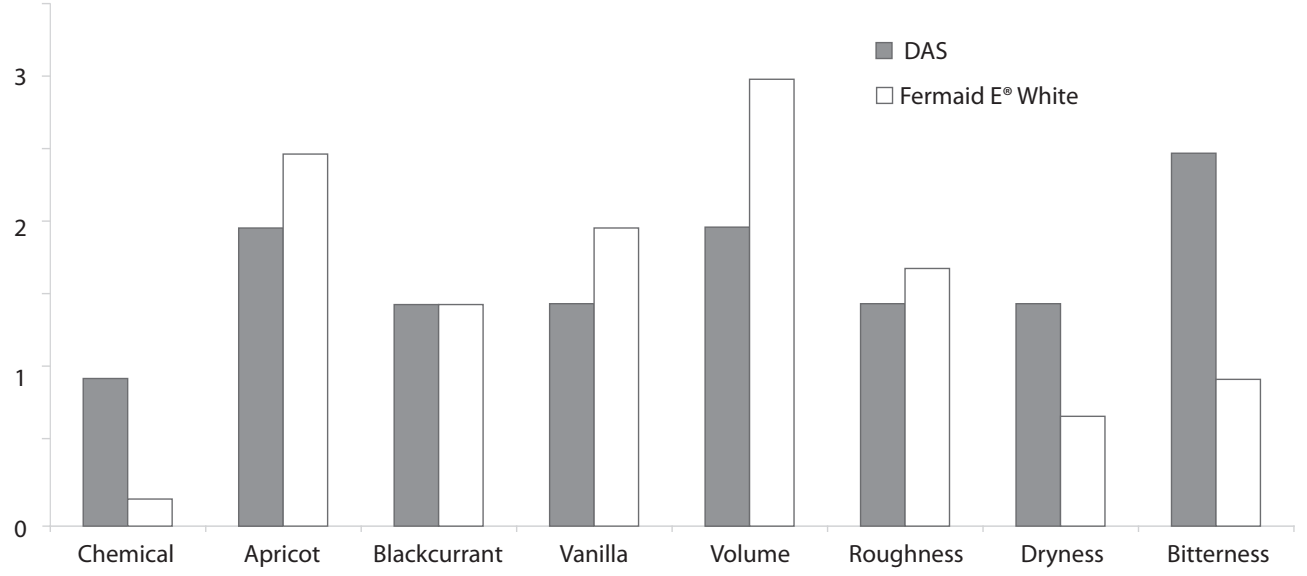
Table 1. Need for yeast-available nitrogen in relation to the alcohol level

Potential alcohol	<12%	12-13%	13-14%	>14%
Average need for yeast-available nitrogen	150 mg/L	180 mg/L	210 mg/L	240 mg/L

Table 2. Dosing the nitrogen additions

Nitrogen deficiency	<30 mg/L	30 to 60 mg/L	>60 mg/L
Addition at beginning of alcoholic fermentation	none	1/3	1/2
Addition at 1/3 to mid-alcoholic fermentation	all	2/3	1/2

Figure 3. Sensory impact of nitrogen addition on Grenache rosé wines



The sensory impact is quite evident, as the descriptive sensory analysis of two Grenache rosé wines resulting from identical musts, but with additions of nitrogen in different forms, shows (figure 3).

Complex nitrogen lets the winemaker obtain less chemical-tasting wines (with less isoamyl acetate), that are softer on the nose and less aggressive on the palate.

Different strategies can be developed, but in any case the winemaker should favour adding complex nitrogen at the start of fermentation and after mid-fermentation.

The alternatives

Nitrogen, as such, has no substitute. Simply inoculating the must with more yeast will not solve all the problems tied to a nitrogen deficit:

- More yeast, even with a mediocre survival rate, may let the must complete AF; but
- The nitrogen stress will almost inevitably lead to sulphur odours.

The weakening of the must during racking may be compensated in part by the reintroduction of fine lees up to 2% of the must's volume, in order to limit the risk of changing the style of the finished product. Yeast hulls do not add enough effective YAN for AF.

2.2 Growth and Survival Factors

Encourage good AF through yeast resistance

Vitamins, minerals and fatty acids/sterols are the main factors for the vitality of the yeast population.

Sterols and fatty acids play a vital role in the yeast's resistance to stress factors, particularly to the initial osmotic shock (which induces the production of glycerol and volatile acidity by the yeast), and to stress related to the presence of alcohol in the medium. It is very important to preserve the potential of the initial yeast population through good yeast rehydration and acclimatization practices, as this potential is diluted over the following generations. The greater the initial potential and the better it is preserved, the better the conditions for AF. In addition, the stresses are amplified by the deficiencies: The yeast concentration varies from one yeast strain to another, musts for rosé wines very often have deficiencies, due to racking, etc., and the quality of the yeast preparation is largely dependent on the winemaker's skill and other factors.

Vitamins, particularly thiamine and biotin, play an important role in cell growth: Thiamine on fermentation activity, and biotin on nitrogen metabolism.

Minerals have an impact on the proper functioning of the yeast, with consequences on the sensory quality of the wine.

Deficiency correction

The winemaker has different and complementary strategies available for correcting deficiencies in growth and survival factors:

- Increase the initial yeast population with the dosing of the yeast;
- Correct sterol deficits by adding a survival factor, such as GoFerm Protect®, to the rehydration water;

- Introduce fine lees (1% to 2%) to significantly increase the turbidity and viability of the medium;
- Adding oxygen during the first half of AF is favourable for the synthesis of sterols and does not pose any risk to the oxidizable aromatic compounds; the cumulative objective is 4 to 8 mg/L of oxygen;
- Add complex nutrients, such as Fermaid E®.

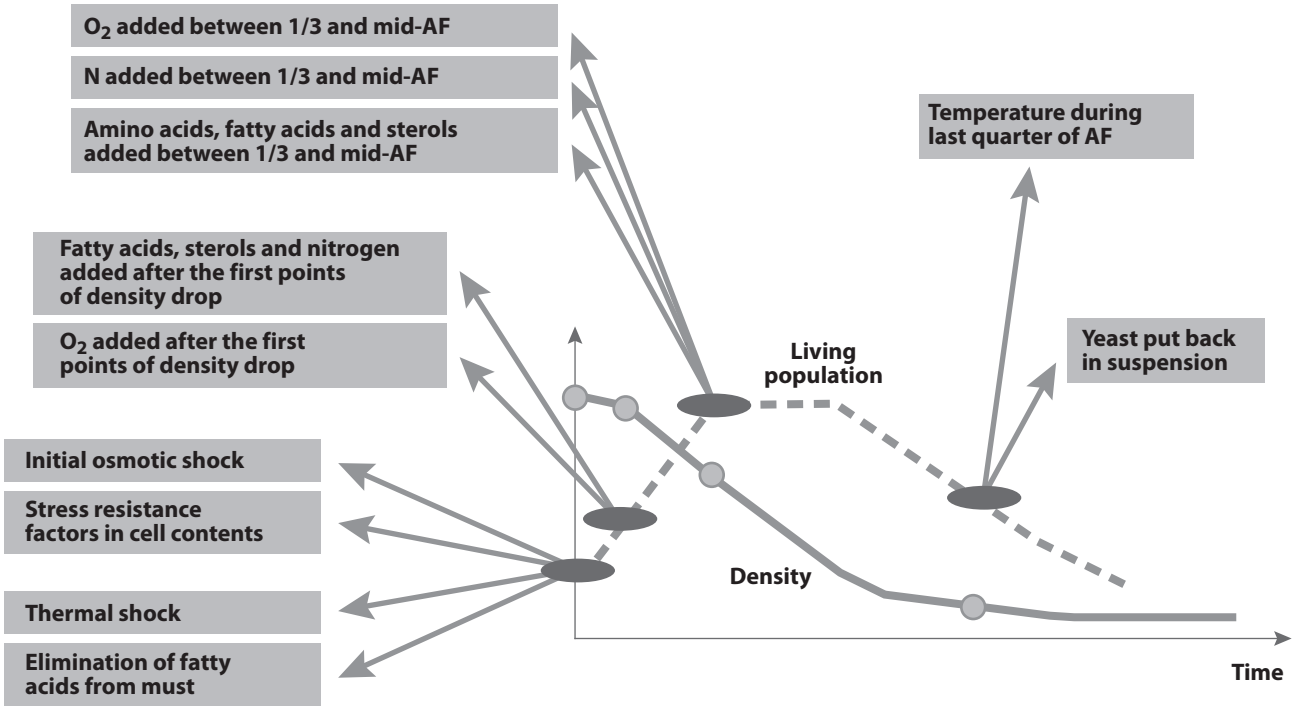
3. Conclusions

It is often necessary to reconsider the process for making rosé wines:

- Lower **turbidity** (<80 NTU) is not obligatory, and is even not advisable when seeking a ripe fruit profile;
- Lower **temperature** (<15°C) is not advisable either, even for "technological" profiles. Opale® yeast, for example, yields more regular profiles of the 16° to 17°C type than 12° to 13°C where fruitiness is more present;
- The quintet **raw material – turbidity – yeast – temperature – nutrition** is the foundation to achieving the style objectives.

Note that, as shown in figure 4, practically everything happens during the first third of alcoholic fermentation, after which is often too late to intervene effectively.

Figure 4. Actions occurring during alcoholic fermentation



INNOVATIVE PROCESSES, EQUIPMENT AND INPUT TO DESIGN ROSÉ WINES FOR DIFFERENT MARKETS

Laure CAYLA and Gilles MASSON

Institut Français de la Vigne et du Vin, Centre de Recherche et d'Expérimentation sur le Vin Rosé,
70, avenue Wilson, 83550 Vidauban, France

accueil@centredurose.fr

Abstract

The Centre de Recherche et d'Expérimentation sur le Vin Rosé and its partners contribute to a better understanding and appreciation of rosé wines. The analysis of a collection of rosé wines from around the world shows a great diversity of products typically categorized according to the geographic area of production. In particular, the colour varies from white stained with light red, to light red. As more than 90% of rosé wines are presented in clear bottles, the colour can impact the purchase decision and is therefore central to concerns in the winery. Numerous tools have been developed to describe this colour palette, and great efforts made to adapt these tools as far as possible to the winemaking processes selected according to the desired colour objective. The control of the colour is based on the choice of varietals and the good management of pre-fermentation operations. The temperature of the harvest, the crushing and how long the skins are in contact with the juice are probably the most important factors. The pressing process and sulphite management can also play a role in colour management. Fining must be considered as a variable for adjusting rosé wine. It is important to know that colour is irreparably lost (by an average of 50%) during alcoholic fermentation, and is partially masked in the presence of free SO_2.

Various volatile compounds originating from the varietal and during fermentation are responsible for the fruity component in rosé wines. The technological decisions made in the winery can favour one or another of the compounds. The winemaker can therefore direct the results through the choice of techniques, input and equipment, so the sensory quality of the wines meets the needs of different markets. Currently, there are also economic and environmental concerns. Above all, the aromatic profile depends on the potential of the raw material. The fermentation conditions (active dry yeast, temperature and activators, etc.) modulate the development of the various compounds. The lee management and the control of micro-oxygenation are favourable for the expression of volatile thiols, but the wines will inevitably evolve as soon as the fermentation has ceased. The conditions for storage, racking and shipping, etc., can also alter the quality.

1. Introduction

Rosé wine is a cultural entity: it has its own history, geography, economy, sociology, codes – and its own colour (R.I.R. 2006). Rosé wine is also a technical entity. Winemakers often say rosé wine is very difficult to make, and how it requires great *savoir-faire* and an entire set of techniques. The process gives it a singular personality, but its variations confer a diversity that is its treasure.

In 2006, the world production of rosé wines was estimated at 21.5 million hL – 9% of the total world production of wine (Aigrain 2009). Production has been increasing for several years; 75% of rosé wines are now produced in European countries, including France (28%), Italy (21%) and Spain (18%). The United States is also a major producer of rosé wines, with a specific production of blush wine (9% alcohol and residual sugars); 75% of rosé wine is consumed by European countries.

2. Materials and Methods

2.1 Experimental wines

For this study, the vinification was, for the most part, carried out in the experimental winery at the Centre du Rosé in 120 L vats, with grapes or musts from the Provence area. The vinification was standardized.

2.2 Rosé wines from around the world

Each year, the Union des Œnologues in France supplies the Centre du Rosé with more than 600 rosé wines from all over the world. These wines come from more than 25 different countries, while French rosé wines make up a large part of the sampling.

2.3 Analyses

- The physicochemical analyses are carried out by the laboratory at the Centre du Rosé. The fermentation compounds are measured by gas chromatography with flame ionization detector (GC/FID), and the specific compounds are entrusted to specialized laboratories.
- The colour measurements are conducted by spectrophotometry. A calculation module extracts the tristimulus coordinates from the spectra. Several colour charts are utilized. The position on the colour chart represents the centroid of the positions assigned by the tasters. The wine is observed in a glass, under controlled light.
- The wines are tasted in a black glass by a panel of technicians and the expert judges trained in the specific quantification of certain aromas.

3. Results and Discussion

3.1 Typology of rosé wines around the world

In the sample studied, 94% of the bottles were made of clear glass and 70% were in the Bordelais shape. As for the stoppers, in 2005 corks were utilized the most (70% of the wines studied). The utilization of synthetic corks and twistcaps is progressing, as they represent 50% of closures in 2008 (Massonet al. 2008).

The rosé wines placed on the colour chart (figure 1) are in the space between the red wines and the white wines. Internationally, the colour of rosé wines is quite varied, from light red to grey. The distribution of the wine colours on the colour chart appears to be related to their geographical origin. Going from north to south, the colour becomes redder and more intense (figure 2). The robe of wines from southern European countries (Spain, Italy and Greece) is more intense than the robe of wines produced in more northern zones, including Switzerland, Austria and Germany (Cayla et al. 2010). France occupies a middle position. The varietals, *terroirs* and climate conditions justify these different colours. Rosé wine is marked by its origin, even when the winemaking techniques bring variations. This work on the colour can be reproduced on the other indicators measured. We can also observe a gradient according to the geographical origin for the total volatile acidity (TVA) and the acidity (Masson 2006). The wines

Figure 1. Representation of 556 samples from 2004 on the colour chart for rosé wines

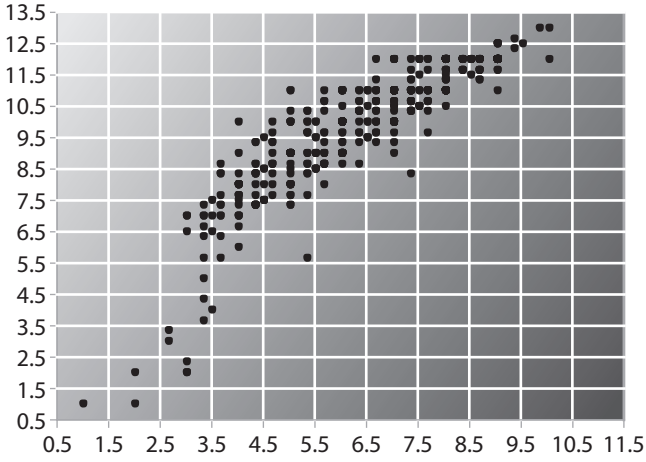
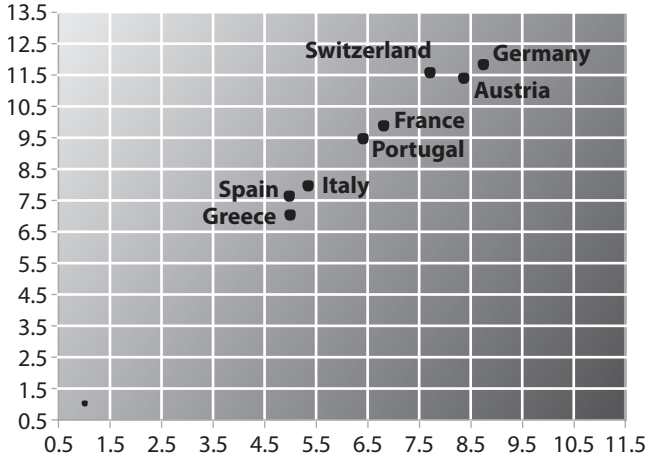


Figure 2. Representation of averages for the countries most represented. Note that these average points hide sometimes a major disparity in the same producing country.



from the south also have higher alcohol levels and less acid.

3.2 Consequences of technical choices on wine profiles

The profile of a wine can be defined by the balance on the palette, the aroma expression and the colour. The winemaking conditions influence these parameters in various proportions, according to the technology utilized.

3.2.1 Well-balanced rosé wines

Based on experiments conducted at the Centre du Rosé, we can affirm that the quantity of residual sugar is clearly the factor that most influences how well-balanced a wine is. It has been shown that the presence of sugar logically increases the perception of fattiness, and also influences aromatic intensity, mouthfeel and the overall score (Pouzalgues 2008). Sixty-two percent of consumers (among 80 people questioned at a trade show) preferred the sweetest wine (7 g/L of sugars compared to tested wines with 4 g/L and 2 g/L), which was judged to be rounder and fruitier. This phenomenon is probably a cognitive reaction, as food is associated with sweetness and fruit since childhood.

The level of maturity at harvest also has a strong influence on the wine's balance. Analytically, maturation has a direct impact on the alcohol level, the malic acid level and, therefore, the pH level. In addition, the wines resulting from the ripest harvests are generally judged to be fatter and less acid, and tend to score more intense aromas (Cayla 2009). However, if the degree of alcohol is very high, the wine may be judged too hot (too high a level of alcohol) and lose points. That is why the lowering of the alcohol level is an interesting alternative.

Pressing also influences the taste of the wines. The *jus de goutte* (the juices that run from the grapes while filling the wine press and generally constitute more than 50% of wine batches) are always more acid. On the other hand, the pressed juices present more intense and fruity aromatic expressions, and offer appealing roundness. Due to the more marked astringency, the blending of both fractions permits the best compromise (Cayla 2005).

Among the other factors that can influence balance in wines, malolactic fermentation attenuates acidity that is too noticeable. Logically, any corrections made to the pH or alcohol level have a direct impact on the physicochemical parameters. In more subtle ways, the choice of yeast strain, the utilization of winemaking products, prolonged aging on lees and the level of CO_2 can also play a role.

3.2.2 The aromatic expression of rosé wine

The aromatic expression is above all the reflection of the raw material. The varietal, the *terroir*, the climate and the growing conditions all play major roles, in particular for the compounds resulting from precursors. It has been shown (Masson and Schneider 2009) that with Syrah, Rolle, Grenache, Mourvèdre and Cabernet varietals the winemaker can more easily develop a wine rich in thiols (3-mercapto-hexanol and its acetate). Indeed, the quantity of fermentation compounds (esters in particular) and furaneols (compounds involved in the aroma of rosé wines) increase when the grape's level of maturity is advanced. In sensory analysis, the wines resulting from the ripest harvests are always judged to be fruitier.

A key to successfully producing fruity rosé wines is the clarification of the must. Trials have shown (Masson 2009a) that the wine must have a turbidity level of about 100 NTU in order to not over-strip the medium and to avoid fermentation problems. At this level of turbidity, the wines have higher levels of esters and present lower levels of higher alcohols.

The quantity of aromatic compounds depends on the conditions of the alcoholic fermentation. First, the availability of selected yeast strains is sufficiently varied to adapt to the potential of the raw material and the sought-after wine profile. Second, it is well understood that rosé wines must ferment at a low temperature. However, a comparison of wines fermented at 13° and 18°C (Cayla and Masson 2010) shows that:

- Systematically, the wines have higher levels of 3-mercapto-hexanol when the fermentation occurs at 18°C;
- Contrary to popular opinion, wines are not always more amylic at 13°C. It is possible that this temperature, maintained throughout fermentation, can sometimes be a limiting factor. An increase in the duration of fermentation can also lead to a more important loss of volatile aromas.

In addition, malolactic fermentation (MLF), conducted post-alcoholic fermentation (AF), strongly modifies the typicity of the wine. From fresh fruit, the wines will show notes of ripe fruit, stone fruit and caramel. With the goal of enriching the thiol levels of the wine, cold stabilization of the juice on lees before racking, or the filtration of the lees, are two techniques that have proven their worth. However, the winemaker must often deal with an increased perception of bitterness. With varietals that are rich in thiol precursors, carrying out the pre-fermentation operations under a controlled atmosphere is a way to preserve this potential. If yeast derivatives can sometimes

lead to more expressive wines, fining – especially during development – means aromatic loss.

Controlling the conditions for development, racking and storage – in terms of oxygen, sulphiting and temperature for the factors that seem to have the greatest importance – ensures the preservation of aromas over time.

3.2.3 The colour of rosé wines

The duration of maceration on skins appears to be the main source of colour variability in rosé wines. Limited contact between the skins and the juice can result in rosé wine that is nearly white, while maceration longer than 24 hours results in wine that is almost red. Other parameters can significantly increase the intensity of maceration, notably the temperature at which it occurs. High temperatures encourage the diffusion of the coloured compounds in the skin. That is why vineyards producing light rosés are increasingly turning to night harvests in order to pick the grapes cool. The mechanical actions that are part of the harvest, transport, transfer to the press, destemming and crushing, and the pressing cycle alter the integrity of the grapes and encourage contact between the skin and the juice. These steps play a crucial role in the extraction of the colour material (Flanzy and Cayla 2006).

It can be interesting to estimate the colour potential of the harvest before picking to better manage the conditions for obtaining the must: by limiting or, on the contrary, encouraging the diffusion of the colour by modifying the schedule and temperature of maceration. The main characteristic of the IFV-Rosé method, which can be routinely carried out by a laboratory, is to simulate on a small scale conditions close to actual winemaking. The method consists of crushing 200 grapes in a reproducible way with a bench press, and to measure the colour of the juice after being in contact with the skins for two hours at room temperature (Cayla 2008).

The colour of the wine is often closely related to the colour and thickness of the grape skin. That is why the pink varietals (Clairette rose) or grey varietals (Grenache gris) generally give very pale rosé wines, while wines from other varietals (Syrah, Carignan and Merlot, for example) rapidly take on more intense colours. The varietal influences not only the intensity of the colour of rosé wine, but its hue as well. The more acid the varietal, the brighter the colour – a bright pink dominates. Less acid grapes yield rosé wines with a yellow-orange shade. Other compounds, such as hydroxycinnamic acids and glutathione (Masson 2009b), are likely to influence the hue of rosé wines, depending on their level in the wine.

There is generally a significant loss of colour during AF, which makes the winemakers' work particularly difficult. They must anticipate this decrease by designing a must with more colour than the final colour objective for the wine. If half the anthocyans are systematically lost in the first three days of AF, this loss of colouring matter does not result in an ongoing loss of colour (Cottereau 2004). The drop in colour intensity during AF is estimated at an average of 50% (Touzand 2008), with great variation from one batch to another. The yeast strain, the alcohol level, the acidity and the tannin concentration could explain certain variations. Decolouration after post-fermentation sulphiting, while partially reversible, must also be considered. The presence de SO_2 will cause decolouration of the anthocyans and the under-evaluation of the colour red. It is possible to avoid the partial decolouration of the anthocyans by adding a few drops of ethanol (Flanzy and Cayla, 2006); the potential red colour is brought out.

A consequence of the fining carried out on rosé wines to ensure good clarification and stabilization is a decrease in colour intensity in musts and wines (Tourrel and Cayla, 2009). The nature of the fining agent utilized, the dose and the timing are all determining factors for the colour of rosé wine.

4. Conclusions

The quality of rosé wines is dependent on natural and human factors. Temperature control is probably among the most influential parameters. Indeed, cold controls the rate of diffusion of the compounds from the skins to the juice, limits oxidation, makes clarification easier and restricts the implantation of undesirable microorganisms. Cold is indispensable for fermentation and maintains rosé wine's qualities during aging and beyond.

The diversity of rosé wines must be considered as a major treasure, which meets the hugely varied demand of consumers and occasions to consume.

References

Aigrain, P. 2009. Le marché international des vins Rosés. *Le vin Rosé: entité, élaboration, perception*. Édition Féret. 33-41.

Cayla, L. 2005. Une phase sensible du pressurage: le remplissage. *Rosé.com*. 8:20-22.

Cayla, L. 2008. Itinéraires d'élaboration des vins Rosés. *Actes de Mondiaviti,* Bordeaux. 153-165.

Cayla, L. 2009. Maturité des raisins et qualité des vins Rosés français. *Le vin Rosé : entité, élaboration, perception*. Édition Féret. 139-141.

Cayla, L., and G. Masson. 2010. Conséquences des fermentations à basses températures sur les vins Rosés. *Rosé.com*. 16:5-7.

Cayla, L., N. Pouzalgues, and G. Masson. 2010. Connaissance et maîtrise de la couleur des vins Rosés. *XXXIII^e Congrès OIV*.

Cottereau, P. 2004. Synthèse des études « vins Rosés », influence sur la couleur des principaux paramètres de vinification. *Entretiens Rhône Méditerranée*.

Flanzy, C., and L. Cayla. 2006. Élaboration des Vins Rosés, résultats d'expérimentation. *Cahiers Itinéraires d'ITV–France*. 11-40.

Masson, G. 2006. Panorama des Rosés du monde. *Actes des Rencontres Internationales du Rosé,* Aix en Provence, 30 juin 2006. 33-37.

Masson, G. 2009a. Clarification du moût et valorisation des bourbes. *Le vin Rosé : entité, élaboration, perception*. Édition Féret. 171-175.

Masson, G. 2009b. Oxydabilité des cépages provençaux ; conséquences sur les arômes des vins Rosés. *Rosé.com*. 15:11-13.

Masson, G., L. Cayla, V. Cesari, and N. Pouzalgues. 2008. Panorama des vins Rosés du monde, Premiers résultats d'analyses. *Rhône en VO*. 3:66-70.

Masson, G., and R. Schneider. 2009. Key aroma compounds of Provence Rosé wines. *Am. J Enol. Vitic*. 60:1.

Pouzalgues, N. 2008. Évaluation sensorielle des vins Rosés, exemple d'application. *Actes de Mondiaviti,* Bordeaux. 165-171.

2006. *Actes des Rencontres Internationales du Rosé,* Aix en Provence. June 30, 2006. 63.

Tourrel, B., and L. Cayla. 2009. Collages des vins Rosés. *Le vin Rosé : entité, élaboration, perception*. Édition Féret. 177-181.

Touzet, F. 2008. Couleur des rosés : des repères à connaître, des pièges à éviter. *Rosé.com*. 14:12-14.

BIOCHEMICAL AND SENSORIAL IMPACT ON THE FRUITY NOTE IN RED WINES DURING MALOLACTIC FERMENTATION: THE SPECIAL ROLE OF ESTERS

Guillaume ANTALICK
Winner of the Prix *Michel-Feuillat-Entretiens Scientifiques Lallemand 2011*

Université de Bordeaux II, France

Summary of Doctoral Thesis

The preservation of the fruity aroma in wines has become a major issue for the quality of wines and their acceptance by consumers. The expression of the fruity aroma is shaped during the vinification process through the activity of yeast and lactic bacteria (LB) – the main microorganisms in wine. Although the literature has a wealth of data regarding the modulation of the fruity note in wine during alcoholic fermentation (AF) carried out by the yeast, there is much less documentation regarding the malolactic fermentation (MLF) conducted by the LB – an essential step in the development of red wines. LB significantly modify the composition of wines, but there is no consensus specifically concerning their impact on fruity aroma.

Thus, the objective of this thesis was to study the role of MLF on the fruity aroma of red wines and report on the analytical and sensorial ways LB activity impacts the composition of the aromatic markers potentially involved. Measuring the quantity of 70 molecules, including esters, C13-norisoprenoids, lactones, sulphur compounds, diacetyl, branched-chain amino acids (BCAA) and linalool, in some 100 wines was made possible thanks to the prior development of rapid and high-performance analysis techniques, such as gas chromatography and mass spectrometry (GC/MS), solid phase micro-extraction (SPME) and stir bar sorptive extraction (SBSE).

Contrary to empirical ideas regarding MLF, this study showed the short-term absence of a "lactic mask," although such an olfactory interaction could appear at a later time. However, the existence of an aroma close to the reduction note, a smoky/grilled type, was shown, but its characterization was not carried out within the parameters of this study. It was also shown that certain sulphur compounds, such as thiols and dimethyl sulphide (DMS), are more involved in the bacterial modifications of the fruity note in red wines in the short term than certain compounds released by β-glycosidase activity (e.g., C13-norisoprenoids and lactones). The modifications in the ester levels, mainly of yeast origin, are shown as a major process in the balance of the fruity note during MLF. An "ester database" (32 compounds measured in 200 wines) increased the number of variations observed during the development of the LB. In the short term, MLF permits both the synthesis as well as the hydrolysis of esters thanks to the esterase activity, and, in the long term, the late formation of BCAA ethyl esters generated by the catabolism of certain amino acids. The variations in ester levels during MLF are the result of a balance between the hydrolytic activity of esters, and the esterification activity of fatty acids, apparently more by the alcoholysis of glycerides than by the esterification of the corresponding simple fatty acids. The specificity of the esterases vis-à-vis the nature and the length of the ester carbon chain is emphasized, as well as the importance of the availability of the substrates, related in part to the yeast activity.

If the LB strain is a factor to take into account, the substrate composition of the wine is even more important. The study of the influence of the interactions between the yeasts and the LB on the modulations of the fruity note has shown to what extent the microbiological fermentation processes are complex. Indeed, the strain of LB and the timing of inoculation have a huge influence on the aromatic and biochemical profile of the wines. But these variations are specific to each yeast/LB pair in a given medium. Thus, the phenomena at the base of the great variability of impacts MLF has on the fruity note of red wines are more complex than a simple strain effect. These variations reflect the bacteria-yeast-wine triad where each component has its importance. The utilization of a given strain of LB cannot ensure the expression of the fruity note in red wines. On the other hand, the study of yeast-LB interactions on the production of esters could reveal certain pairings that are potentially more interesting than the bacteria strains alone.

This thesis was prepared under the direction of Professor Gilles de Revel (USC 1219 Œnologie, équipe chimie analytique, ISVV-Faculté d'œnologie, 33882 Villenave d'Ornon, France) and the author gratefully acknowledges research funding from the Aquitaine regional council and the Conseil Interprofessionnel des Vins de Bordeaux.

THE EVOLUTION OF ROSÉ WINE STYLES AND CONSUMER PREFERENCES GLOBALLY OVER THE PAST FIVE YEARS

Sharon WILD – Second-year Master of Wine – Australia
Winner of the Lallemand – Institute of Masters of Wine Bursary

The rosé landscape is looking particularly rosy. In the United Kingdom, for instance, rosé wine is now included in the basket of goods that measures inflation, while in France rosé has overtaken white wine as the nation's favoured tipple. Consumers have been embracing a veritable palette of styles over the past five yeas, beyond the sweet, entry-level rosés that represented the category in the 1980s. Producers are demonstrating increased focus on quality and innovation, making rosés that are setting the pace for a rosy future.

Rosés encompass the palest dry blush to the sweetest, strawberry pink-coloured wines. While the category can include sparkling wines, still wines are the focus of this study. Rosé wines are produced from softly pressed red grapes or free-run juice (known as *saignée* in French, meaning bleeding) after a controlled maceration with skins that attains the desired colour. Some basic rosés are created by blending red wine with white wine. Roughly 75% of the world's rosés are produced in Europe – with France the leading producer. The United States makes approximately 20% and the rest of world, 5%.

In the 1980s, the most visible rosés were sweet, basic styles. Prominent examples included white Zinfandels, with sales skyrocketing in its home market, the U.S., to 4.5 million cases – 10% of that country's total wine sales. Produced from Zinfandel grapes and typically with 10% alcohol and 35 to 50 g/L of residual sweetness, the style was originally developed by Sutter Home in the decade prior, to supply market demand for white wines in the face of white grape shortages.

At the same time, the global exports of Mateus Rosé from Portugal boomed. This slightly spritzy, sweet, low-cost wine made from local Portuguese varieties, including Baga and Touriga, achieved sales in excess of 3.2 million cases, 60% in Europe, and was followed closely by another Portuguese rosé brand, Lancers. By the 1990s, however, consumer preferences had shifted, notably to red wines, rendering these styles of rosés unfashionable.

Unfashionable, that is until recently. In 2003, sales of rosé began rising, initially in the U.K. – the world's leading wine importer. The heat wave of that year was a likely trigger for revitalized interest in rosé wines. Adam Lechmere, editor of decanter.com, explained, "It was a long hot summer. People suddenly cottoned on to [rosé] as a very nice summer drink." Sales of rosé grew by 65% in the U.K. from 2003 to 2007 and have been rising steadily since, reaching the £1 billion mark last year – 12% of the U.K.'s total wine market.

The rosés that consumers are cottoning on to this time around are more varied in style compared to those of the 1980s. One trend is towards dry, premium examples, like the classics of Europe. The U.K.-based supermarket giant Sainsbury's, for instance, has steadily increased their range of rosés over the past five years, and notably from 28 to 40 brands last year alone. As a spokesperson explained, "There is a definite move towards fresher, less syrupy styles. Italian varietals such as Sangiovese are becoming very popular." Consumer preferences, it seems, have evolved to embrace rosé's diversity.

Exports of the dry, elegant rosés from Provence have also increased substantially over the past five years, and by a staggering 50% last year alone. The greatest increase has been to the U.S., with rosés commanding over US$8 per bottle (www.vinsdeprovence.com).

Plus, a brand that has risen to prominence recently is "Arrogant Frog" from the Languedoc region. Since Jean-Claude Mas launched the brand in 2004, growth has been phenomenal, making the company a leading French wine exporter. The Arrogant Frog Syrah Rosé, a dry, elegant style has been the best-selling rosé in Australia for the past few

years, outselling locally produced rosés. Other key export markets for the brand include Canada, Japan and the U.S.

Also reminiscent of the 1980s is that sweet, low priced rosés have also regained momentum. Young drinkers and new consumers have been turning to such uncomplicated styles in recent years as an alternative to Alcopops (which are being taxed out of the market in some countries). In the Netherlands, where the majority of wine drinkers are young, rosé now represents 10% of all wine sales (www.dailymail.co.uk). These factors illustrate that there's a proliferation of rosé choices available to fuel the varied and fickle tastes of the modern consumer.

The explosion of rosé styles is matched with an evolution in quality. Since the *Appellation d'origine contrôllée* (AOC) Provence was established in 1993, for instance, over 80% of the region's rosés are now AOC classified. Plus, the region's wine industry association, the *Conseil Interprofessionnel des Vins de Provence* (CIVP), established the world's first Rosé Wine Research & Experimentation Centre in 1999 – further testament to producers' commitment to evolving quality. And it's significant globally as Provence makes around 8% of the world's rosés.

Also in Europe and significant is that attempts in 2009 at changing the laws in the European Union (EU), which would have allowed blending of red and white wines to produce rosés outside the Champagne region, were thwarted. These are signs the quality platform for rosés is being raised and the integrity of classic styles protected.

At the same time, a producer is attempting to create a whole new benchmark for rosé. Striving to produce the world's best rosé, Sacha Lichine –a Bordeaux winemaker and son of the great vintner and writer Alexis Lichine – established Château d'Esclans in 2006 in the heart of Provence. Enlisting the help of Patrick Léon (esteemed winemaker of Château Mouton Rothschild and Opus One), the Garrus rosé was born.

Garrus is made from old-vine Grenache grapes that are handled with meticulous care and fermented and matured in individually temperature-controlled 600 L oak barrels – an innovation from Bordeaux. After only a few vintages, Garrus is commanding attention beyond the super yachts of the Côte d'Azur; the U.S.'s Wine Spectator Magazine, for example, awarded it 90/100 points in 2009. And, at around €80, it is currently the world's most expensive still wine rosé. Garrus represents the evolution of a new iconic category for rosé.

It's really not surprising that rosés have become so popular in recent years. As taste preferences have evolved, so too have rosés. Consumers have discovered and embraced rosé's diversity and left limiting preconceptions in the past. It's because rosés truly offer a versatility no other category can contest; suiting red and white wine drinkers, matching with a vast array of foods or without, at any time of the day, on casual or formal occasions, for any wallet. Rosé wines will likely continue to tickle our palates various shades of pink for years to come.

Original Cover Design: Bruno Loste – Layout and Printing: MODULI INC.

LALLEMAND S.A.S. – 19, rue des Briquetiers - B.P. 59 - 31702 Blagnac CEDEX – Tel.: +33 (0)5 62 74 55 55 – Fax: +33 (0)5 62 74 55 00
www.lallemandwine.com

PART 1: SENSORY DEVELOPMENT OF HOT-CLIMATE RED VARIETALS DURING FERMENTATION
PART 2: ROSÉ WINE FERMENTATION MANAGEMENT AND THE CURRENT MARKET SITUATION

DUBROVNIK
APRIL 28, 2011

LALLEMAND

18