

NEWS FLASH

❖ The **Lalvin ICV oKay®** winemaking yeast received an Innovation Award at the SITEVI 2013 international exhibition. **Lalvin ICV oKay®** is the result of collaborative research by the scientific group of the Institut Coopératif du Vin (ICV), Lallemand, SupAgro and INRA Montpellier. The innovation relies on successive back-crosses between a particularly effective yeast strain from a winemaking point of view and a yeast presenting a very low capacity for producing such sulphur compounds as H<sub>2</sub>S and SO<sub>2</sub>. The outcome is **Lalvin ICV oKay®**, which combines excellent fermentation capacity with very low SO<sub>2</sub>, H<sub>2</sub>S and acetaldehyde production. **Lalvin ICV oKay®** is recommended for aromatic white and rosé wines, where it reduces volatile acidity levels, reveals aromatic esters and enhances freshness and balance on the palate.

❖ The **Lallemand Wine App** is now compatible with iPad and Android devices. Integrating all the functions offered for the iPhone, the iPad and Android versions of the application put all the information you need regarding Lallemand fermentation products – from wine yeast and bacteria, to their nutrients, protectors and enzymes – at your fingertips and help you make the best selection.



WINEMAKING UPDATE

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# Varietal Aromas Resulting from Alcoholic and Malolactic Fermentations

The interaction between microorganisms and the grape must is a complex mechanism involving a multitude of substrates and products, including some that have major sensory impacts. The nutrients present in the grape must (e.g., carbon, oxygen, nitrogen, sulphur and phosphorus compounds, vitamins, minerals and trace elements) create the conditions necessary to ensure the proliferation of microorganisms. Particularly, microorganisms use the sugars and amino acids, which have the metabolic effect of producing multiple metabolites from non-volatile esters (mostly polyols and acids) and volatile esters (e.g., alcohols, aldehydes and ketones, fatty acids, sulphur and phenols), which also impact the aroma and the “*bouquet du vin*” (Swiegers et al. 2005). The microorganisms interact with, notably, the various aroma precursors present in the grape by targeting, more specifically, the glycosides, conjugated cysteine and the phenolic compounds (Eglinton et al. 2004, and Dillon et al. 2004).

This issue of *Winemaking Update* summarizes the XXIV<sup>es</sup> *Entretiens Scientifiques Lallemand*. These scientific meetings, held in 2013, discussed the impact of wine yeasts and bacteria on varietal aromas.

## 1. The Aromatic Potential of Warm Climate Wines

Professor Eduardo Agosin, from the Pontificia Universidad Católica de Chile, presented a summary of his research team’s work on aroma precursors. Yellow Muscat and Early White Muscat, two rather rare varieties in northern Chile, where Pisco (a young brandy with a fruity nose) is produced, are especially rich in terpene precursors. In the same region, the study of aroma potential on more than 50 samples of Muscat of Alexandria and Muscat Rosé revealed that these varieties are high in aglycones from the terpene family, as well as C13-norisoprenoids, which confer floral notes. Carménère (figure 1), the varietal emblematic of Chile, is high in C13-

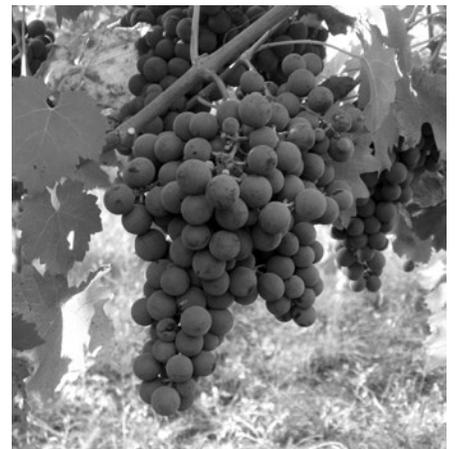
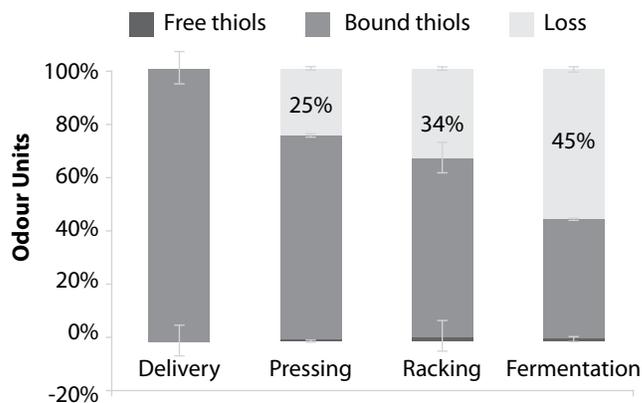


Figure 1. Carménère varietal

norisoprenoid precursors, which encourage the development of a rich aroma profile.

The influence of the *terroir* on the aroma potential of Sauvignon Blanc grapes was also shown. For example, in the Casablanca valley (a temperate climate and later harvest), produces grapes that were two and four times higher in 4-methyl-4-mercaptopentanone (4MMP) and 3-mercapto-hexanol (3MH) than those of Curicó valley, where it is very hot and vines are planted very densely.

Other research has revealed that mechanical harvesting causes a greater loss of aroma precursors than hand harvesting. At the pre-fermentation stages, the thiol precursors continue to degrade (figure 2). By comparing the measurements taken on the grapes at their delivery to the winery and in the final wine, immediately after fermentation, we can see that only 2% to 5% of the initial level of active and odorant thiols remain in the final wine. The level of the thiol precursors in the grape transforming into free thiols is very low. Even though the yeasts, during fermentation, release thiols, the concentration varies considerably from one strain to another, and we observe a loss of 25% to 45% of the initial value. Understanding the evolution of the thiol precursors at the pre-fermentation stage and during alcoholic fermentation



**Figure 2.** Evolution of free and bound 3-mercapto-hexanol (3MH), as a percentage of total 3MH at delivery to the winery and during different stages of winemaking with Sauvignon Blanc grapes from Casablanca (2006 harvest)

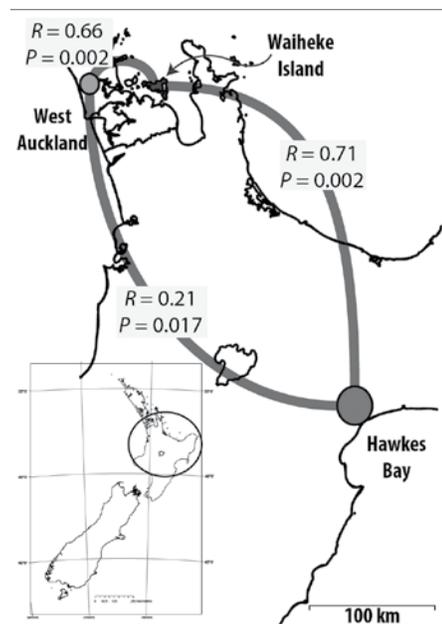
(AF) is essential to optimizing their impact on the final quality of the Sauvignon Blanc wines, much more than seeking to increase their concentration in the grape.

## 2. How to Exploit the Microbial Ecosystem of the Wine

Professor Matthew R. Goddard, from the University of Auckland, New Zealand, presented concepts and techniques from the ecology of microorganisms, explaining their particular interest in the field of oenology. Although we have considerable knowledge about different strains of *Saccharomyces cerevisiae*, there is still much to learn about the phenotypic and genetic diversity of

yeasts, as well as their distribution in space and in time. For example, are the yeast species associated with vines and wines the same in every region of the world? If not, how and to what extent do they differ from one region to another? Are yeasts involved in defining *terroir*? To better understand the distribution of yeasts in space and time and what links them to the vines and wines, Professor Goddard proposes sampling procedures, statistically useful tests and appropriate analysis techniques.

Figure 3 outlines observations from research by Gayevskiy and Goddard (2012) on six vineyards located in three regions in northern New Zealand. During this study, 1,566 colo-



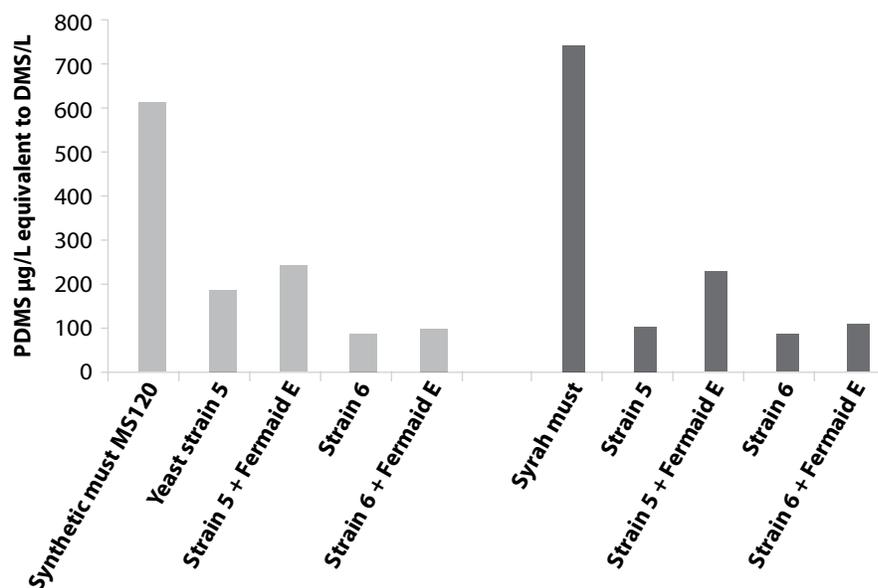
**Figure 3.** Outline of observations from the study by Gayevskiy and Goddard (2012) on six vineyards located in three regions in northern New Zealand

nies were identified and underwent analysis for similarities (ANOSIM), which revealed that, objectively, for the 2010 vintage randomly sampled fungal communities from ripe Chardonnay grapes showed a much greater difference than would be expected by chance (R values are significant according to pairwise comparisons). The biodiversity of microorganisms, linked to different regions and *terroirs*, is still to be explored as much remains unknown.

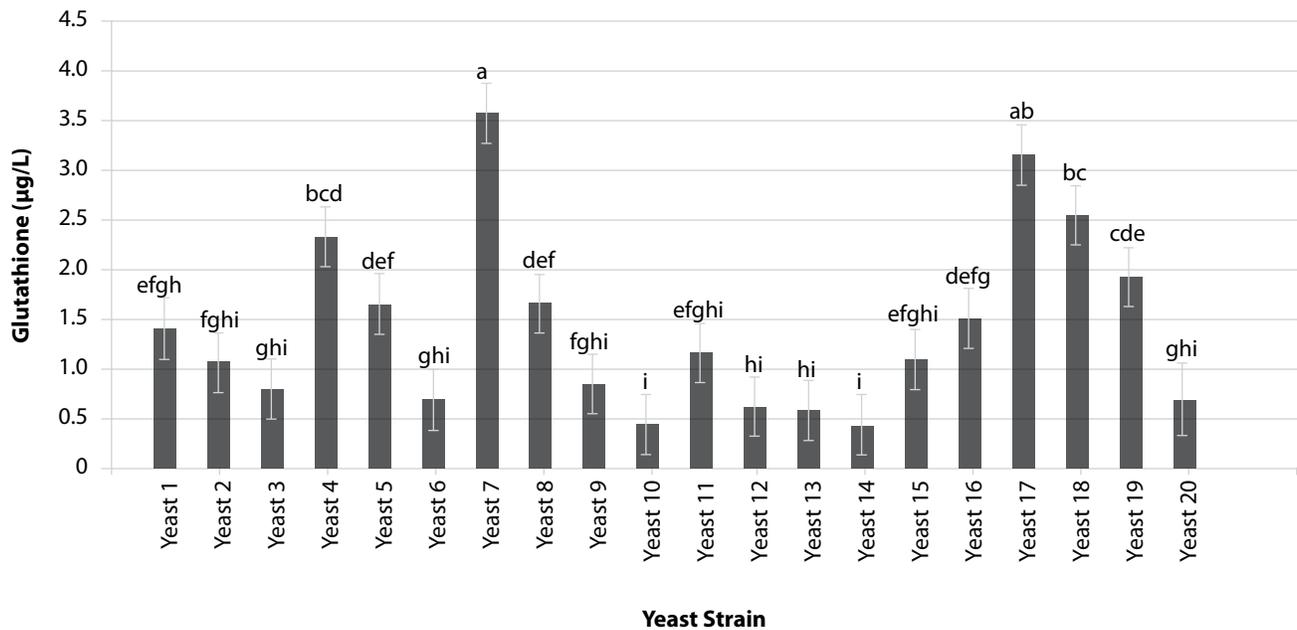
## 3. Influence of Enzymes on the Aroma Typicity of Touriga Nacional

The “bergamot” aroma signature of the Touriga Nacional varietal is attributed to terpenol, linalool and their acetates. Research by Frank Rogerson and Charles Symington (Symington Family Estates, Portugal) showed the interest of pectolytic enzyme preparations rich in  $\beta$ -glucosidase activity on the level of key odorant molecules of Touriga Nacional grapes. The red wines treated with these preparations present a greater floral and citrus aroma complexity, which is maintained throughout aging in bottles for 2.5 years. These results suggest that not only the terpenols, but other aglycone precursors as well, are released and play an important role in the aroma complexity of the varietal. Winemakers should learn to master the potential of using enzymes by determining the appropriate dose and the best timing to obtain the desired wine style.

The best moment to add an enzyme prepara-



**Figure 4.** Influence of the addition of Fermaid E on the consumption of potential dimethyl sulphide by different strains of yeast during alcoholic fermentation



**Figure 5.** Reduced glutathione (GSH) concentrations at the end of alcoholic fermentation after adding 20 different commercial *Saccharomyces cerevisiae* strains. Vertical bars denote 95% confidence intervals for the means. Letters indicate significant differences on a 5% ( $p < 0.05$ ) significance level (Kritzinger et al. 2013b, reproduced with the permission of the Australian Journal of Grape and Wine Research).

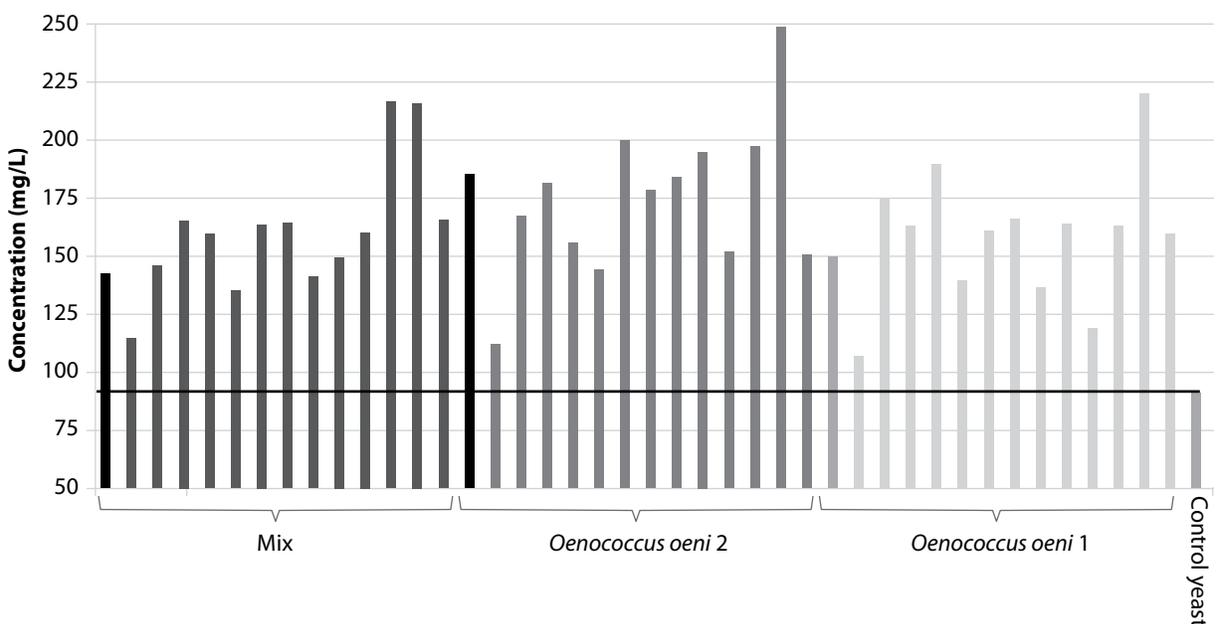
tion high in glycosidases is following sulphiting and after malolactic fermentation (MLF), when the winemaker is ready to evaluate the aroma impact of AF and MLF on the wine profile, and to judge the remaining need to enhance the complexity of the wine.

#### 4. Managing the Dimethyl Sulphide Concentration in Bottles

The work of Laurent Dagan (Nyséos) and Rémi Schneider (Institut Français de la Vigne

et du Vin [IFV]) focused on dimethyl sulphide (DMS), associated with the perception of herbaceous notes, truffle, cabbage, undergrowth and green olive in wine aromas, where it also enhances the fruity character. Most of the DMS disappears with the CO<sub>2</sub> during fermentation; the levels at the time of racking are generally situated under the perception threshold. The goal of Dagan and Schneider is to identify and evaluate the winegrowing factors (e.g., hydric stress,

maturity, production site, foliar spraying and varietal) that influence the potential DMS (PDMS), of which the most important are maturity and the varietal. However, during the study, the fermentation parameters appeared to have a more determining impact on the concentration of PDMS at racking. At the pre-fermentation stages of operations, such as macerating on skins and *stabulation* on lees, the solution for extracting PDMS, located mainly in the skin, remains to be



**Figure 6.** Total ester concentration in wine after malolactic fermentation using 14 different yeast strains and three lactic acid bacteria starter cultures in co-inoculation and a control that was not inoculated for MLF, in a 2011 Merlot (Schöltz 2013)

explored. The fermentation conditions (e.g., yeast strains, nitrogen nutrition, fermentation temperature, fermentation adjuvants) also have an impact on PDMS. The choice of yeast strain and the management of nitrogen supplementation, notably, are the key parameters to limit PDMS consumption by the yeast during fermentation (figure 4).

Two strains of yeasts, L5 and L6, were inoculated into MS120 synthetic must and a Syrah must with and without the addition of Fermaid E, a nitrogen nutrient designed to prevent a possible deficiency in the must. Adding this nutrient (5 g/hL) contributed to preserving a part of the PDMS. In the must with the L5 yeast, the percentage of residual PDMS increased from 12% to 29%, which shows that the addition of nitrogen has a positive influence on the consumption of PDMS by the yeast. With L6, the increase was not as marked, which confirms the specificity of certain strains to assimilate the PDMS.

Indeed, the mastering of DMS in the wine can also occur through the management of PDMS during blending. As for aging, the correlations obtained allow us to predict the approximate percentage of releasable DMS, but this model must be refined through better knowledge of the influence of storage conditions on the percentage of DMS released. Already, new data on managing DMS levels in wine allow us to imagine integrated production processes better adapted to the style of the final product.

### **5. Glutathione – An Important Antioxidant**

E. C. Kritzinger presented a study she conducted in collaboration with Dr. Wessel du Toit, C. Coetzee, and M. Gabrielli, from the University of Stellenbosch, South Africa, and with D. Fracassetti, from the University of Milan, Italy, with the aim of better understanding the evolution of glutathione (GSH) during AF and verifying the effects of a wide range of oenological factors on the levels of GSH in Sauvignon wine, which is subject to oxidation. GSH has a protective effect of the wine aroma during aging. The researchers examined the effects of adding various combinations of O<sub>2</sub> and SO<sub>2</sub> to the must on the GSH concentration in the must and in the Sauvignon wine.

The wine made with oxidized juice and no SO<sub>2</sub> presented significantly lower GSH con-

centrations. Twenty commercial strains of *Saccharomyces cerevisiae* were inoculated into synthetic solutions of grape juice, and GSH levels were measured after AF. Significant differences were observed among the results obtained for the different strains (figure 5).

Sauvignon juices containing various levels of GSH were also analysed. During AF, significant trends appeared regarding the evolution of GSH, according to the yeast strain utilized and the initial GSH concentration in the juice. Sometimes the GSH concentration increased over the initial level, which appears to indicate that the GSH is synthesized and secreted by the yeast.

Commercial preparations of GSH-enriched inactivated dry yeasts (GSH-IDY) were added at different stages of fermentation. The antioxidant potential of these preparations is highly variable. The analyses showed that the addition can lead to higher GSH levels in the wines, as long as the addition is made during the first third of fermentation.

The mechanism by which these preparations influence the yeast metabolism and contribute to increasing GSH levels, as well as their impact of the sensory profile of the wine, remains to be explored.

### **6. The Impact of Wine Bacteria and Different Malolactic Fermentation Scenarios on the Aroma Profile**

Professor Maret du Toit, from the University of Stellenbosch, South Africa, presented the results of trials carried out in collaboration with Caroline Knoll and Doris Rauhut, from the Geisenheim Institute in Germany, for the purpose of evaluating the impact of wine bacteria and different MLF scenarios on wine aromas.

Changes to the aroma profile during MLF depend on different factors, such as the varietal and the aroma precursors, and on specific parameters of the wine (notably the pH and ethanol level, which exerts a strong influence on the bacterial viability and activity). The choice of yeast strain for co-inoculation also has a major impact on the final aroma and style of the wine (figure 6). In addition, wine bacteria synthesize and hydrolyze the aromatic esters in various ways during MLF, according to the strain utilized. Among the four species of oenological bacte-

ria generally present in wine, *Oenococcus oeni* is the bacteria best adapted to this hostile environment and the one most often utilized. However, *Lactobacillus plantarum* is the one most often utilized in fermentations destined for high pH wines and for co-inoculation. *L. plantarum* produces different ester concentrations. What's more, this bacteria has the capacity to release monoterpenes through its  $\beta$ -glycosidase activity. The aroma profile depends on the timing of inoculation. The winemaker can get the most from all these factors to adapt the wine style to consumer preferences.

### **7. Volatile Compounds and the Perception of the Aroma Attributes of Wine**

Dr. Ana Escudero, from the Universidad de Zaragoza, Spain, reviewed current knowledge and presented the latest findings on the aroma chemistry of wine.

Her research focuses on the key odour molecules perceived in wine. The aromas extracted through gas chromatography-mass spectrometry (GC-MS) are the basis for a list in which they are divided into two groups – qualitative aromas and quantitative aromas. A series of sensory analyses followed with the purpose of reconstructing the wine aroma, which gave rise to a new classification system.

According to this system, all the wines share a common basic aroma structure that includes ethanol and 27 aroma compounds, most of which are by-products of fermentation. When combined, these compounds release the aroma characteristic of wine and form an “aroma buffer” that has the capacity to buffer several existing odorants, particularly those associated with fruity notes. The ability of the different aromatic chemical compounds to break this olfactory buffer and modulate the aroma profile of the wine, as well as the link between the aroma note transmitted and the aroma of the chemical compound from which it results, are the basis for determining the contribution of the aroma compounds in the aroma profile of the wine. Thus, a compound could be qualified as a genuine (high-impact) contributor, a major contributor, a secondary (subtle) contributor, an aroma enhancer or an aroma depressor in the wine.

## **TO SUMMARIZE...**

All new knowledge, every discovery related to wine aromas and the multiple factors that modulate their expression and participate in their balance make us realize the remarkable complexity of this field of research. The perspectives offered by current research are fascinating. The constant improving of our understanding of the effects of microorganisms and their derivatives on the varietal aromas of wine generates the ideas and the tools that will help winemakers modulate and define the style desired for their final product.

References available upon request.

You can obtain a copy of the proceedings of the XXIV<sup>es</sup> *Entretiens Scientifiques Lallemand* from your Lallemand representative.