

The Infinite Choices

The microflora of grapes form a complex biological system where a great variety of bacteria, yeasts and other fungi interact in multiple ways. Then, during the pressing of the grapes, the biofilm on the grapes' surface is put in contact with the juice. With the contribution of the microorganisms in the winery, this new environment is a fully active medium. Through the complex interactions of inhibition and stimulation occurring among the yeasts and bacteria, the must is gradually transformed into wine.

Although the process has not changed since ancient times, at the end of the 19th century – thanks to the research on microorganisms and fermentation by Charles Cagniard de La Tour (1838) and, of course, Louis Pasteur (1860, 1867) – empiricism yielded to modernism and the development of the first techniques to control winemaking. Our knowledge of winemaking microorganisms has surged. By identifying them and studying their metabolisms from the vine to the wine, we have established the fundamental rules of the transformation of must and the contribution of microorganisms to the quality of wine. This microbial consortium depends above all on its environment – the conditions of the climate, soil, grapevine and the *terroir*. It evolves over time, during the ripening of the grapes, in the must and during alcoholic fermentation.

Certain species are omnipresent; others develop in very special conditions. Research on the evolution of non-*Saccharomyces* yeasts shows that they can persist. Researchers are learning that other species can be involved in the transformation of must in a complementary way. Indeed, some species enrich the must with metabolites that have a positive impact on the aroma profile of the wine. Non-*Saccharomyces* yeasts were long considered harmful to alcoholic fermentation, but recent research shows they can have real potential for differentiating wines. Recent progress in molecular biology has led to the discovery of the roles played by various non-*Saccharomyces* yeasts and the realization that they can have an exciting impact on the final composition of wines,

even enhancing the complexity of the aroma profile. Interest in non-*Saccharomyces* yeasts has been growing for several years, and the increased understanding of their behaviour has also brought us to a greater appreciation of the potential of all yeasts. Wine microbiologists continue to study how something so small can transform a simple grape must, with all its potential, *terroir*, history – and the winemaker's magic touch – into such precious nectar.



The choice among different microorganisms broadens as our knowledge broadens, for non-*Saccharomyces* and *Saccharomyces* yeasts at their best.

In this issue of Oenomag, Lallemand invites you to discover original yeasts with incredible oenological potential as well as a specialty inactive yeast that is just revolutionary. And this is just the beginning of our investigations into this new approach to winemaking . . .

Cheers!

The Biodiversity and Microbial Flora of Wine: The Infinitely Small Is Still Infinitely Surprising!

The diversity of *Saccharomyces cerevisiae* may have been at the heart of oenological advances and have permitted a large choice for winemakers for many years, but exploring the realm of non-*Saccharomyces* is newer and offers still undiscovered possibilities.

A short history lesson

The growing number of articles and publications about non-*Saccharomyces* yeast in the last few years (from 50 in 2005 to more than 300 in 2011!) is proof enough that the subject has become the latest craze in oenological research. But it would be wrong to assume that the biodiversity of wine microflora is a recent issue. By the end of the nineteenth century, the work of several scientists (Koch, Malvezin, Muller-Thurgau, etc.) already showed the diversity of microorganisms, using the means available. In 1967, at the 2nd Symposium International de Bordeaux-Cognac, Castelli said, "*Torulaspora rosei* is of interest due to its low production of acetic acid. Using *Torulaspora rosei*, either in pure fermentations or in fermentations mixed with other species, is now so widespread that it seems unnecessary to say more." *Torulaspora rosei* is also known as *Torulaspora delbrueckii*. More than forty years ahead of his time, Castelli was already talking about what some now consider to be one of the greatest advances in modern technology—the use of selected non-*Saccharomyces* yeasts.

Intra-species variability: the case of *Torulaspora delbrueckii*

The decrease in the production of volatile acidity that was talking about almost half a century ago is now a reality thanks to the improvement in microbiological techniques and the expertise of manufacturers like Lallemand, who have provided winemakers with this type of yeast with the same ease of use as classic yeasts. But just as there are a multitude of strains of *Saccharomyces cerevisiae* with high genetic diversity, *Torulaspora delbrueckii* also has the same type of diversity, with the same consequences in terms of behavioural and characteristic differences. The ability to withstand osmotic stress in musts with high sugar content, which explains the very low levels of acetic acid, varies from one yeast to another, and it is important to differentiate between each strain of the same species. As

seen in figure 1, the use of *Torulaspora delbrueckii* allows for a significant reduction in levels of acetic acid produced compared to the *Saccharomyces cerevisiae* control; it is, however, interesting to note that there is also a clear difference between the two strains of *Torulaspora delbrueckii* that were tested. Using the strain TD291™ allows for an even more significant reduction in volatile acidity in this case.

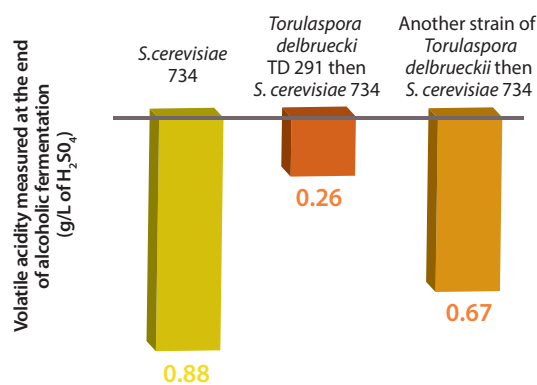


Figure 1: Variability of *Torulaspora delbrueckii* on the production of volatile acidity

Biodiva™, a pure culture of *Torulaspora delbrueckii* TD291™, can reveal less common but essential esters (when all are present at the same time) that will express certain fruity notes such as propionate and ethyl 2-methyl propionate. These two esters are often found in spontaneous alcoholic fermentations where they add to the complexity of the wine. Using Biodiva™ can achieve even greater complexity without the risk of fermentation problems or deviations.

Varietal aromas: *Metschnikowia pulcherrima* (Flavia™) opens up new opportunities

The uniqueness of these microorganisms offers infinite possibilities because of the amazing diversity of behaviours and metabolisms. Take, for example, varietal aromas. They are particularly sought after in wine, but the mechanisms resulting in their production in wines still hold many mysteries.

next page

The Infinitely Small Is Still Infinitely Surprising! (cont'd)

They include terpene compounds that produce floral aromas; norisoprenoids that are described as aroma enhancers and that are responsible, in part, for the perception of fruity notes; and positive varietal thiols that produce citrus and exotic fruit aromas. These compounds are mainly found in the bound form that require a step in which they are released into a free form, and thereby contribute to the wine aroma. The large majority of terpenes, for instance, are bound to two sugars – glucose and arabinose (Günata *et al.*, 1988) – and for them to be revealed requires the combined action of two hydrolytic enzymes – α -arabinofuranosidase and β -glucosidase – that will allow, by cutting the bonds, the release of free odorant terpenes (figure 2).

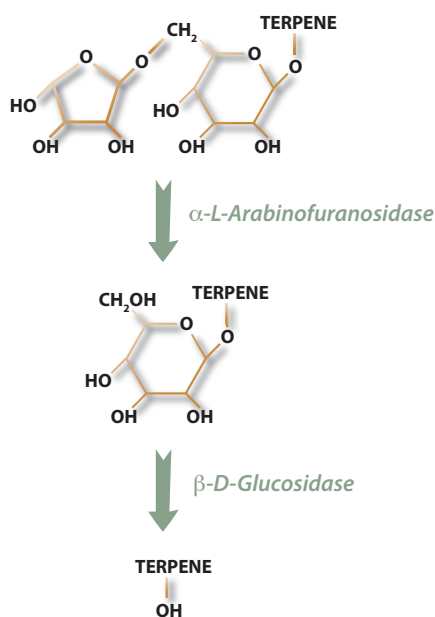


Figure 2: Enzymatic terpene-release mechanism

The α -arabinofuranosidase activity was identified and measured as particularly active in Flavia™, a *Metschnikowia pulcherrima* yeast selected by the University of Santiago (US-ACH) in Chile. Used at the start of alcoholic fermentation with sequential inoculation using the appropriate selected *Saccharomyces cerevisiae* yeast, Flavia™ enhances the release of certain varietal aromas. This ability was verified in many winemaking trials. Figure 3 illustrates

the case of a Muscat in which the use of Flavia™ allowed for a significant increase in the levels of linalool and geraniol, which are responsible for floral notes, and β -damascenone, an aroma enhancer that contributes to fruity aromas.

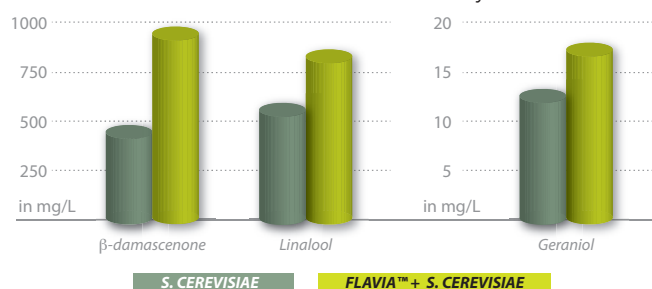


Figure 3: Terpene compounds and norisoprenoids revealed by Flavia™ (2011 Muscat)

Is there a similar interest in varietal thiols?

The mechanisms behind varietal thiols also remain largely unknown. Even if some of their precursors have been identified, all researchers agree that there is still a lot to discover on this topic. And even among the mechanisms that are known, only a fraction of precursors are revealed by the yeast. Various studies show that *Metschnikowia pulcherrima* has a strong aptitude to reveal certain thiol precursors (Zott *et al.*, 2011; Parapouli *et al.*, 2010), so we can assume that Flavia™ also allows the revelation of varietal thiols during alcoholic fermentation. To validate this characteristic, various trials were done in wineries. They showed that indeed, under certain conditions, the levels of thiols obtained with Flavia™ with sequential inoculation using a *Saccharomyces cerevisiae* are much greater than with the same *Saccharomyces cerevisiae* on its own (figure 4).

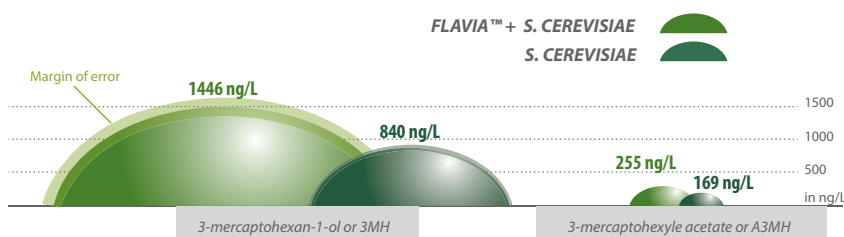


Figure 4: Varietal thiols revealed by Flavia™ (2011 Colombard)

INNOVATIONS

Glutathione-rich specific inactivated yeast : a good allied for clean and fruity wines.

A great number of winemakers seek to provide clean fruity wines to their customers. Specific inactivated yeast rich in glutathione are the latest trends in reliable natural tools to ensure aroma and color preservation.

Oxidation of wines tends to change aromas of fresh fruits to cooked, dried fruits. Specific inactivated yeast rich in glutathione are interesting tools to provide glutathione to the must in order to help protection against oxidation. As glutathione can't be added to must or wine, the use of specific inactivated yeast rich in glutathione (GSH-rich SIY) becomes an interesting natural alternative to optimize the quality of wines. Among Lallemand portfolio, specific wine yeast are chosen for the production of Opti-White®, OptiMUM White® and Booster Blanc® (patent N°WO/2005/080543) and the process from yeast multiplication to inactivation and drying is also adapted in order to get a high content of soluble reduced glutathione (GSH) in the corresponding biomass.

GSH-rich SIY will also vary in the quantities and quality of GSH that they contain. The most important GSH that is needed to be active must be in the reduced form as this is the active anti-oxidant form. Optimum White® has been shown to have the highest quantity of the reduced form (most efficient quality) therefore making it the most suitable to protect against oxidation.

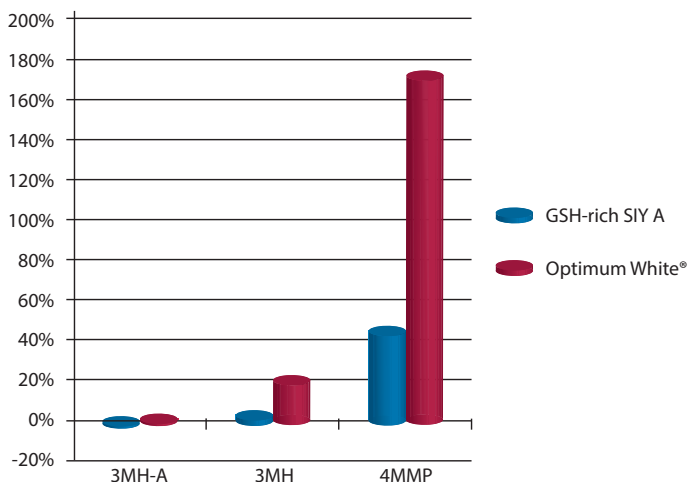


Figure 5: Aromatic thiols levels in 2013 Sauvignon Blanc (New Zealand)

This results in wines that are richer in fruity aroma compounds and more interestingly, thiols compounds (see figure 5). In a trial done in 2013 New Zealand Sauvignon Blanc, higher levels of the aromatic thiols such as 3-mercaptohexanol (3MH) and 4-mercapto-4-methylpentan-2-one (4MMP) are found in the wines where 30 g/hL of Optimum White® was added at the beginning of alcoholic fermentation compared to another GSH-rich SIY. The richer thiols bring to Sauvignon Blanc sought after notes of citrus, grassy, passion fruit aromas.

Add Optimum White® at the beginning of AF, it's better than at the end!!

The timing of addition is particularly important with this tool. In a trial conducted in French Colombard in 2012, it was found that the sooner the Optimum White® is added, the better the impact. Figure 6 shows the impact of the different addition time on the total levels of thiols found in the wines. This trial confirmed the importance of adding the Optimum White® at the beginning of fermentation.

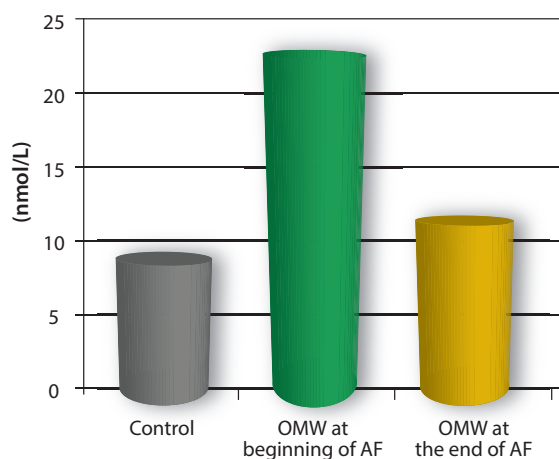


Figure 6: Sum of thiols in 2012 French Colombard with different timing of addition of Optimum White®.

When winemakers seek to preserve those fragile, fruity components in aromatic varieties, the innovative Optimum White® rich in GSH is a proven winemaking aid.

"Wine is bottled poetry."

Robert Louis Stevenson



The popular **Lallemand Wine** app has been optimized for the iPad and is also available for Android users. Please look under Lallemand wine to download this useful tool. The app provides the same set of information corresponding to each market, as well as an advanced search function, and recommendations for varietal pairings.

The sensory profile tool for winemaking bacteria is popular among winemakers, making it easier to choose the right wine bacteria for malolactic fermentation based on winemaking conditions, the style of wine, and the desired sensory profile.

The app also includes a new section called the Knowledge Centre, where you can find technical information and the latest protocols by market. New features will be available shortly, as well as an iPad version.

Next up in Lallemand's oenom@g

A yeast that's at the cutting edge of biotechnological innovation



Following the collaborative work of Lallemand, the ICV, the INRA, and SupAgro, an innovative technology has led to the selection of a new generation of yeast.

Find out in the next Oenomag just how much this innovation at the cutting edge of research can help the 2014 harvest meet winemaking requirements, notably by limiting SO₂ and sulphuric notes produced during fermentation and by controlling the sensory profile of wines.

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Lallemand – a leading producer of wine yeast and bacteria selected from nature, and their nutrients, and a developer of specific enzyme applications – is a privately owned corporation with divisions operating around the world. The Oenology Division, based in Toulouse, France, has a major focus on research and development, both in-house and in collaboration with renowned research institutes.