



SPECIFIC INACTIVATED YEASTS FOR RED WINEMAKING



... TRANSCEND your wine

LALLEMAND

INTRODUCTION

Consumers' demand for fruit driven red wines with deep color and soft tannins is continuing to grow. Meeting this market demand presents a real challenge for winemakers, given that it relies on grape polyphenolic maturity. The balance between technological maturity and polyphenolic maturity is hard to achieve due to climate changes. Viticultural and winemaking practices are evolving, and finding for ways to reach this balance and improve wine quality.

Aging on lees is well established as a great tool to increase the mature character of wines. It's a traditional practice in many areas, for both white and red wines. During this stage of the process, winemakers take advantage of dead and dying yeasts' potential, which is known to involve many contributions related to their autolysis:

- ▶ Increased mouthfeel
- ▶ Protection against oxidation
- ▶ Smoothing of the astringency
- ▶ Color stabilization
- ▶ Improving integration of wood and other inputs
- ▶ Developing new flavors
- ▶ Increased persistence

However this technique also presents some serious drawbacks:

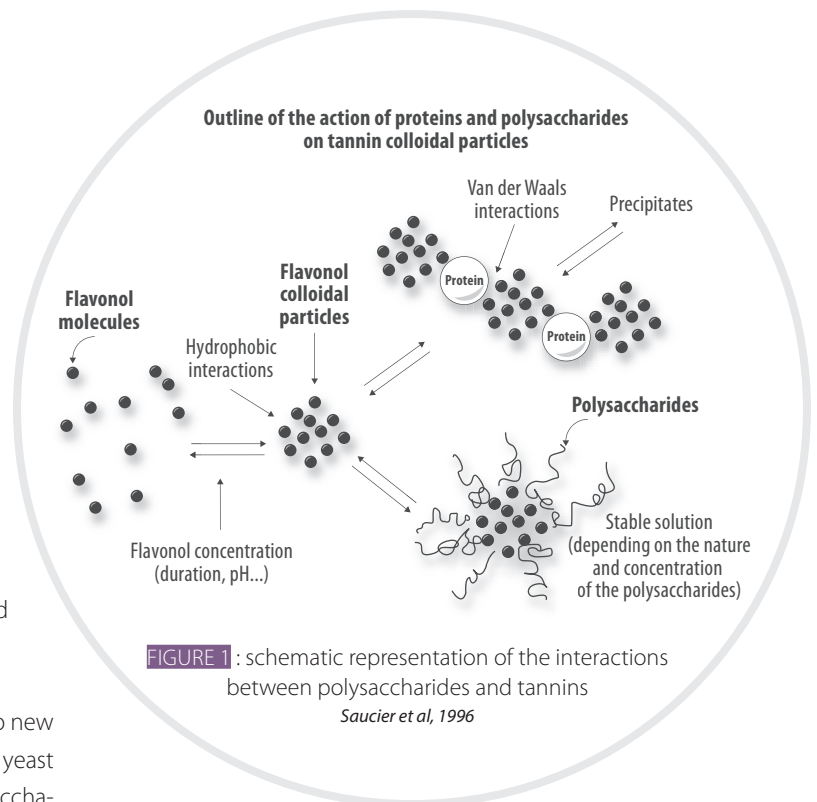
- ▶ Increased risk of *Brettanomyces* spoilage
- ▶ Increased risk of negative sulfur like compounds
- ▶ Slower evolution of the wine

Aging on lees results in wines with a significant decrease in their astringency and an increase in smooth mouthfeel perception (Rodriguez *et al.*, 2005). These effects are due to the enrichment of mannoproteins released by yeasts during autolysis (Klis *et al.*, 2002). Mannoproteins are polymers of mannose with other branches of monosaccharides that contain less than 30% of peptide fractions and are considered like polysaccharides.

In order to avoid the drawbacks of aging on lees, two new strategies have recently appeared. First is the use of yeast strains with a higher capacity for producing polysaccharides (Gonzalez 2006). The second is the addition of specific inactivated yeast that favors the release of polysaccharides (Guadalupe *et al.*, 2007, and Rodriguez-Bencomo *et al.*, 2010), taking into account that this addition can be made at different stages of the winemaking process, namely at the beginning of maceration or towards the end of alcoholic fermentation and throughout aging.

POLYSACCHARIDES AND THEIR IMPACT ON WINE QUALITY

Polysaccharides and tannins: it has long been speculated whether certain polysaccharides might complex with tannins, thus altering the astringency of wines. A model of polysaccharide-tannin interaction has been proposed (Saucier *et al.*, 1996): see FIGURE 1. This model would explain why specific inactivated yeasts, rich in polysaccharides, have the ability to make a smooth wine, increase its volume in the mouth, and stabilize color.



Specific inactivated yeasts (SIY): what are we talking about?

YEAST DEDICATES ITS ENTIRE LIFE TO WINE QUALITY DUE TO:

- ▶ **Their metabolic activity** (all the transformations related to alcoholic fermentation)
- ▶ **Their biochemical composition** (constituting a natural source of wine quality factors): amino-acids, peptides, nucleic acids, aromatic compounds, polysaccharides (mannoproteins & glucans), and micronutrients (vitamins, minerals), which may be beneficial for wine quality throughout the autolysis as illustrated by **FIGURE 2**. Yeast cell-wall structure is of major interest when it comes to an application to increase mouthfeel and soften tannins as the cell-walls are rich in polysaccharides, namely mannans, glucans and mannoproteins **[SEE FIGURE 3]**

SPECIFIC YEAST STRAINS

Yeast gives off polysaccharides during alcoholic fermentation (*Llauberes et al., 1987*); and during aging on lees as a result of cell autolysis (*Feuillat et al., 1989*). It has recently been shown that different strains of yeast vary in their tendency to produce these polysaccharides (*Rosi et al., 1998*).

Use of yeast strains which produce an abundance of polysaccharides, alters the colloid structure of wine and increases the length on the palate, roundness, and sensation of volume. Based on this knowledge and the characterization of the yeast strains in Lallemand's portfolio, specific yeast strains were chosen in order to develop SIY as new tools for winemaking.

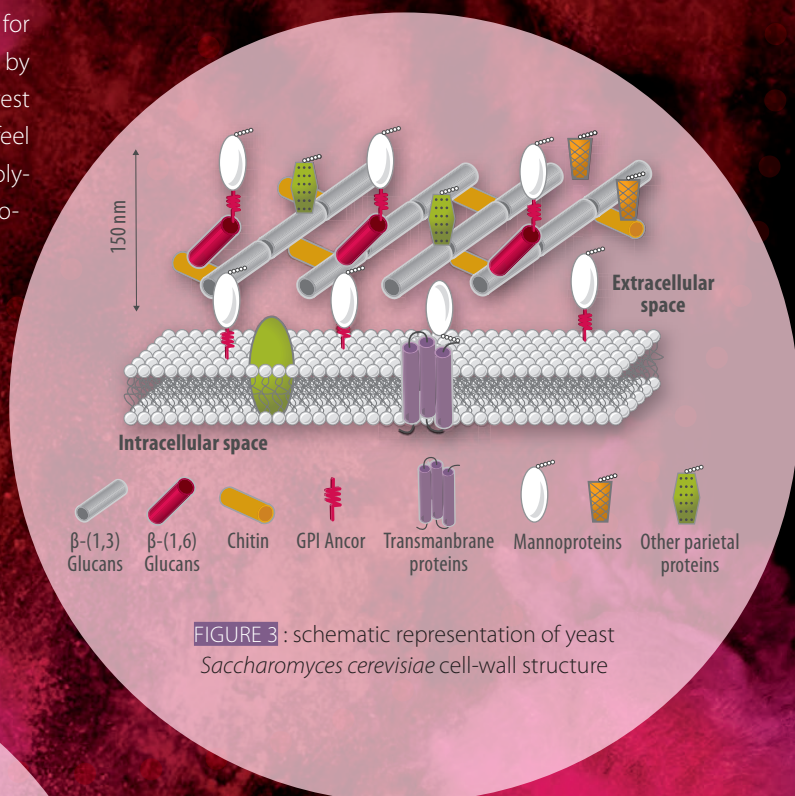


FIGURE 3 : schematic representation of yeast *Saccharomyces cerevisiae* cell-wall structure

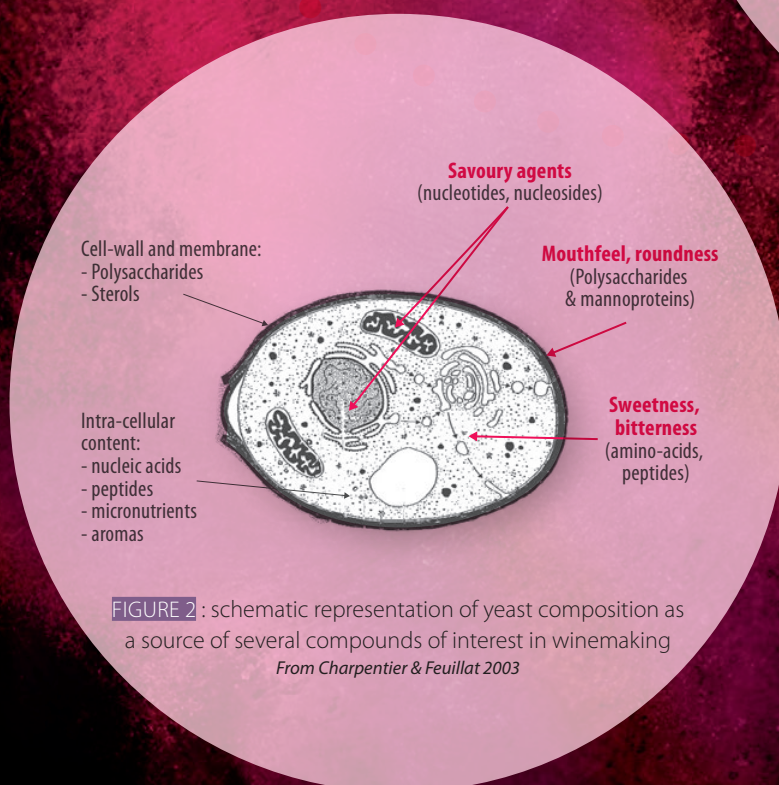


FIGURE 2 : schematic representation of yeast composition as a source of several compounds of interest in winemaking
From Charpentier & Feuillat 2003

SPECIFIC INACTIVATED YEASTS (SIY):

There are diverse applications of SIY in enology:

- ▶ Yeast and bacteria protectors and nutrients;
- ▶ Stabilization and aging tools.

Each Specific Inactivated Yeast developed for winemaking:

- ▶ Is a particular enological yeast strain (non-GMO);
- ▶ Differentiates itself through its qualitative and quantitative content in cell-wall components, soluble mannoproteins and specific components (micronutrients, sterols, glutathione etc.);
- ▶ Is the result of a particular preparation and inactivation process: which enables a quick release of the components of interest.

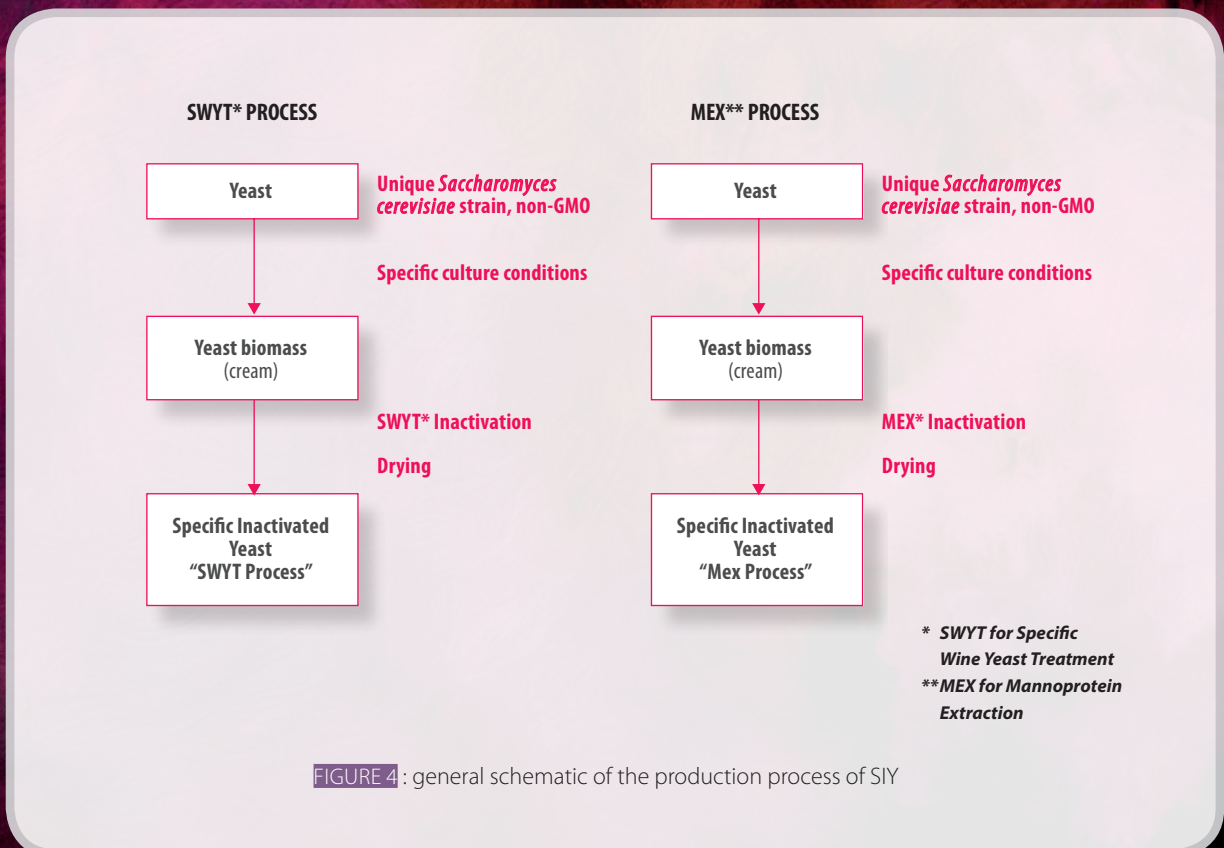
FIGURE 4 shows the main steps for preparing SIY for winemaking. As you can see, there's a common line consisting of:

- 1 ► Production of biomass from a unique enological non-GMO yeast strain, under specific medium and culture conditions.
- 2 ► Towards the end of the exponential growth of the biomass, inactivation is realized in order to stop metabolic activity.
- 3 ► Drying of the inactivated biomass to obtain the SIY product.

Lallemand has developed two specific processes, **MEX** (for "Mannoprotein Extraction") and **SWYT** (for "Specific Wine Yeast Treatment") in order to modulate the final SIY's composition and impact on wine. These processes are applied to the biomass of unique enological yeast strains already known for their polysaccharides production ability.

MEX process was developed and optimized in order to weaken the yeast cell-wall structure to facilitate the availability of high molecular weight compounds.

SWYT Process was developed and optimized in order to preserve cell-wall structure and favor yeast autolysis in order to get a higher release of the lower molecular weight compounds.



COMPARISON OF OUR DIFFERENT LALLEMAND SIY FOR RED WINEMAKING

In a research program carried out by Fernando Zamora in URV Tarragona, a characterization of several of our SIY was performed (*Proceedings of Entretiens Scientifiques Lallemand, Zamora, 2011*).

FIGURE 5 shows the results corresponding to the maceration in a wine model solution with 5 different inactivated yeasts: one inactivated standard yeast, MEX SIY 1, MEX SIY 2, SWYT SIY 3, SWYT SIY 4.

This data confirms that all the specific inactivated yeast products release more polysaccharides and quicker than the standard inactivated yeast. It also seems that MEX SIY 1 and MEX SIY 2 quickly release polysaccharides of high molecular weight (10 to 1100 kDa), whereas SWYT SIY 3 and SWYT SIY 4 release greater amounts of lower molecular weight fractions (<10 kDa).

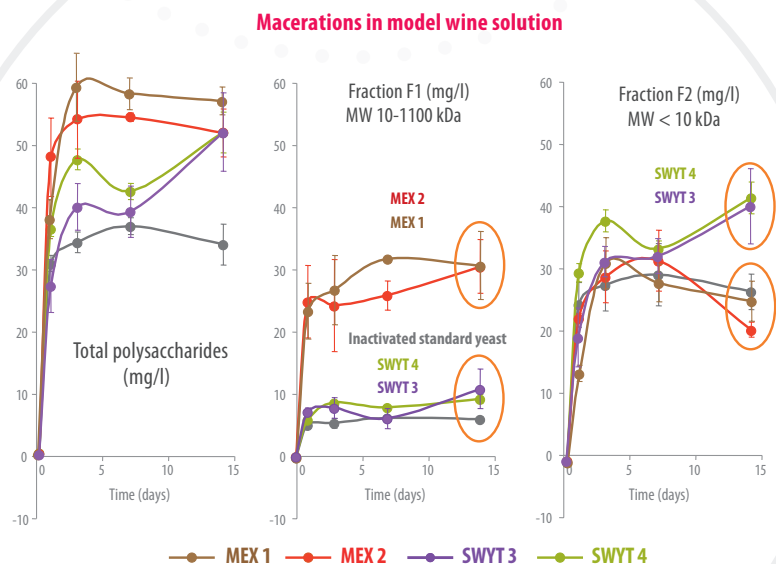
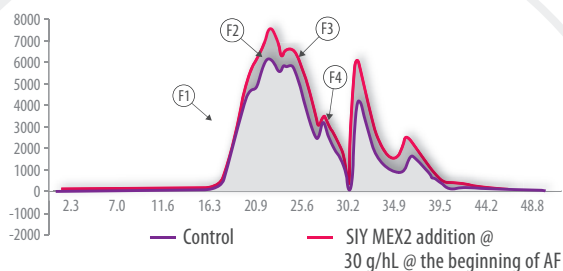


FIGURE 5 : Characterization of the released polysaccharides along maceration into wine model-solution for different types of SIY



Fraction	Control	SIY MEX 2	Variation(%)
(F1) (144-1100 kDa)	132.6 ± 5.7	148.5 ± 4.4	11.9
(F2) (40-144 kDa)	187.6 ± 1.3	254.3 ± 3.8	35.5
(F3) (6-40 kDa)	258.1 ± 13.9	297.9 ± 6.1	15.4
(F4) (1-5 kDa)	95.7 ± 10.7	96.3 ± 25.9	0.7
Total	674.1 ± 46.3	770.3 ± 2.4	14.3

FIGURE 6 : Polysaccharidic profile of the wines fermented with or without the addition of SIY MEX 2 at the beginning of AF

These solutions were later lyophilized and dissolved in mineral water and tasted informally by a panel that judged the MEX SIY 1 and MEX SIY 2 products increased mouthfeel, whereas SWYT SIY 3 and SWYT SIY 4 products provided sweetness. Simultaneously, different trials were carried out under winemaking conditions using Cabernet sauvignon grapes with and without the addition of the three different SIY products.

The results confirm that SIY use, especially SIY from MEX process during alcoholic fermentation, is useful to enrich the wine with high molecular weight polysaccharides as shown for MEX SIY 2 in **FIGURE 6**.

Application of SIY IN RED WINEMAKING

Applying MEX SIY at the beginning of red wine maceration/alcoholic fermentation leads to an increased content of high molecular weight polysaccharides that are involved in the formation of stable complexes with anthocyanins and tannins. This leads to more stable polyphenols (FIGURE 7). The resulting wines have better color, increased volume and lower astringency (FIGURE 8).

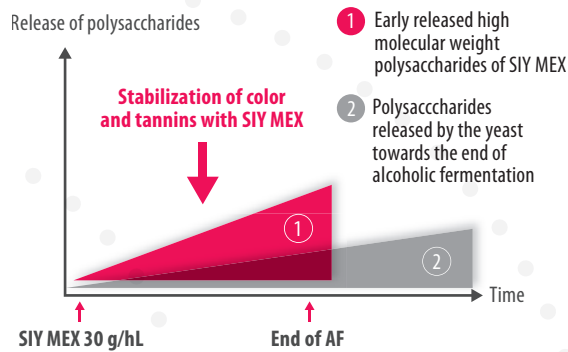


FIGURE 7 : Schematic representation of the advantage of SIY MEX addition at the beginning of alcoholic fermentation in order to get early impact of polysaccharides on color and tannins thanks to the early release of high molecular weight polysaccharides, namely mannoproteins

During aging, the polysaccharides released by SWYT SIY, especially those of low molecular weight, have an impact on the tannin perception. The resulting after months of contact show a higher polysaccharidic content (FIGURE 9) and the sensory analysis revealed a higher voluptuous character of the tannins and a lower astringency (FIGURE 10).

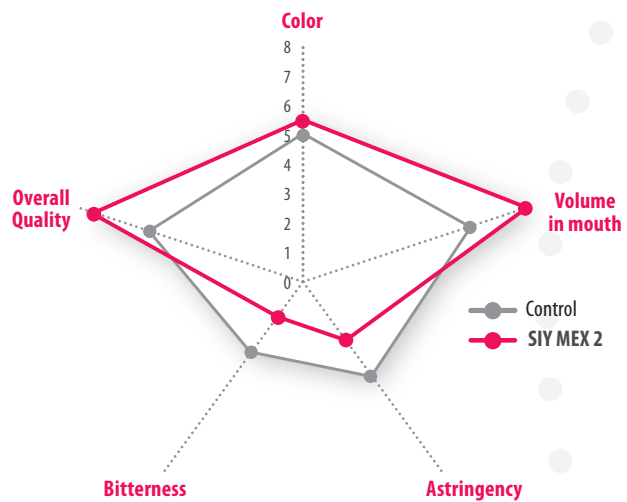


FIGURE 8 : Experimentation on Grenache red winemaking, in collaboration with INRA-Montpellier (France). Comparison of the control wine (no SIY addition) and the treated wine (addition of **SIY MEX 2**) @ 30 g/hL at the beginning of maceration. Sensory analysis by a professional tasters panel of 14 persons.

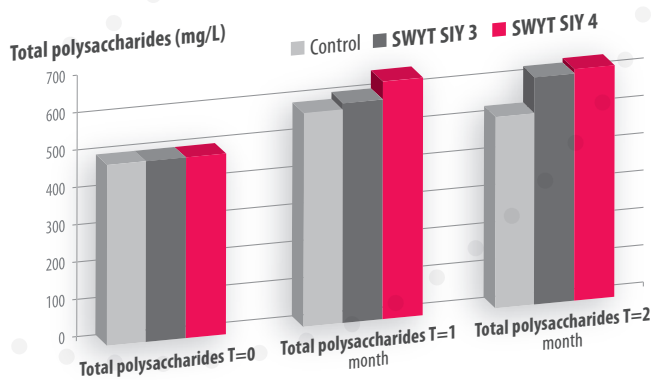


FIGURE 9 : Experimentation on a Merlot Syrah wine, DOC Priorato: Comparison of control and addition of **SWYT SIY 3 & 4** @ 20 g/hL. Analysis of the total polysaccharides content of the wines at the beginning of the experiment and 3 & 4 months after.

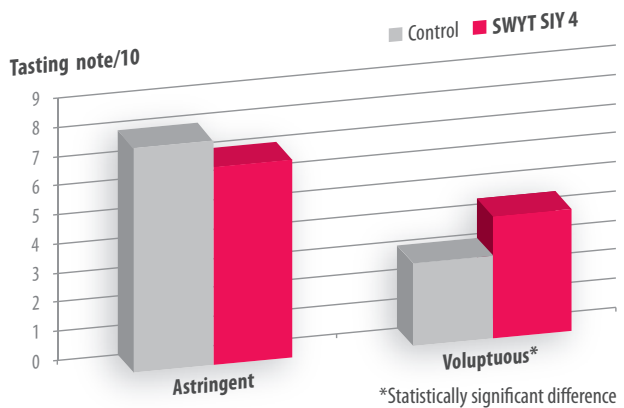


FIGURE 10 : Experimentation on a Tempranillo wine, DOC Ribera del Duero: Comparison of control and addition of **SWYT SIY 4** @ 20 g/hL. Sensory analysis of the wines after 2 months by a trained tasters panel (INRA Montpellier) on "astringent" and "voluptuous/gras" descriptors.

Testimony



Fernando Zamora Marín

Doctor in Chemistry (University of Barcelona, 1989) and Diplôme National d'Oenologue (University of Bordeaux II, 1992). He is now Professor of the Enology Faculty of the Rovira i Virgili University in Tarragona (Spain).

His main research topics are:

- Phenolic compounds, color and astringency of red wines.
- Proteins and polysaccharides of wine; sensory and technological implications.
- Influence of climate change on wine composition and quality; Adaptation of winemaking techniques to global warming conditions.

He has directed 9 PhD theses; he has published 45 scientific articles (peer-reviewed), more than 100 articles in other revues, numerous book chapters and several congress communications.

He exerts as enologist consultant in some wineries in AOC Priorat, Montsant and Cava. He is co-owner of the renowned wine "Espectacle del Montsant".

“ I am convinced that specific inactivated yeasts are an interesting tool to improve wine quality. Our experiences have shown that the employment of specific inactivated yeasts during winemaking increases the wine polysaccharides concentration which has positive effects in some sensory attributes. Specifically we have observed that they are useful for increasing mouthfeel, for smoothing astringency, bitterness and herbaceous characters, and for improving the foamability of sparkling wines. I consider therefore that their use can be suitable especially when grapes are not ripe enough. ”

BIBLIOGRAPHY

Rodriguez M., Lezaun J., Canals R., Llaudy M.C., Canals J.M. and Zamora F. 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.

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

Gonzalez-Ramos D. and Gonzalez R. 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., Palacios A. and Ayestaran B. 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.

Rodriguez-Bencomo J.J., Ortega-Heras M. and Perez-Magarino S. 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.

Saucier C., Roux D. & Glories Y. 1996. Stabilité colloïdale de polymères catéchiques - Influence des polysaccharides. *Conférence proceeding*.

Llaubères R-M., Dubourdieu D., Villettaz J-C. 1987. Exocellular polysaccharides from *Saccharomyces* in Wine. *Journal of the Science of Food and Agriculture*. Vol. 41, Issue 3, pages 277-286, 1987

Feuillat M., Freyssinet M. et Charpentier C. 1989. L'élevage sur lies des vins blancs de Bourgogne. II. Evolution des macromolécules: polysaccharides et protéines. *Vitis*. Vol. 28, 161-176

Rosi I., Gheri A. & Ferrari S. 1998. Effets des levures produisant des polysaccharides pariétaux sur certaines caractéristiques des vins rouges pendant la fermentation. *Revue Française d'Oenologie*. N°172, 24-26.

Zamora F. 2011. Adapting winemaking to warm climate conditions. *Entretiens Scientifiques Lallemand*. N°18, Part 1, Pages 17-24.

Gonzalez-Royo E., Urtasun A., Gil M., Kontoudakis N., Esteruelas M., Fort F., Canals J-M., and Zamora F. 2013. Effect of Yeast Strain and Supplementation with Inactive Yeast during Alcoholic Fermentation on Wine Polysaccharides. *American Journal of Enology and Viticulture*. 64:268-273.

Lallemand SAS

19, rue des Briquetiers - BP 59 - 31702 Blagnac Cedex
FRANCE

Lallemand Inc. Succ. Italiana

Via Rossini 14/B - 37060 Castel D'Azzano - Verona
ITALY

Lallemand Península Ibérica

C/Zurbano 71, Oficina 6 - 28010 Madrid
SPAIN

Lallemand Fermented Beverages

Zeiselberg 18 - 3550 Langlois
AUSTRIA

Ferment Zagreb

Vincenta iz Kastva 17 - 10 000 Zagreb
CROATIA

Lallemand North America

PO Box 5512 - Petaluma - California 94955
USA

Lallferm S.A.

Pedro Molina 433 - Primer Piso, Oficina 2 - Mendoza 5501
ARGENTINA

Lallemand Inc. Chile

Camino Publico Los Siete Puentes s/n - Bodega N°6
Los Lirios - Requinoa - Rancagua
CHILE

Lallemand Australia Pty Ltd

23-25 Erudina Ave, Edwardstown, South Australia 5039
PO BOX 210, Edwardstown, SA 5039
AUSTRALIA

Lallemand South Africa

31 Blousuikerbos Street - Proteavalley - BELLVILLE - 7530
REPUBLIC OF SOUTH AFRICA