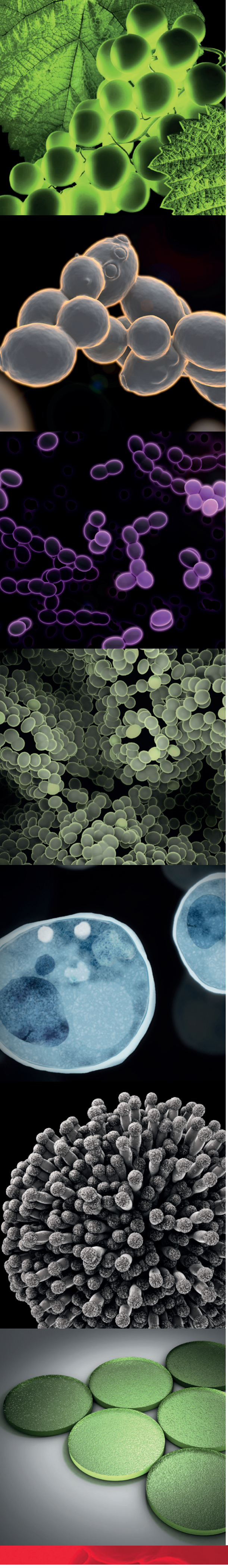

Unique specific autolysate to help with Pinot noir colour and texture management

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Introduction

Consumer demand for fruity red wines with intense colour and good mouthfeel continues to grow. Meeting this demand in Pinot Noir winemaking is challenging, especially in terms of colour and texture management. Pinot Noir grapes exhibit a peculiar polyphenolic composition: low total anthocyanins, no acylated or coumarylated anthocyanins, and a high tannin content that comes mostly from seeds (Mazza et al., 1999). Winemaking practices such as cold maceration and aging on lees are well-established methods for dealing with the characteristics of this varietal. Indeed yeast and Pinot Noir wines have been closely linked for decades for better (colour stabilization, texture/balance improvement…) and for worse (*Brettanomyces* and other undesirable microorganisms, sulfur offflavours).

With respect to colour and texture, research on the impact of different yeast strains has illustrated how yeast impact on tannin content and colour intensity is strain-dependent (Carew et al., 2013). As such, yeast-derived winemaking and aging tools offer an opportunity for colour and texture improvement.

Aging on lees is a widespread traditional winemaking technique aimed in part at reducing astringency and bitterness while increasing body and aromatic length and complexity. Aging on lees can also help stabilize the colour of red wines. During this step, winemakers reap the many well-known benefits—including the release of mannoproteins— provided by adding dead or dying autolyzed yeast (Rodriguez et al., 2005). To avoid the inconvenience of traditional aging on lees, a practice has developed over the past 15 years where specific inactivated yeasts are added to promote the release of polysaccharides (Guadalupe et al., 2007, and Rodriguez-Bencomo et al., 2010). The concept that certain polysaccharides can bind with tannins and thereby reduce the astringency of wines has been around for a number of years.

In the past two decades, specific inactivated yeast (SIY) products have been developed in order to provide tools to modulate wine astringency and improve wine texture. More recently it has been evidenced that, depending on the process applied to yeast biomass, yeast-derivate fractions can differ in terms of composition and solubility, thus impacting wine quality differently (Mekoue et al., 2015). Polyphenols can interact in different ways with these fractions. Phenolic oligomers and polymeric tannins are the major polyphenols involved in interactions with SIYs. The initial finding that polyphenols are absorbed in the yeast cell wall gave way to the more recent discovery of their massive trapping in the yeast's internal space, followed by the precipitation demonstrated by Mekoue et al in 2015. Intracellular compounds can lead to the formation of either large aggregates with a following precipitation, or the formation of soluble complexes that remain in solution.

A recent study focused on the interactions between mannoproteins and grape or wine polyphenols was conducted at the INRA Montpellier (Science Pour l'Oenologie research unit) (Mekoue et al., 2016). Interactions in solution between grape skin tannins with an average degree of polymerization of 27 and yeast parietal mannoproteins led to the formation of finite-size submicronic aggregates that were stable over time and remained in suspension. These findings support the hypothesis that mannoproteins released by specific inactivated yeasts can help improve the taste of red wine by binding with tannins. It is likely that using this type of product (high in mannoproteins) at the beginning of the winemaking process will limit aggregation of tannins and anthocyanins early on, thus improving the colour and mouthfeel of red wine. Recent scientific advances have provided more precise tools for characterizing wine yeasts and their products, leading to the development of a new yeast autolysate (MEX-WY1) with unique mannoprotein properties based on an innovative combination of a special strain of Saccharomyces cerevisiae (WY1) and a specific inactivation process (MEX).

Development of the specific yeast autolysate

Physico-chemical characterization of the specific yeast autolysate (MEX-WY1)

Specific yeast strain with special parietal mannoprotein properties evidenced by atomic force microscopy

In recent research conducted in partnership with INSA Toulouse, atomic force microscopy (AFM) was used to characterize properties of wine yeast cell walls (Schiavone et al., 2014). Wine yeasts that displayed strong mannoprotein- producing capacity were selected and AFM used to explore the unique properties of the WY1 strain of *Saccharomyces cerevisiae*. Figure 1 shows AFM topographical images of two cells of the WY1 and WY2 strains (Fig. 1A and 1B) and corresponding images of their adhesion (Fig. 1C and 1D). WY1 was particularly adhesive, and due to its high mannoprotein content and the length (average length: 96.9 nm) of its mannoprotein chains stretched on the cell wall (Fig. 1E and 1F), it interacted strongly with the lectin Concanavalin A.

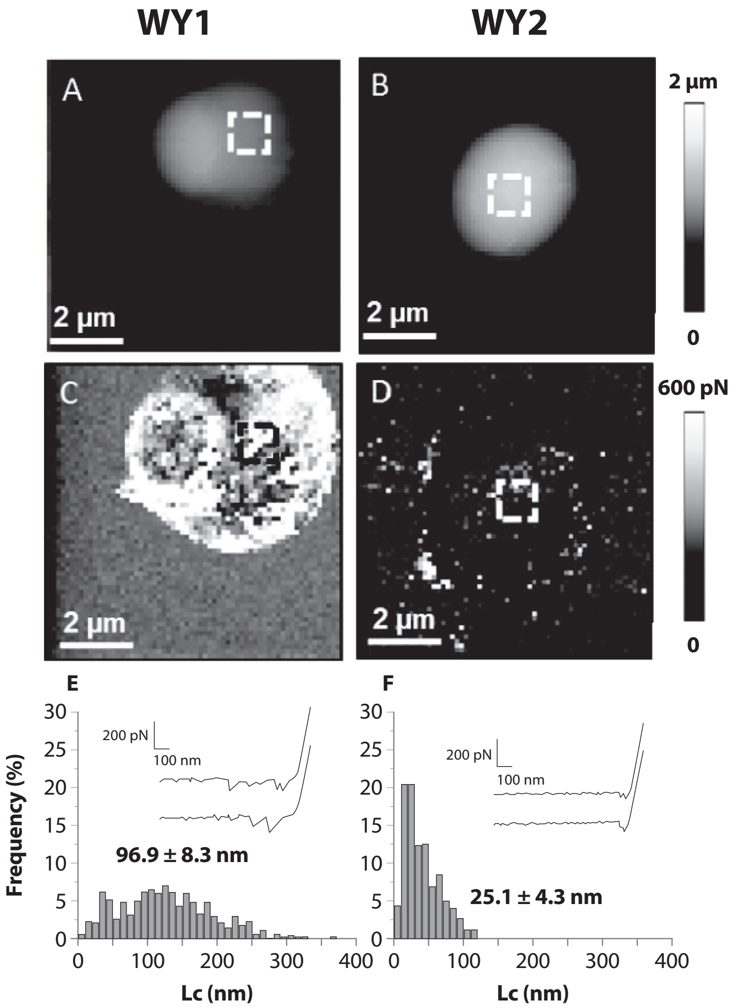

Figure 1. AFM images of the height (A, B) and adhesion (C, D) of strains WY1 and WY2. Distribution and average total length (Lc) of mannoproteins fully stretched on yeast cell walls.

An innovative inactivation process combined with a unique yeast strain leading to an original autolysate with specific properties

Various autolysis conditions and thermal or physicochemical inactivation procedures were applied to the WY1 yeast to release its high content and long chain mannoproteins. Following several screening and optimizations in the lab, a specific physicochemical treatment was selected (MEX process) for its ability to disrupt yeast and release high molecular weight parietal mannoproteins. Figure 1 shows transmission electron microscopy (TEM) images from autolysates obtained through a classic thermal process (Fig. 2.A = SWYT-WY1) in comparison to the MEX treatment (Fig. 2.B = MEX-WY1). The autolysates obtained through thermal and physicochemical treatments had very different appearances. Although thermally inactivated WY1 yeasts maintained a certain cellular integrity and were more than 60% insoluble, physicochemical inactivated yeasts using the MEX process released more components that were 80% soluble. Size exclusion chromatography (SEC) confirmed that the MEX soluble fraction contained a high level of high molecular weight polysaccharides compared to the classical thermal process (Figure 3).

2.A: SWYT-WY1 **2.B: MEX-WY1**

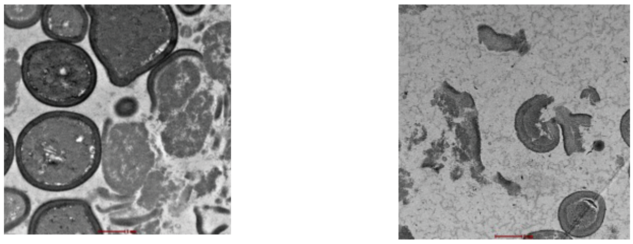

Figure 2. Microscopic (TEM) images of yeast derivatives produced either with a classical thermal process (A, SWYT-WY1) or a specific inactivation process (B, MEX-WY1).

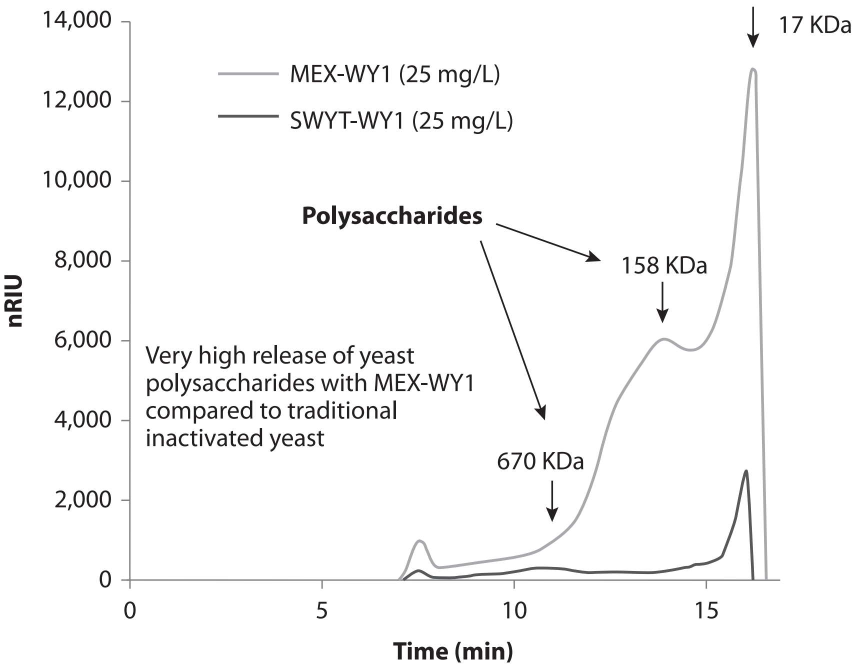

Figure 3. Size exclusion chromatography of SWYT-WY1 and MEX-WY1 soluble fractions

Exploring into the action mechanism

Further experiments were undertaken at lab-scale in order to determine the mechanism of action of MEX-WY1 autolysate interactions with polyphenols extracted from Merlot grape skin. Interaction experiments were performed in a synthetic must with added Merlot grape skin polyphenols and the soluble fraction of the yeast autolysate (MEXWY1- S) at a dose rate equivalent to the application of 30 g/hL of the total MEX-WY1. After 24 h contact (stirred at ambient temperature), samples were centrifuged and the supernatants were analyzed. Total Polyphenols (TP) and Total Red Pigments (TRP) were determined using UVvisible spectrophotometry, and BSA precipitable tannins and polymeric pigments were determined according to the procedure described by Boulet et al. (2016). Absorbency differences at 280 (ΔA 280) between the untreated and BSA-treated wines indicate the amount of tannins and pigments the protein (BSA) precipitated.

Interactions between polyphenols and MEX-WY1 soluble components did not lead to visible aggregation and precipitation. Only a small measurable decrease of the TP and TRP indexes was observed (around 5% of TP and 6% of TRP) between the control (synthetic must + polyphenols alone) and the samples after interactions.

BSA precipitation determination showed a lower precipitation of tannins with the addition of the whole MEX-WY1 soluble fraction (Fig. 4 A) compared to the control. This would suggest a reduction of astringency with the addition of the specific autolysate. The very low PT and TRP decrease indicated the formation of stable complexes with high molecular weight tannins and pigments. This stabilization of polyphenols in solution by MEX-WY1-S could enable colour stabilization during fermentation and a reduction in astringency, as their complexation with autolysate's soluble components would make tannins unavailable to interact with salivary proteins that are involved in astringency perception.

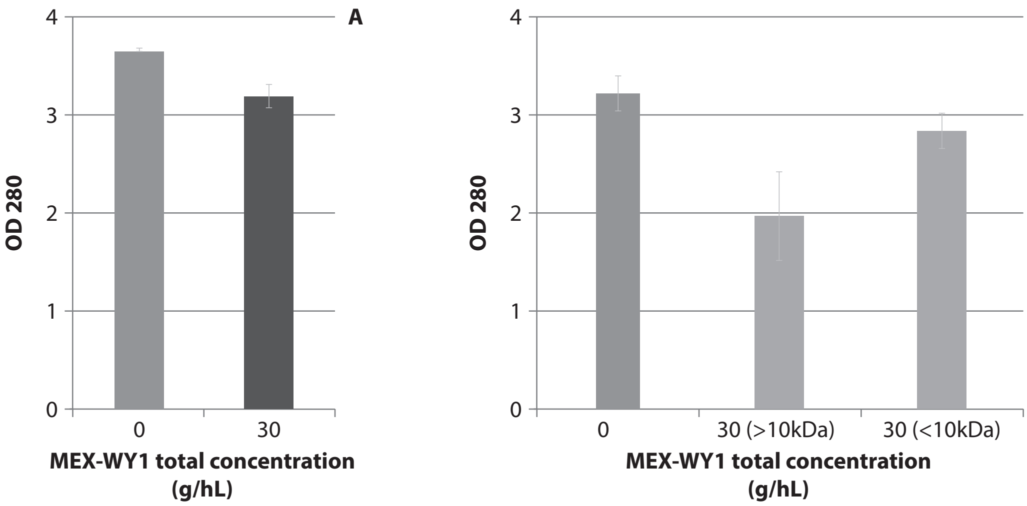

Figure 4. Evaluation of BSA-precipitable tannins (OD 280 nm) after polyphenol interactions with whole MEX-WY1 soluble fraction (A), low molecular weight (< 10 kDa) and high molecular weight (> 10 kDa) soluble fractions (B). MEX-WY1 soluble was added for interaction experiments at an equivalent concentration of total MEX-WY1 of 30 g/hL.

To identify the specific soluble component involved in these interactions, MEX-WY1-S was fractionated into low (< 10 kDa) and high (> 10 kDa) molecular weight fractions and interactions with polyphenols were performed.

The MEX-WY1-S autolysate was able to reduce tannin precipitation after BSA addition. This would indicate a lower precipitation with salivary proteins, thus a lower astringency. When fractionated, the high molecular weight components were more effective regarding the reduction of tannin precipitation. (Fig. 4 B).

Thus, these studies have demonstrated the role of macromolecules in MEX-WY1 autolysate in wine quality improvement, specifically colour stability and astringency. These macromolecules are mainly composed of mannoproteins with unique properties, obtained through the combination of a special yeast strain and a specific inactivation process.

Beyond the science, proof of impact in winemaking conditions

The final step in this study was to evaluate the performance of the MEX-WY1 specific autolysate under red winemaking conditions.

To study the effect of adding the specific autolysate MEXWY1under large-scale production conditions, numeroustrials were conducted at pilot scale (1 hL) and production(50-200 hL) scale on various grape varieties in differentgrape growing areas in both hemispheres. For each trial,the objective was to compare standard red wine production (control) with MEX-WY1 autolysate (addition rate of 30 g/hL at the beginning of alcoholic fermentation) under the same winemaking process. Fermentation kinetics were monitored and the resulting wines were analyzed at different stages (post-alcoholic fermentation, post-malolactic fermentation, and post-stabilization). Batch homogeneity was checked by measuring classic physicochemical parameters. The colour of the wines was evaluated through spectrophotometry and by measuring tristimulus values (CieLab). The wines were subjected to a post-stabilization sensory analysis and the saliva precipitation index (SPI) assay. Fermentation kinetics in the numerous trials were not affected by the addition of MEX-WY1. The effect of MEXWY1 on colour stability and wine sensory qualities are described below.

Effect on the colour of red wine

In numerous trials, the addition of the specific autolysate MEX-WY1 at the beginning of fermentation was observed to have a positive effect on wine colour. An example is shown in Figure 5, which shows the colour (parameters L, a, b) measured in Pinot Noir wines from trials conducted in New Zealand (Marlborough, 2016). The wine from the fermentation using MEX-WY1 had a darker, redder colour. The ΔE calculated based on the three parameters was 4.7. It is widely recognized that a trained professional is able to detect an average ΔE of 3 in red wine.

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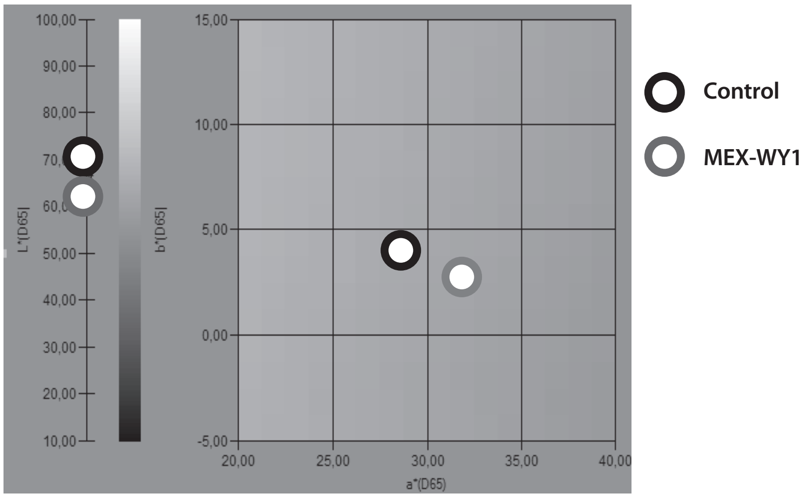

Figure 5. Wine colour as determined by CieLab measurements (L, a, b parameters) in Pinot Noir wines (Marlborough, New Zealand, 2016) from MEX-WY1 (MEX-WY1 added at the beginning of fermentation) and Control fermentations.

Another example is shown in Figure 6., which highlights the impact the addition of MEX-WY1 has on wine colour parameters after alcoholic fermentation (Fig. 6. A.) and on the corresponding wines after bottling (Fig. 6.B.). Colour intensity was higher after alcoholic fermentation when compared to the control and this improvement of colour was confirmed even after malo-lactic fermentation and after bottling, with a ΔE of 2.5.

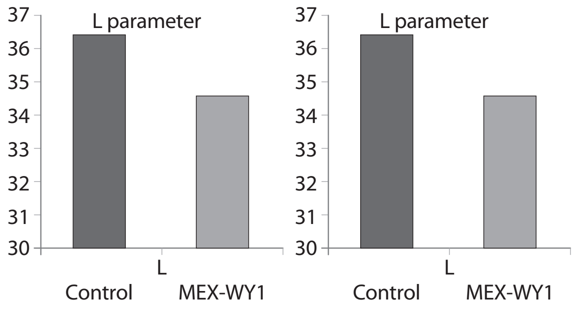

Figure 6. Pinot Noir, Burgundy, 2017, comparative trial: L Analysis (L, a, b) after alcoholic fermentation (6. A.) and after bottling (6. B.).

Effect on the sensory qualities of red wine (fruitiness, mouthfeel, overall quality)

Trials using the specific autolysate MEX-WY1 demonstrated that several sensory characteristics of red wine can be improved: reduced astringency, better overall mouthfeel, and riper, fruitier aromas.

- Significant reduction in astringency:

The Saliva Precipitation Index (SPI) measures the reactivity of salivary proteins to polyphenols in wine and it is a good estimate of wine astringency (Rinaldi et al., 2012). Figure 7 shows SPI of Grenache wine made with the Thermo Flash process, which is known to promote significant phenolic extraction and can lead to pronounced astringency. We can see that wine fermented with MEX-WY1 has significantly lower SPI compared with the control (38 versus 52). This difference directly correlates with reduced astringency in the MEX-WY1 wine.

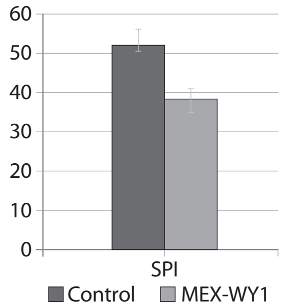

Figure 7. Saliva Precipitation Index (SPI) measured in Grenache wine (France, Côtes du Rhône, 2016). The only variable was the addition of the specific autolysate at 30g/hL at the beginning of fermentation in the MEX-WY1 treatment compared to the Control without MEX-WY1.

The release of volatile thiols is also influenced by environmental factors such as the nutrient and micronutrient content of grapes.

- Overall improvement in the mouthfeel and structure of red wine:

Apart from the reduced astringency observed, most of the trials demonstrated an overall improvement in the perceived wine structure and mouthfeel.

Figure 8 illustrates the impact the early addition of the specific autolysate has on the sensory attributes of a Tempranillo treated wine compared to the control: higher tannic structure and fuller body.

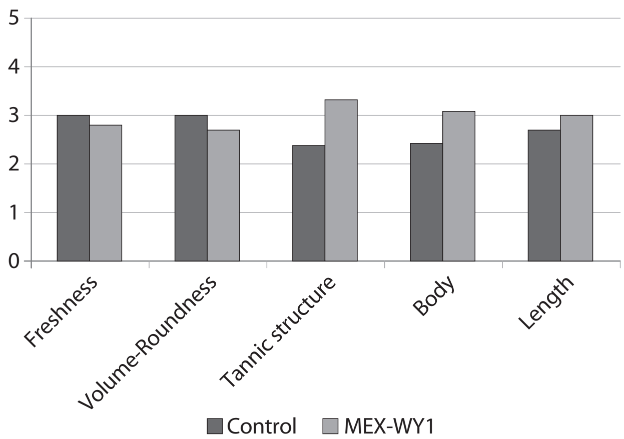

Figure 8. Winery-trial, Tempranillo wines made with the specific autolysate MEX-WY1 added (30g/hL) at the beginning of fermentation (MEX-WY1 treatment) or without (Control treatment), 2016. Sensory analysis by a panel of professionals.

Thus, the mechanisms and interactions observed in the model studies above have an impact not only on wine astringency, but also on other taste characteristics related to the wine's mouthfeel and structure.

- Enhanced fruit maturity:

In a number of the winery trials, some unexpected differences in aroma were noted, including fruit maturity and vegetal and grass characteristics. For example, Cabernet Sauvignon (Bordeaux, France, 2016) wine made from grapes harvested and fermented under the same condition, either with or without the addition of the specific autolysate MEX-WY1 at a rate of 30 g/hL at the beginning of fermentation, showed a different aroma sensory profile (Figure 9). The MEX-WY1 treatment produced a significant difference (10% confidence level) in "fruit maturity," i.e., more mature fruit notes, compared to the control. The control wine was considered to be slightly more vegetal and the MEX-WY1 wine to have more red/black fruit notes.

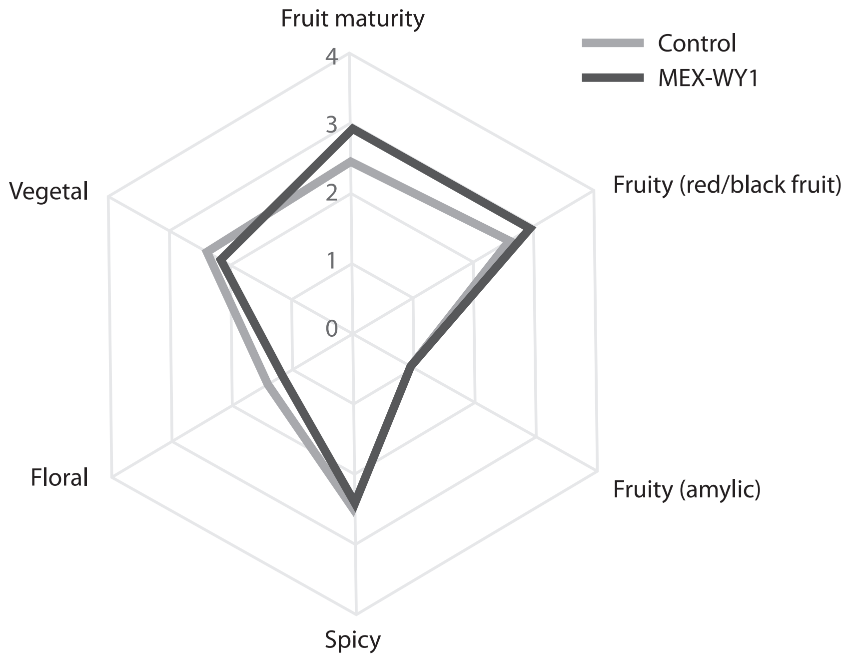

Figure 9. Aroma analysis by a panel of second-year student enologists (Toulouse, France, March 2017) of a Cabernet Sauvignon (Bordeaux, France, 2016) made with the specific autolysate MEX-WY1 added (30g/hL) at the beginning of fermentation (MEX-WY1 treatment) or without (Control treatment).

Summary

Recent research has given us a much better understanding of how yeast and phenolic compounds interact in red wine, enabling us to better characterize the biochemical and biophysical properties of yeast with unique wine-relevant characteristics. We have described the development of a specific yeast autolysate with unique wine sensory impacting properties. A yeast autolysate (MEX-WY1) was prepared from a wine yeast with distinctive characteristics. Studies using model grape must revealed the involvement of mannoproteins in the soluble fraction of the autolysate in the formation of stable complexes that contribute to colour stabilization and reduction in wine astringency.

Winery trials demonstrated that adding the specific autolysate MEX-WY1 at the beginning of fermentation had a positive effect on wine sensory characteristics such as colour, mouthfeel, and fruitiness in red wine, especially Pinot Noir wines. Thus, the new specific autolysate constitutes a unique tool to improve colour and texture management in Pinot Noir.

MEX-WY1 has been released as commercial product, ***OPTI-MUM RED™***.

The research work described in this article is from a collaboration between INRA/Montpellier Supagro, LISBP/INSA Toulouse, and Lallemand.

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