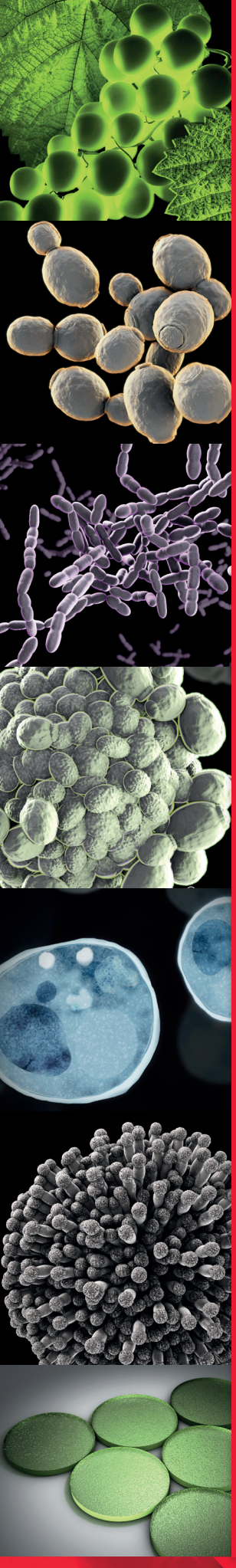

RELATIONSHIPS BETWEEN THE WINE YEAST INOCULATION METHOD AND THE COMPOSITION AND SENSORY PROFILE OF WINES

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Wine yeasts play a crucial role in the winemaking process and are essential to induce and achieve alcoholic fermentation (AF). Different options are available to winemakers, including spontaneous fermentation or fermentations with the use of selected wine yeast. This latter option offers better control of the AF, the quality and the style of the wine, while still respecting the typicity of the grapes and avoiding spoilage development. Selected yeast can be inoculated from different preparations, but the most common and the most efficient, and safe is by using selected yeasts under various forms (active dry yeast, frozen, compressed) as described in the International Oenological Codex. Multiplication of active dry yeast in the winery is also used by some producers despite various quality issues.

Inoculation method of selected wine yeast during the winemaking process has been known to influence the behaviour of the yeast. As early as 1981, Kraus et al. showed the importance of temperature, and the type of rehydration media impacted on fermentative activity and the health of the cell membrane as well as on the maintenance of the yeast cell constituents. Later, Soubeyrand et al. (2006) confirmed the importance of the rehydration procedure on not only the viability, but also on the fermentation activity.

With this knowledge, specific best practice instructions are given to winemakers on how to use selected wine yeast in the dry form (WADY) to maximize their efficiency and fully reveal the sensory potential of the grapes. If the action of the yeast on the aromatic compound production is compromised, then all the efforts undertaken by the winemaker, on the grape and vineyard management are diminished.

The secondary metabolism (revelation of aromatic and sensory related compounds) of wine yeast is complex (Figure 1) and as important as the sugar-alcohol conversion. A proper rehydration is key to retain the capacity of wine yeast to be fully functional.

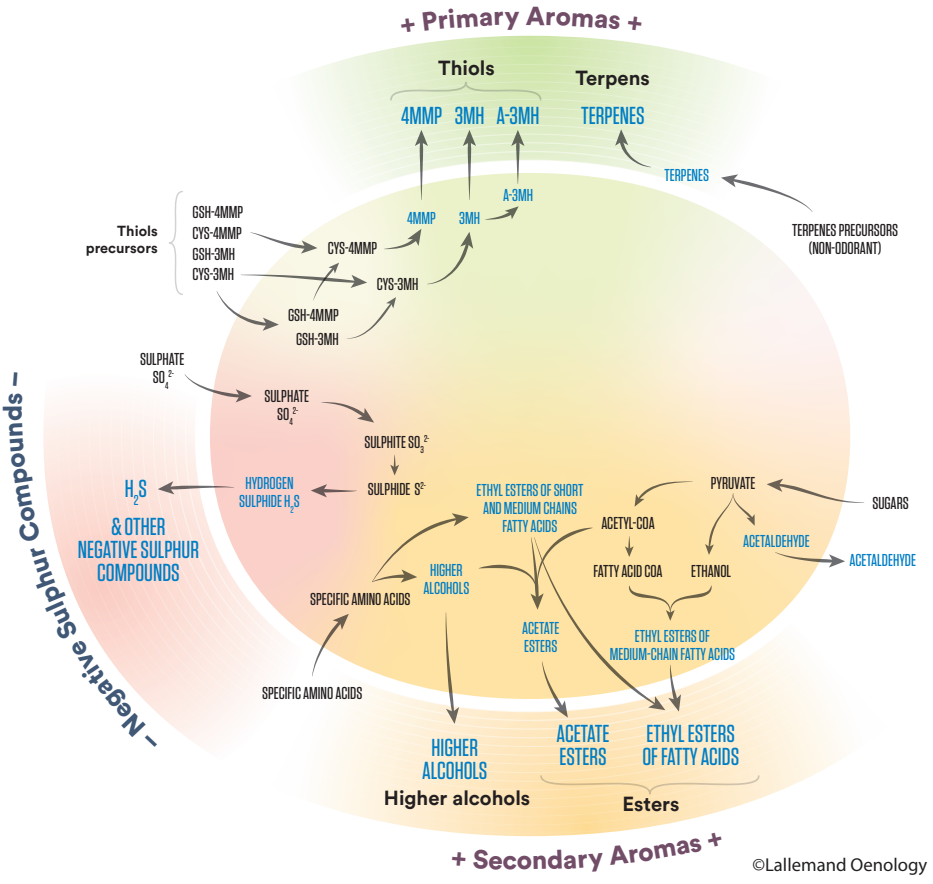

Figure 1. Secondary metabolism of wine yeast

IMPACT OF REHYDRATION ON ALCOHOLIC FERMENTATION

A winery-scale study was undertaken to validate that there are significant differences in bacterial contamination levels in yeast starter cultures prepared via two different methods. Two selected dry wine yeast strains were prepared either by rehydration following the manufacturer's instruction or as an inoculum using a multiplication system, with a dedicated equipment and process. The winery was very familiar with the process and had a dedicated team managing it.

Bacteria (lactic and acetic acid bacteria) concentrations in the two yeast culture preparation were found to vary greatly (up to two logs higher). The yeast culture obtained from multiplication had high levels of bacteria contamination which were well out of OIV specification (OENO 576A/2017 ; F-COEI-1-SACCHA). Upon microscopic examination, bacilli-shaped bacteria were found, in pairs or chains in the yeast inoculum obtained after yeast multiplication.

	Total bacteria	Lactic acid bacteria	Acetic acid bacteria
	CFU/g or CFU/mL		
Specifications	Internal Lallemand spec (no OIV specs existing) = **<1x10^5**	OIV Specs **<1x10^5**	OIV Specs **<1x10^4**
Recommended rehydration Yeast S	<1x10^5	<1x10^5	<1x10^4
Same yeast S obtained after multiplication in the winery	1.62x10^6	1.13x10^7	3.05x10^4
Recommended rehydration Yeast X	< 1x10^5	<1x10^5	<1x10^4
Same yeast X obtained after multiplication in the winery	1.27x10^7	3.79x10^6	2.46x10^7

Table 1. Bacteria concentrations found in two selected wine yeast strain cultures (S and X) prepared either rehydrated classically or used in the multiplier process. The same production lot of yeast was used for the recommended rehydration and for the multiplication process by the winery.

In terms of fermentation kinetics, several trials were undertaken in different varieties, and the results consistently showed that there were no differences in the lag-phase, and that the rehydrated selected yeast maintained the fermentation speed during the stationary phase, and resulted in a shorter fermentation: 10 days vs 17 in the Merlot and 15 days versus 23 days in the Maccabeu (Figure 2A and 2B). Genetic analysis was used to determine the implantation rate (the successful establishment of the yeast in the must) at 1/3 through AF. The rehydrated selected yeast showed 100% implantation, whereas with the multiplied yeast, the success was 0%, indicating that the winemaker does not know which yeast is fermenting the wine, and certainly not the one they were intending to multiply.

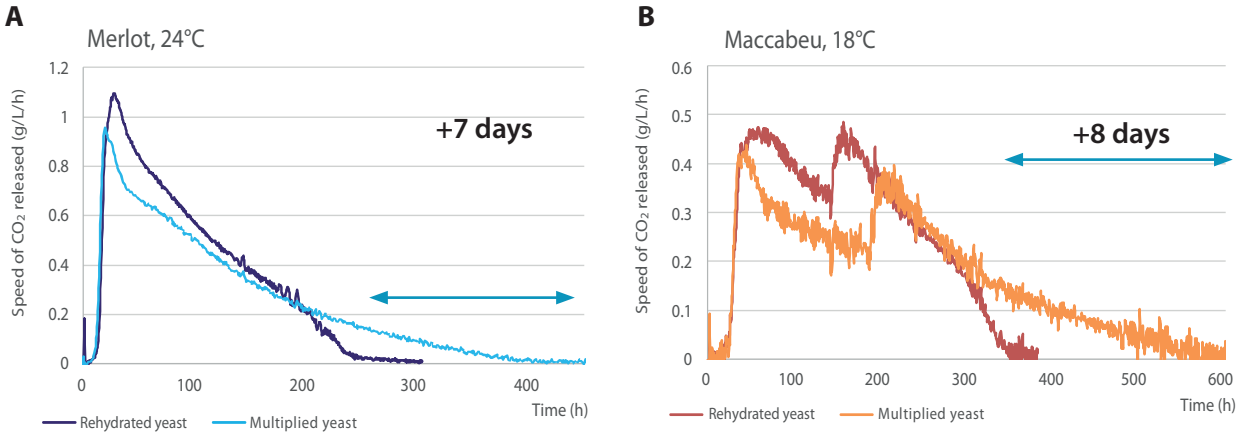

Figure 2. Fermentation kinetics of Merlot with YAN: 138mg/L ; Sugar 244 g/L; pH 3,5 (A) and Maccabeu with YAN: 109mg/L ; Sugar 220 g/L; pH 3,54 ; total SO_2: 25mg/L Nutrition: 30g/hL of Fermaid E at 1/3AF (B) wines fermented by the same yeast which was either multiplied in the cellar or rehydrated prior to inoculation (same active dry yeast from the same production lot).

IMPACT OF REHYDRATION ON AROMA AND FLAVOUR COMPOUNDS

In a study conducted by Bordet et al. (2023), the researchers were able to show that the inoculum preparation method (either through recommended rehydration or by multiplication) impacted not only fermentation kinetics but also the aroma and flavour compounds produced by *Saccharomyces cerevisiae*, and therefore the composition and quality of the final wine. They used different selected wine yeast strains prepared in dry form and compared them to the same yeast strains, coming from multiplication (mimicked as done in wineries through multiplication systems). All the fermentations were conducted in pasteurized Chardonnay juice.

Bordet and colleagues characterized the volatilome (67 volatile compounds were quantified by GCMS and GCFID gas chromatography), and exo-metabolome (measurement of the chemical composition of the non-volatile fraction of the wine (at a time t) using an untargeted approach) of the wines.

The PCA analysis (Figure 3) of the 67 volatile compounds characterized in both modalities, showed that there were differences in concentration and discrimination of the two inoculation modes along the second axis (PC2) and that whichever strain was used, the mode of inoculation impacts the composition of volatile compounds.

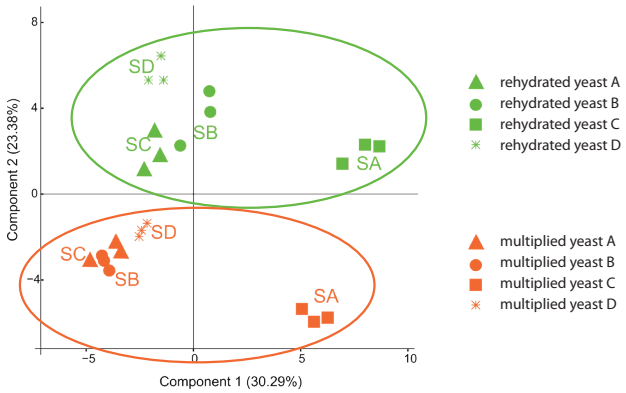

Figure 3. Principal component analysis (PCA) of volatile compounds profiles of wines prepared with four different yeast strains (A, B, C, D) and using two types of yeast preparations (rehydrated (green) or multiplied (orange)) for inoculation.

In terms of aromatic impact, there was a significant decrease of positive esters when the selected yeasts were multiplied instead of rehydrated (Figure 4).

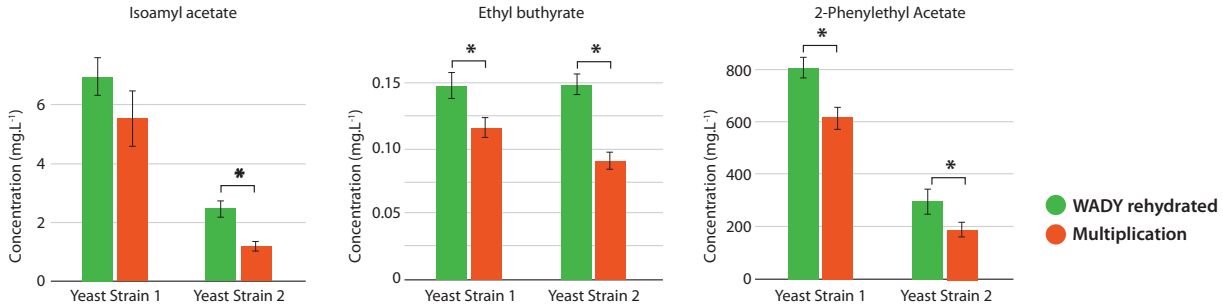

Figure 4. Three main esters measured with 2 different selected wine yeasts, either classically rehydrated or multiplied.

From the metabolomic analysis, it was shown that there was a difference between the two inoculation strategies with regards to number, composition, and nature of the compounds. It showed that for the selected yeast properly rehydrated, differences between the wines were seen with the two yeast strains, whereas the corresponding multiplied yeast wines were very similar; their metabolomic fingerprint was very uniform, not only in terms of specific compound groups, but also in terms of their intensity as seen in Figure 5. These differences between the two modalities are not due to an eventual indigenous flora as all the inoculations were into pasteurized musts. It is really due to a shift in the yeast metabolism which loses its specificity and characteristics when multiplied under those conditions.

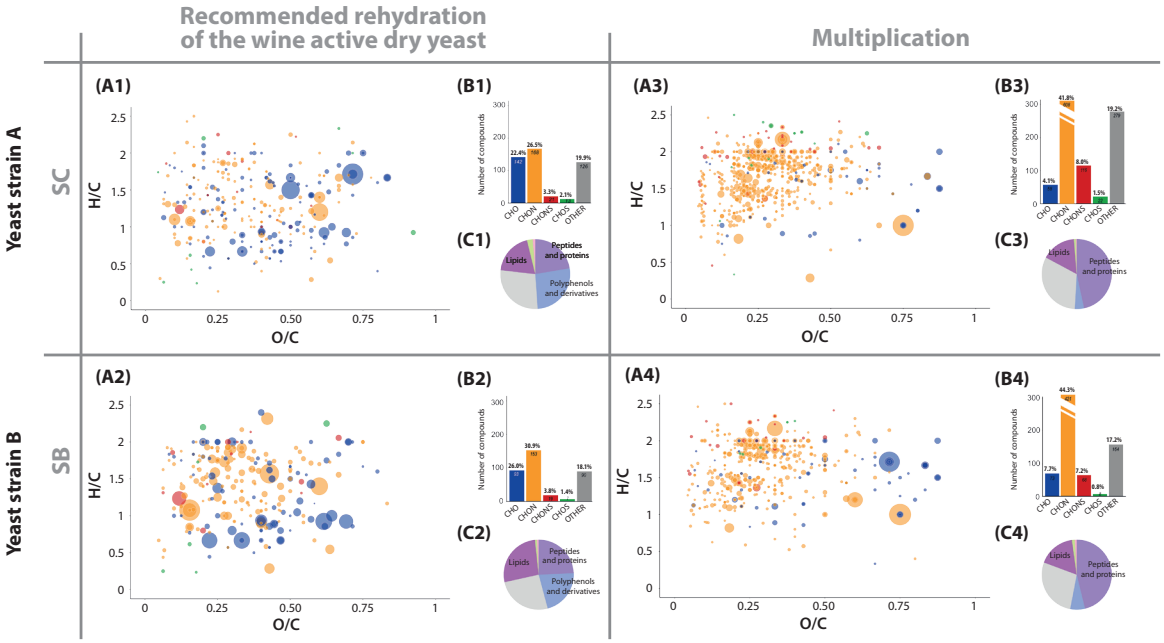

Figure 5. Exometabolomic studies showing the differences in composition of the Chardonnay wine either inoculated by a multiplied yeast or by a classically rehydrated yeast

CONCLUSION

The classical method of rehydration of selected wine yeast for inoculation into juice or must is a time proven technique. Bacterial contamination (LAB and AAB) has been shown to be high in yeast multiplied cells prior to inoculation. In addition, multiplied yeast cells have been shown to no longer be a pure single yeast strain which can impact fermentation kinetics and will impact the sensory of the wine.

The most important criteria of a yeast strain other than robust fermentation kinetics is impact on wine sensory and quality. The study of Bordet (2024) has clearly shown that the resulting wine from a multiplied selected yeast has an aroma compound signature that is clearly different from properly rehydrated yeast with less diversity of aroma compounds composition. These observations were very consistent between the different wine yeast strains used.

In a highly competitive wine market, it is of the essence to have an efficient fermentation producing a microbiological stable wine with sensory that reflects the grape. The use of properly rehydrated yeast will ensure the optimum expression of the grape variety and wine aromatics.

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