Optimizing the aromas of wines made from Sauvignon Blanc and other grape varieties with «Sauvignon» aromas: impact of a yeast rehydration nutrient and inoculation with mixed yeasts

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INTRODUCTION

Saccharomyces cerevisiae winemaking yeast is the key factor in enabling must to express its aromatic potential (***Murat et al., 2001, Swiegers et al., 2005; Dubourdieu et al., 2006***). Several metabolic pathways are involved in forming aromatic compounds, such as fatty acids and higher alcohols. Some specific pathways are responsible for releasing aromatic compounds from their odourless precursors in grapes. Volatile thiols are a good example of this phenomenon. Indeed, 4-mercapto-4-methylpentan-2-one (4MMP) and 3-mercaptoketone-1-ol (3MH) are released from their odourless precursors by yeast during alcoholic fermentation (AF), while mercaptohexyl acetate (3MHA) is derived from 3MH.

Volatile thiols are extremely odoriferous molecules, which give wines their fruity aromas, even at very low concentrations (***Dubourdieu et al., 2006***). Volatile thiols (4MMP, 3MH, and 3MHA) were initially identified in Sauvignon Blanc wines and are still mainly associated with this grape variety. They also participate in the aromas of wines made from Alsace grape varieties: Pinot Gris, Riesling, and Gewürztraminer, and a few other white grape varieties, such as: Colombard, Chenin Blanc, Rolle, Petit Manseng, and Gros Manseng (***Tominaga et al., 2000***), as well as the fruity aromas of rosé wines made from Merlot, Cabernet, Syrah, and Grenache (***Murat, 2001, 2005; Ferreira et al., 2002***). They contribute to the aroma of these wines in synergy with other aromatic compounds produced by the yeast metabolism, such as esters, higher alcohols, and/or of grape origin, e.g. methoxypyrazines, terpenes, etc. (***Ferreira, 2007***). As routine assays for these compounds are now available, they are commonly used as aroma markers for identifying wines made from the grape varieties mentioned above.

Not all strains of S. ***cerevisiae*** have the same capacity to reveal these compounds (***Murat et al., 2001; Dubourdieu et al, 2006; Augustin et al., 2006***). As a result, several different technical options may be envisaged for producing the most intensely aromatic wines. Inoculation with yeast strains produced by breeding, selected for specific traits inherited from their parent strains, considerably improves aromatic expression. For example, wines fermented with the ZYMAFLORE X5® yeast have intense varietal aromas and a high 4MMP content (***Augustin et al., 2007***). Another alternative consists of inoculating the must with several different yeast strains at the same time. This article also examines the second option, known as inoculation with mixed yeasts.

Independently of the yeast strain, all the parameters which affect fermentation kinetics have an impact on a wine's volatile thiol content. For example, ***Masneuf et al. (2006)*** demonstrated that their release was facilitated by higher temperatures. However, while the negative effect of nitrogen deficiency in the vineyard on the aromatic potential of the must (***Peyrot des Gachons, 2000***) and aroma stability in the resulting wine was known (***Pons, 2006***), the impact of nitrogen levels during fermentation on the release of volatile thiols had not yet been clearly elucidated. Winemakers are well aware that poor management of yeast nutrition has a negative impact on wine aromas. One new concept consists of adding nutrients to Active Dry Yeasts (ADY) during the rehydration phase (***Dumeau et al., 2004; Van der Westhuizen, 2006***). This new generation of yeast rehydration nutrients brings considerable improvement to membrane structure and fluidity, thus enhancing yeast viability. The original formula **DYNASTART®**, on sale since 2004 and patented for use in water to rehydrate yeasts, includes growth factors (vitamins and minerals, but no nitrogen!) and survival factors (sterols and fatty acids). This yeast preparator is increasingly popular with winemakers all over the world. While its effect on fermentation kinetics is clearly recognized, there has been very little scientific research into the impact of **DYNASTART®** on the release of volatile thiols. Initial results published by ***Swiegers et al (2008)*** revealed that it had a positive effect on volatile thiol release and fermentation ester production.

This article examines the impact of the yeast strain, inoculation with mixed yeasts, and the use of a yeast rehydration nutrient (**DYNASTART®**), on the release of varietal volatile thiols and the production of fermentation esters in Colombard and Sauvignon Blanc.

MATERIALS AND METHODS

Colombard test

This test, carried out in southwest France, consisted of fermenting Colombard must (2005) in 50 000L tanks. The characteristics of the must were as follows: potential alcohol content 9.76% v/v., pH 3.00, Turbidity < 50 NTU, Free SO_2 25 mg/L, Total SO_2 77 mg/L, Yeast Available Nitrogen 110 mg/L, indigenous microflora before inoculation (counted by epifluorescence microscopy): yeasts 1.1.104 cells/mL; bacteria 6.7.104 cells/mL. The must was quite acid, with relatively high SO_2 levels, low turbidity, a marked nitrogen deficiency, and considerable pressure from indigenous flora. The must was chaptalized to reach an alcohol content of 12 % v/v. and the nitrogen deficiency was corrected by adding ammonium salts in two stages. Two tanks were compared: rehydrated ADY with or without **DYNASTART®** (300 ppm), using the same yeast strain in both cases (200 ppm). The temperature was maintained at 16°C (61°F) in both tanks throughout fermentation.

Sauvignon Blanc test

The musts were fermented in Australia, in an independent research centre located on the campus of Adelaide University (Provisor), using Sauvignon Blanc (***2007, Adelaide Hills provided by Yaldara Estate***) with the following characteristics: Sugars 218 g/L, potential alcohol 12.9% v/v, pH 3.19, Turbidity < 50 NTU. Duplicate fermentations were conducted in 500 L stainless steel tanks at a constant temperature of 14.5 °C +/- 0.5 (58°F), maintained until the end of fermentation.

Yeast strains / ADY rehydration methods for the Sauvignon Blanc test.

This test consisted of duplicate samples for 6 conditions. The winemaking parameters were identical in all samples, except for the yeast strain(s) used for alcoholic fermentation:

- ZYMAFLORE VL3®, a strain selected from the 'terroir' for its capacity to reveal volatile thiol aromas,
- ZYMAFLORE X5®, a strain isolated from breeding, selected for its fermentation performance, as well as its marked capacity to release varietal volatile thiols and produce fermentation esters,
- ZYMAFLORE X5®, rehydrated with 30 g/hL (300 ppm) **DYNASTART®**, a specific yeast rehydration nutrient,
- Yeast strain A, a commercial strain widely used to produce Sauvignon Blanc wines,
- «Mix» 1, inoculating with a blend of two commercial strains used to produce Sauvignon Blanc wines (50/50),
- «Mix» VL3/X5®, inoculating with a blend of ZYMAFLORE X5® and VL3® yeast strains (50/50).

In the samples seeded with mixed yeasts, the two strains were rehydrated separately, and then inoculated into the must in equal proportions at the same time. Yeast was added at 20 g/hL (200 ppm). All the yeast strains used belonged to the same species: *Saccharomyces cerevisiae*.

The fermentation kinetics in both series of tests were monitored by measuring density. Development of the yeast strains inoculated in both tanks was confirmed by genetic analysis (PCR), half-way through fermentation. The volatile thiols and fermentation esters were assayed by **SARCO** laboratory after post-AF sulfiting.

RESULTS AND DISCUSSION

Colombard test

Fermentation kinetics (figure 1)

Fermentation in the control tank (ADY rehydrated without preparator) stopped after 15 days (fermentation temperature: 16°C). The wine contained 16.5g/L reducing sugars with a volatile acidity of 0.21 g/L H_2SO_4. The sample with **DYNASTART®** completed fermentation in 12 days and the wine had volatile acidity of 0.12 g/L H_2SO_4, indicating that the yeasts had not suffered any major stress. These results showed that, under difficult conditions (large volume, clarified must, high acidity, sulfiting, pressure from indigenous microflora), merely supplementing with nitrogen did not ensure completion of AF. Using a yeast rehydration nutrient minimized the stress on the yeast throughout fermentation.

Varietal and fermentation aromas (figure 2)

As far as aroma was concerned, using **DYNASTART®** raised varietal aroma (3MH, 3MHA) concentrations by 13 to 30% and increased isoamyl acetate production (AI, reminiscent of banana and sweets) by 18%.

Therefore, this activator had a significant impact on yeast cell viability, even after several generations, improving the yeast's general metabolism, thus increasing its resistance and aromatic expression.

Sauvignon blanc test

Fermentation kinetics (figure 3)

Fermentation temperature was maintained at 14.5°C (no increase at the end of AF). Fermentation kinetics were very similar for all the samples, except those containing VL3® and X5® + **DYNASTART®**.

It was noted that ZYMAFLORE VL3® did not have optimum fermentation kinetics. This strain, included in the test as an undisputed commercial benchmark for Sauvignon Blanc, is known to be better suited to different fermentation conditions from those in this test (best results are obtained at 16-18°C with higher turbidity, around 100 NTU). On the contrary, ZYMAFLORE X5®, rehydrated with yeast rehydration nutrient, completed fermentation much more rapidly, confirming the results of the Colombard test.

Under these test conditions, fermentation kinetics were not affected by the presence of two yeast strains inoculated in equal proportions at the same time ("mix" tanks).

Current analyses of the wines did not reveal any significant differences between the samples (data not shown), apart from the fact that the wine fermented with ZYMAFLORE VL3® had residual sugar, for the reasons mentioned above.

Varietal aromas (figure 4)

Analyses of the aromas of the finished wines confirmed previous observations (data not shown) that Sauvignon Blanc grapes from the Adelaide Hills area produced wines with a low 4MMP content (high temperatures, full sun exposure, and water stress). However, it is interesting to notice that, with few precursors, ZYMAFLORE X5® yeast only produced a wine with a high 4MMP content (considerably above the perception threshold for this compound). Furthermore, rehydration with **DYNASTART®** yeast activator doubled the concentrations of 4MMP and 3MHA, and significantly increased 3MH release.

Samples inoculated with mixed yeasts produced wines with very similar volatile thiol concentrations (particularly 3MH and 3MHA). The release of varietal aromas with the X5®/VL3® blend was better than that obtained with VL3® alone (N.B. The test conditions were not perfect for this strain to produce optimum results), but not as good as in the X5® sample.

Fermentation aromas (figure 5)

The differences in fermentation aroma concentrations between the samples were much more marked than the variations in varietal aromas.

Strain A produced large amounts of isoamyl acetate (IA, banana aroma), which correlates with its reputation on the market. Rehydrating ZYMAFLORE X5® with **DYNASTART®** significantly increased the production of fermentation esters: 25 - 45% more isoamyl acetate and hexyl acetate (HA, pear aroma) than in the control without nutrient.

Under these test conditions, inoculating the samples with mixed yeasts did not increase the production of fermentation aromas as compared to the two controls (single yeasts). With the exception of HA, where yeasts mix 1 produced more fermentation aromas (+ 18%), both mixed-yeast samples gave similar results.

Sensory analysis

In addition to these analysis results, to confirm the impact of using an activator to increase a wine's aromatic intensity and complexity, the Sauvignon Blanc wines obtained during this test were tasted by panels of French, New-Zealand, and Australian winemakers.

In France, 39% of the wine tasters (n=24) preferred the wine fermented with ZYMAFLORE X5®, rehydrated with **DYNASTART®**. The wine produced with ZYMAFLORE X5® alone was ranked second by 26% of the tasters.

The differences between the wines made with ZYMAFLORE X5® and ZYMAFLORE X5® rehydrated with **DYNASTART®** were statistically significant: 92% of the tasters found differences between the wines, and 68% of those preferred the **DYNASTART®** sample.

The two wines produced with mixed yeasts ranked last.

The tasting in New-Zealand (***Marlborough and Hawkes Bay***) involved 69 winemakers. 100% of the tasters found differences between X5® and X5® + **DYNASTART®** and 80% preferred the **DYNASTART®** sample. These results are particularly interesting in view of the world-class reputation of New-Zealand Sauvignon Blanc wines.

The tasting in Australia involved 73 winemakers. Once again, 100% of the tasters found differences between X5 and X5 + **DYNASTART®** and 78 % preferred the **DYNASTART®** sample. In general, this wine ranked first among the 6 samples.

The wines in this test were also tasted in other regions of Australia. It is interesting to note that preferences varied considerably from one region to another: ZYMAFLORE X5® rehydrated with **DYNASTART®** in some areas and strain A in others! Thus, the concept of aromatic quality in Sauvignon Blanc varied depending on the geographical area: some preferred varietal expression (these winemakers were in favor of X5 + **DYNASTART®**), while others preferred strong fermentation aromas combined with varietal aromas (e.g. the wine obtained with strain A). It is, therefore, essential to identify the winemakers' expectations in order to propose appropriate technical solutions: yeast strains that release varietal thiols, produce fermentation aromas, or both.

Aroma stability (figures 6 and 7)

In order to assess how the various aromas behaved over time, the wines made during this test were analyzed again 8 months after bottling (screw caps, free SO_2 levels maintained at 30 mg/L, with a pH of 3.28).

In general, the concentrations of varietal and fermentation aromas were lower than in the first analysis, with a more marked decrease for the esters (IA, PEA, HA, 3MHA). These results were not surprising, considering the instability of these compounds. However, concentrations of 4MMP and 3MH remained high and in the same relative proportions as those found in the post-AF assay: for example, the X5 + **DYNASTART®** sample still had the highest volatile thiol content.

Comparative test of several yeast rehydration nutrients

Yeast rehydration nutrients have a marked impact on fermentation kinetics and aromas under difficult conditions (see results presented above).

In a comparative test, duplicate samples of Sauvignon Blanc were fermented under non-limiting conditions with three nutrients: the ZYMAFLORE X5® yeast strain was rehydrated with **DYNASTART®** or one of two other commercial products recommended for protecting yeast during rehydration. Fermentation took place in small, 20L vessels at 18°C (64°F).

Fermentation kinetics were entirely comparable with all three products (results not shown). However, considerable differences were observed in the expression of varietal aromas (Figure 8). **DYNASTART®** enhanced the expression of varietal aromas by an average of 30% for MMP, 55% for 3MH, and 89% for 3MHA, as compared to the other preparations tested (statistically significant results, considering the measurement uncertainty factor).

CONCLUSION

Many parameters have an impact on a wine's aromatic profile. These may originate in the vineyard: terroir, degree of maturity, condition (disease); or in the winemaking process: pre-fermentation operations (skin contact), yeast strains and species, fermentation temperature, yeast nutrient management, etc. This research shed new light on the impact on wine aromas of adding yeast rehydration nutrient, inoculating with a yeast strain developed by breeding, or inoculating with mixed yeast. It revealed that:

- The ZYMAFLORE X5® yeast, an intraspecies (*S. cerevisiae* x *S. cerevisiae*) cross, had excellent fermentation performance and a tremendous capacity for releasing volatile thiols.
- **DYNASTART®** yeast rehydration nutrient enhanced aroma expression and fermentation kinetics. This activator was responsible for an overall improvement in the yeast cell metabolism and is certainly suitable for use with all activated dried yeast strains. Tests revealed that the various commercial activators available did not all result in the same enhancement of aroma expression by the yeast: the **DYNASTART®** formula was uniquely effective. It had the same impact on the aromas of white, rosé, and red wines.
- Inoculating with a mixes of different strains of Saccharomyces cerevisiae did not achieve the desired objective of producing more intense, complex wines, at least with the blends studied under these test conditions. Research continues in this area.

Future investigations will explore various ways of enhancing the expression of volatile thiols, as well as the quality and complexity of the wine, particularly in conjunction with the Provisor independent research centre in Australia (test in progress for the 2008 vintage). Furthermore, a thesis currently in preparation examines the effect of **DYNASTART®** on a molecular level.

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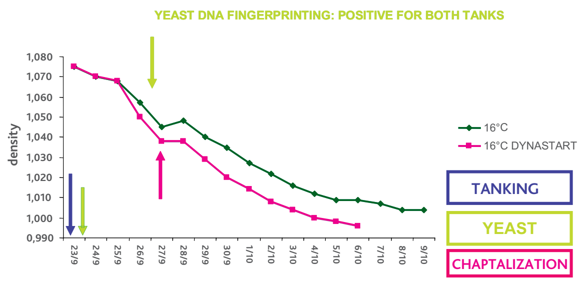

Figure 1: Impact of rehydrating active dry yeast with a yeast rehydration nutrient on fermentation kinetics (Colombard test). AF stopped after 15 days in the sample without yeast rehydration nutrient and was completed in 12 days in the sample with yeast rehydration nutrient.

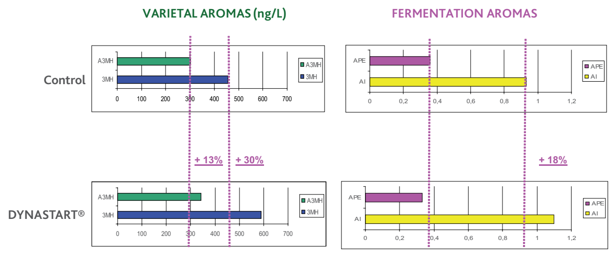

Figure 2: Impact of rehydrating active dry yeast with a yeast rehydration nutrient on varietal and fermentation aroma concentrations (Colombard test).

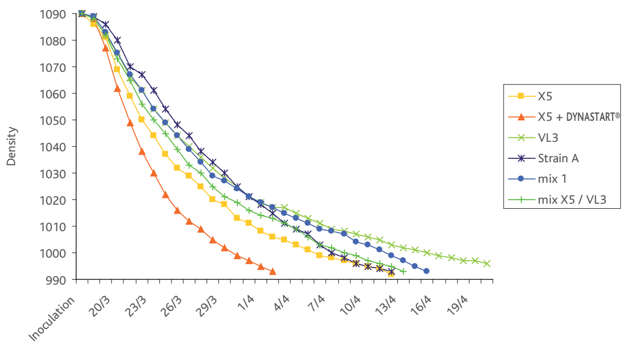

Figure 3: Impact of rehydrating active dry yeast with a yeast activator and inoculating with mixed yeasts on fermentation kinetics (Sauvignon Blanc test, AF: 14.5°C, turbidity < 50 NTU).

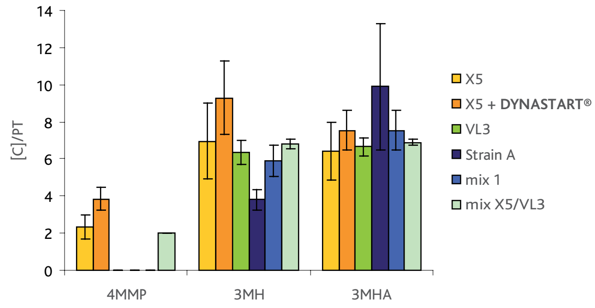

Figure 6: Assay of volatile thiols (varietal aromas) 8 months after bottling (Sauvignon Blanc test).

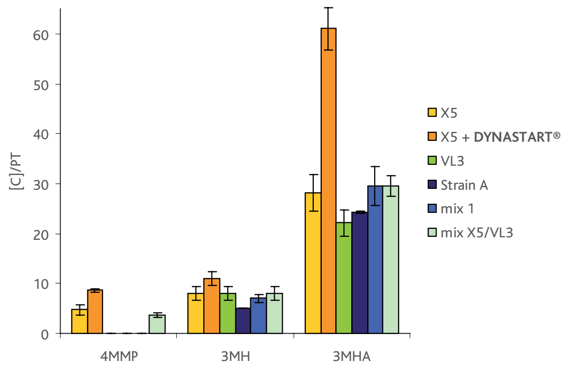

Figure 4: Impact of rehydrating active dry yeast with a yeast rehydration nutrient and inoculating with mixed yeasts on varietal aroma in Sauvignon Blanc wines: 4MMP (boxwood, broom), 3MH (grapefruit), and A3MH (passion fruit).

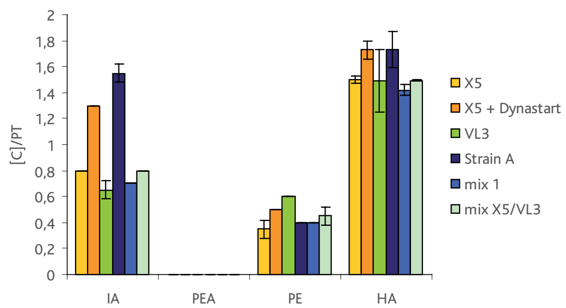

Figure 7: Assay of fermentation aromas 8 months after bottling (Sauvignon Blanc test).

Figures 4 - 7: Results are expressed in terms of aroma index: [C]/PT (Concentration / Perception Threshold). The error bars indicate variations between the duplicate tests.

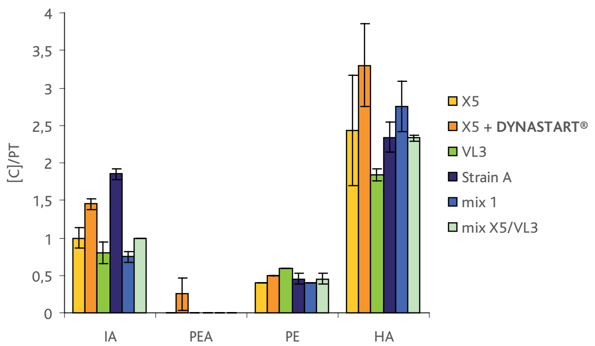

Figure 5: Impact of rehydrating active dry yeast with a yeast rehydration nutrient and inoculating with mixed yeasts on fermentation aromas in the finished wines (Sauvignon Blanc test): IA: isoamyl acetate (bananas); APE: phenylethanol acetate (roses); PE: phenyl-2-ethanol (roses); HA: hexyl acetate (pears).

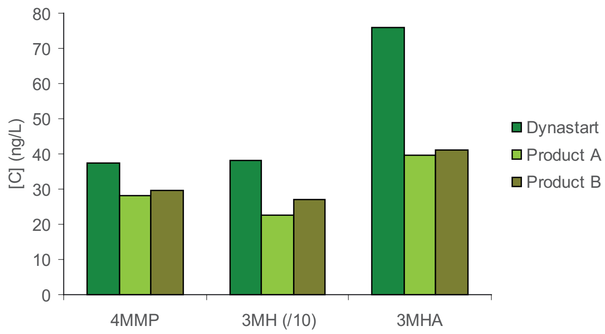

Figure 8: Comparison of the expression of volatile thiols by yeast re-hydrated with DYNASTART® and two other commercial rehydration products. The values represent the means of duplicate experiments (Measurement uncertainty: 6% for MMP, 10% for 3MH, and 14% for A3MH).

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