Yeast strain and nutritional modulation of aroma intensity, longevity and winemaker preference in Sauvignon Blanc wine*

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INTRODUCTION

Recently we have focused much research on the impacts that yeast strain and yeast nutritional status have on the production of both varietal and non-varietal (ie fermentation-derived) aroma production in Sauvignon Blanc (***van der Westhuizen et al., 2008; Swiegers et al., 2008***). *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-mercaptohaxan-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 particular wines significant fruity aromas, even at very low concentrations (***Dubourdieu et al., 2006***). The volatile thiols (4MMP, 3MH, and 3MHA) were initially identified in Sauvignon Blanc wines and are still mainly associated with this grape variety, yet they also contribute to the aromas of wines made from the Alsatian 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***). They are also responsible for the fruity aromas of rosé wines made from Merlot, Cabernet, Syrah, and Grenache (***Murat, 2001; 2005; Ferreira et al., 2002***).

Volatile thiols contribute to the aroma of these wines in synergy with other aromatic compounds produced by yeast metabolism such as esters, higher alcohols, and those of grape origin, such as methoxypyrazines and terpenes. (***Ferreira, 2007***). Since 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.

It is highly significant that not all strains of *S. cerevisiae* have the same capacity to express these compounds (***Murat et al., 2001; Dubourdieu et al, 2006; Augustin et al., 2006***). As a result, several different technical options may be undertaken to produce the most intensely aromatic wines. Inoculation with yeast strains produced through *breeding*, which are selected for specific traits inherited from the parent strains, considerably improves aromatic expression. For example, wines fermented with ZYMAFLORE X5® yeast have intense varietal aromas and a high 4MMP content (***Augustin et al., 2007; Swiegers et al., 2008***). Another alternative consists of inoculating the must with several different yeast strains at the same time, known as inoculation with mixed yeasts.

Independently of the yeast strain, all parameters which affect fermentation kinetics have an impact on a wine's volatile thiol content. For example, ***Masneuf et al. (2006)*** demonstrated that the release of volatile thiols 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 is known ***(Pons, 2006)***, the impact of nitrogen levels during fermentation on the release of volatile thiols has 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. DYNASTART®, patented for use in water to rehydrate yeasts, includes growth factors (vitamins and minerals, but no nitrogen) and survival factors (sterols and fatty acids).

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 significant effect on both volatile thiol release and fermentation ester production. Aside from chemical measurement of aromatic chemicals, ultimately it is a combination of winemaker and consumer preference that dictates the success of any given wine. Moreover, the aromatic composition of wines is known to evolve noticeably over time, with new characters being produced and others decreasing in intensity (***Ribéreau-Gayon et al., 2006a***). The question remained: are winemakers able to perceive the differences in these wines based on observable aromatic differences, justifying the use of a rehydration nutrient? Additionally, do the aromatic characters responsible for the perception of Sauvignon Blanc typicity correlate with wine region, country and/or other factors?

To investigate these matters and the validity of mixed cultures in fermentation, tastings were conducted on a regional basis using the wines from the 2007 trial (***van der Westhuizen et al., 2008 and Swiegers et al., 2008***). The results were compared on a regional basis and are presented, in addition to some suggested correlating factors. The tasting feedback provided valuable insight into what aromatic characteristics most strongly signify Sauvignon Blanc typicity for winemakers (through correlation with the aromatic data), which was found to vary depending on the wine region in which the tasting was conducted. The results also provide an understanding of how the modulation of yeast nutrition and/or strain correlates ultimately with winemaker preference for the wine.

MATERIALS AND METHODS

The fermentations were conducted in Australia, in an independent research centre located on the campus of Adelaïde University (Provisor), using Sauvignon Blanc juice (2007, Adelaïde Hills provided by Yaldara Estate) with the following characteristics: Sugar 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), which was maintained until the end of fermentation (to eliminate the variance in aromatic production observed with temperature fluctuation). Yeast was added at 20 g/hL (200 ppm). Fermentation kinetics were monitored by measuring density. Development of the yeast strains inoculated in both tanks was confirmed by genetic analysis (ie DNA authentication), half-way through fermentation. The volatile thiols and fermentation esters were assayed by the **SARCO** laboratory (Bordeaux) after post-AF sulfiting. All winemaking parameters were identical in all samples, except for the yeast strain(s) used:

- 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;

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- ZYMAFLORE X5®, rehydrated with 30 g/hL (300 ppm) DYNASTART®, a specific yeast rehydration nutrient;
- ZYMAFLORE VL3®, a strain selected from the 'terroir' for its capacity to reveal volatile thiol aromas;
- Strain A, a commercial strain widely used to produce Sauvignon Blanc wines;
- Mix 1, inoculated with a blend of two commercial strains used to produce Sauvignon Blanc wines (50/50), rehydrated separately;
- VL3/X5®, inoculated with a blend of ZYMAFLORE X5® and VL3® yeast strains (50/50), rehydrated separately.

Bottling: screwcap closure; pH 3.26 ± 0.02; TA 6.7 ± 0.3 g/L tartaric acid equivalents; FSO_2 28 ± 2 ppm; TSO_2 142 ± 20 ppm). Tastings of all experimental wines were conducted in the following wine regions: McLaren Vale, Barossa Valley, Clare Valley, Langhorne Creek, and the Limestone Coast (Australia), Marlborough and Hawkes Bay (New Zealand) and South Africa (winemakers from various regions). All wines were masked. The tasters were all winemakers from the local region (responses from winemakers external to the region were excluded), and were directed to concentrate on wine aromas, then instructed to: (1) rank the wines in order of decreasing Sauvignon Blanc typicity (ie 1 = most typical Sauvignon Blanc, 6 = least typical Sauvignon Blanc); (2) indicate their preferred wine from a masked pair in terms of aromatic typicity and intensity, corresponding to those made from ZYMAFLORE X5 and ZYMAFLORE X5 rehydrated with DYNASTART®. Preference data were compiled thusly: the rank of each wine was totalled, hence a lower score indicates higher preference.

RESULTS AND DISCUSSION

kinetics and initial aromatic measurements

For a full discussion of the kinetic data (figure 1) initial aromatic measurements (figures 2 and 3) see ***van der Westhuizen et al (2008)*** and ***Swiegers et al. (2008)***.

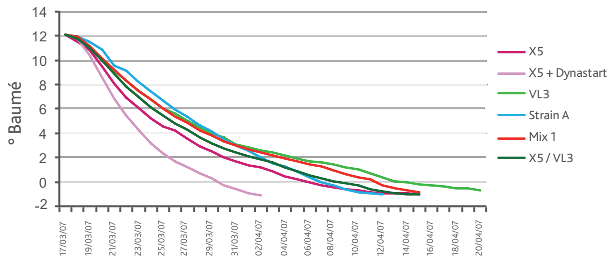


Figure 1: Kinetic data for the fermentations. Note the faster completion of the yeast rehydrated with DYNASTART®, denoted by the dark pink trace.

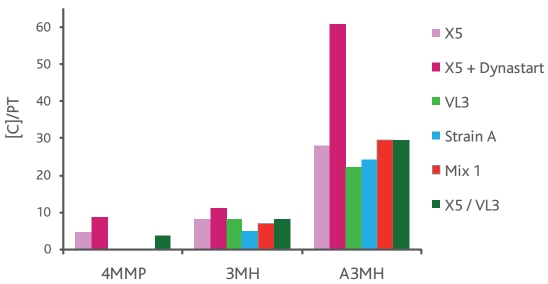


Figure 2: Results of the different yeast strains on the release of the varietal volatile thiols 4MMP (broom, box tree) and 3MH (grapefruit) and on the conversion of 3MH to 3MHA (passionfruit), expressed using the aroma index [C]/PT (**C**oncentration / **P**erception **T**hreshold) in June 2007. **Note that 3MHA is plotted at 1/5th actual intensity for reasons of visual clarity.**

Aromatic longevity

In order to examine the impact of bottle ageing on the aromatic composition of Sauvignon Blanc, the wines from the 2007 trial was re-analysed for volatile thiol and fermentation ester content in January 2008. The data are presented in figures 3 and 4.

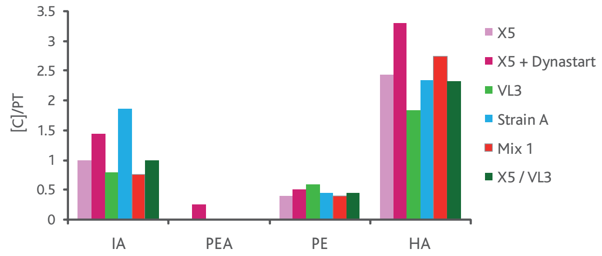


Figure 3: Results of the different yeast strains on the concentration of fermentation esters expressed using the aroma index [C]/PT (**C**oncentration / **P**erception **T**hreshold). (IA) Isoamyl acetate (banana); (PEA) Phenyl ethyl acetate (tea); (PE) Phenyl ethanol (rose); (HA) Hexyl acetate (pear). Note the difference in ordinate axis scales between varietal aromas (figure 2) and non-varietal aromas. **Note also that HA is plotted at 1/2 actual intensity for reasons of visual clarity.**

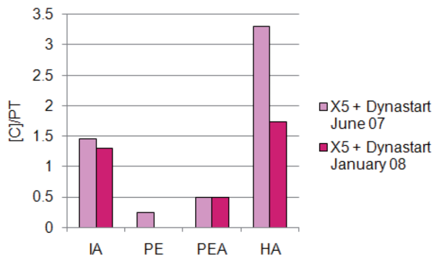


Figure 4: A comparison of the non-varietal (fermentation ester) aromas measure in June 2007 and January 2008 in the wines made with ZYMAFLORE X5 rehydrated with DYNASTART®. Aroma intensity expressed as Concentration/Perception Threshold, where a value of ≥1 indicates contribution to wine aroma. IA = banana; PEA = tea; PE = rose petal; HA = pear.

Clearly there is a difference in the relative stabilities of the three varietal aroma marker chemicals over time. The varietal character 3MH appears to be relatively stable under the conditions of bottling, showing a decrease of around 15 %. The relative stability of 3MH in some wines has been noted previously (Ribéreau-Gayon et al., 2006b). 4MMP and 3MHA, however, appear to be particularly susceptible to degradation post-bottling, in agreement with previous observations (Ribéreau-Gayon et al., 2006c). Within a 7-month time span the boxwood/broom (4MMP) and passionfruit (3MHA) aromas had dropped dramatically in both wines, corresponding to the decreases indicated in figure 3.

In terms of the non-varietal fermentation esters, some similar observations were made, as indicated in figure 4. The rose petal character phenyl ethanol (PE) appears to be particularly stable. Phenyl ethanol acetate (PEA) was found initially only in the wine made using DYNASTART®, yet in the second series of analytical measurements it was absent from this wine. Isoamyl acetate (IA, banana aroma) showed a modest decrease in concentration, whilst hexyl acetate (HA, pear aroma) showed the most instability, dropping 48 %. Similar proportional decreases of all aromatic characters were observed in the other wine samples (data not shown).

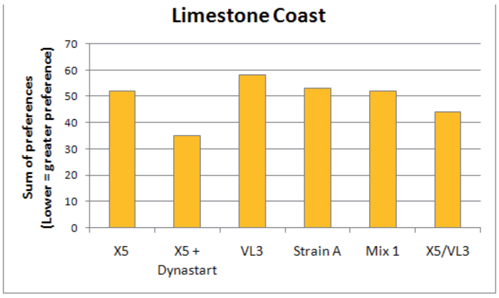
Comparing the ordinate axis scales in figures 3 and 4, it is simple to see that the varietal aromas contribute much more strongly to the wine's aroma than the fermentation esters. In terms of wine aromatic Sauvignon Blanc typicity, these changes indicate that in a 7-month period post-bottling a winemaker could expect to see the aromatic profile of Sauvignon Blanc move from a nose dominated by the varietal passionfruit character of 3MHA to a nose without significant domination of any particular varietal character. In the older wine one would thus expect to notice greater proportional nuance from grapefruit (3MH) over the younger, whilst in terms of the fermentation esters proportionately stronger influence could be expected from banana (IA) and tea (PEA).

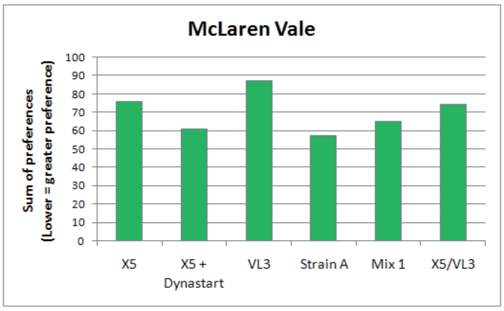
The significance of these aromatic observations is clear: for early-consumption wines where aromatic intensity is the priority, DYNASTART® gives significant advantage.

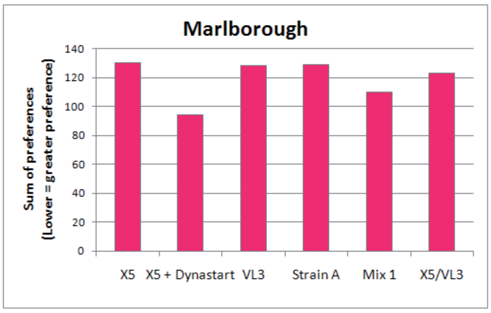
If varietal typicity is a priority, as it is for most winemakers, then the only strain capable of generating all three Sauvignon Blanc varietal thiol characters was ZYMAFLORE X5. In order that these wines retain the most aromatic intensity for the longest shelf-life, initial aromatic intensity must be boosted as much as possible, hence yeast nutrition at rehydration should be used.

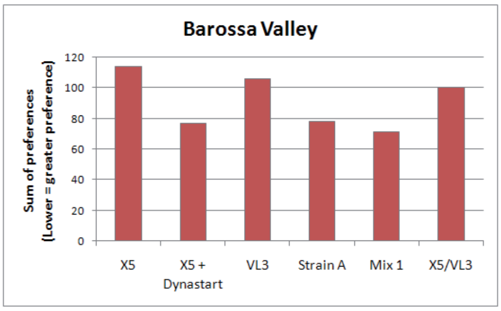
WINEMAKER TASTING PREFERENCES

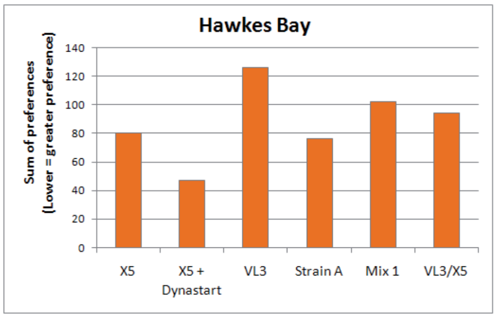
The results of tastings by winemakers of the experimental wines are presented in figure 5. In total, 144 winemakers tasted the wines.

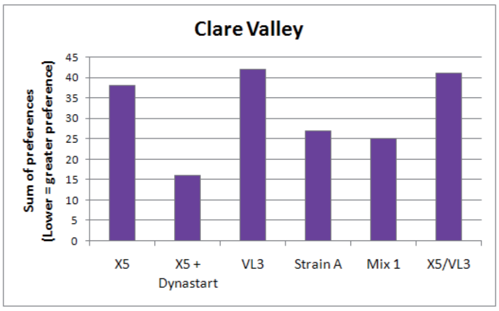


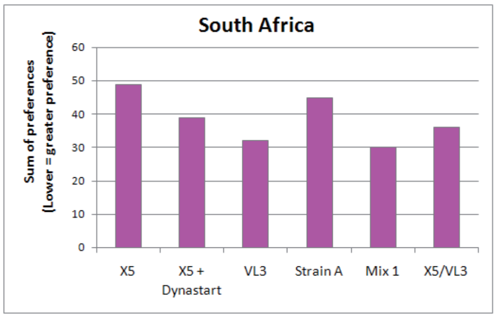












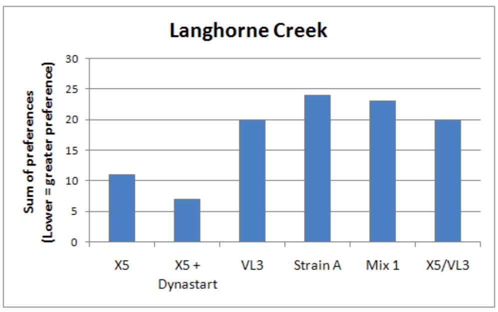


Figure 5: A comparison of the tasting preferences for winemakers in the Australian regions of McLaren Vale (n=20), the Barossa Valley (n=26), the Clare Valley (n=8), Langhorne Creek (n=5), the Limestone Coast (n=14), the New Zealand regions of Marlborough (n=34) and Hawke's Bay (n=25), and the South African regions of Olifantsriver (Northen Cape), Stellenbosch, Paarl, Helderberg and Wellington (n=11). Columns indicate the sums of preferences, hence a lower score indicates higher preference.

McLaren Vale, Barossa and Clare Valleys

In the regions of McLaren Vale, the Barossa Valley and the Clare Valley (figure 5) wine typicity and preference was split roughly into two groups. The favoured wines were those made with ZYMAFLORE X5 + DYNASTART®, Strain A and Mix 1. Correlating these preferences with the analytical data (figures 1 and 2) indicates important differences between the preferred wines. The X5 + DYNASTART® wine is the highest in varietal aromas of 4MMP, 3MH and 3MHA (the latter by a margin of some 100 %). Mix 1, aromatically, does not appear to be especially distinctive when compared with either X5 + DYNASTART® or Strain A, excepting that it lacks the varietal thiol 4MMP. The aromatic profile of Strain A, interestingly, differs from X5 + DYNASTART® and Mix 1 in several respects: it produced no 4MMP, contains the lowest concentration of 3MH and has the second lowest concentration of 3MHA. Conversely, Strain A produced the highest level by some margin of the non-varietal ester isoamyl acetate (IA, banana), a character which was a clearly distinguishing feature for this wine.

Examining these results leads to a simple conclusion: winemakers prefer these wines for different aromatic characteristics. Those favouring the wine made with ZYMAFLORE X5 + DYNASTART® clearly show preference for the varietal Sauvignon Blanc characters 4MMP, 3MH and 3MHA, whilst those favouring the wine made from Strain A show preference for non-varietal characters such as isoamyl acetate.

Langhorne Creek and the Limestone Coast

In both Langhorne Creek and the Limestone Coast the highest preference was given to the wine made from ZYMAFLORE X5 + DYNASTART® (figure 6). In the case of Langhorne Creek, the second most preferred wine was that made from ZYMAFLORE X5, with no significant preference thereafter, whilst in the Limestone Coast no great distinction was indicated by the tasters between all wines excepting that made from ZYMAFLORE X5 + DYNASTART®.Correlating these preferences with the aromatic profiles of the wines (figures 1 & 2) indicates that the winemakers in Langhorne Creek preferred the varietal characters 4MMP (which was only produced by ZYMAFLORE X5), 3MH and 3MHA. The wine made from Strain A, containing the highest concentrations of the non-varietal fermentation ester isoamyl acetate, was least preferred in Langhorne Creek and the equal second least preferred in the Limestone Coast, in sharp contrast to McLaren Vale, the Barossa Valley and the Clare Valley, where it was favoured.

Marlborough and Hawke's Bay

Marlborough winemakers (figure 7), in a region considered by many to be the home of "new world" Sauvignon Blanc, indicated that the highest Sauvignon typicity was displayed by the wine made from X5 + DYNASTART®, with the second most preferred wine made from Mix 1. No significant preference was indicted between the other 4 wines.

These results correlate well with measured aromatic Sauvignon typicity, in that the wine made from X5 + DYNASTART® displays the highest levels of the varietal aroma compounds 4MMP, 3MH and 3MHA (figure 1a). Mix 1, on the other hand, contains no 4MMP and similar levels of 3MH and 3MHA as the wines made from X5 and the mixture of X5/VL3, nor does it contain higher levels of fermentation esters than the other wines in the trial. Given that the winemakers were instructed to focus on Sauvignon Blanc aromatic typicity, Mix 1's high rank in this region is without obvious aromatic correlation.

South Africa

Sauvignon Blanc typicity data for South Africa do not reflect those of any Australian wine region, nor those of New Zealand (figure 8). The South African winemakers involved indicated the greatest Sauvignon typicity to be found in the wines made from Mix 1 and VL3. This indicates a good level of aromatic analysis consistency on their part, given that the concentrations of all measured aromatic components of the wines made from VL3 and Mix 1 are very similar, excepting only 3MHA and hexyl acetate (HA, pear aroma), in which Mix 1 dominates the VL3 wine. Least typicity was indicated for the wines made from X5 and Strain A, with X5 + DYNASTART® and the mixture of X5/VL3 in between. Clearly the South African winemakers involved did not strongly equate Sauvignon Blanc aromatic typicity directly with the measured highest levels of the varietal aroma compounds 4MMP, 3MH and 3MHA. While it is possible that these winemakers were swayed more by textural features, this aspect was beyond the scope of this study. Since the sample size of each of the South African regions was very small, further studies of this type are required to gain an idea of the preferred Sauvignon Blanc characteristics.

Combined regional preference

When all regions are combined the overall responses are indicated in figure 9. Highest Sauvignon Blanc typicity overall was found in the wine made using ZYMAFLORE X5 rehydrated with DYNASTART®. Roughly equal second preference was given to the wines made from Strain A and Mix 1, with approximately 3rd equal preference given to those made using ZYMAFLORE X5 and the mixture of ZYMAFLORE X5 and VL3. The least preferred wine overall in the experiment was that made from ZYMAFLORE VL3.

The relatively low ranking of ZYMAFLORE VL3 is interesting given the strong commercial position that ZYMAFLORE VL3 commands globally. The elevated volatile acidity in this wine compared with the others (data not shown) appeared to detrimentally affect the preference ranking in this trial. The higher VA production of ZYMAFLORE VL3 in this trial was a direct result of the extended low temperature of the fermentation, which is a condition not recommended by the manufacturer. Although VL3 produces comparable levels of 3MH and 3MHA to other strains, and excels in the production of the floral PE aroma, it does not produce high levels of the fermentation esters under the conditions of this trial.

Correlating factors of perceived Sauvignon Blanc typicity

It is clear that what a winemaker perceives to be Sauvignon Blanc typicity varies from wine region to wine region, and between countries also. Some insights into why this might be so are indicated by the aromatic data, and whether preference correlates with varietal aromas or non-varietal aromas. Other similarities between the regions grouped above exist, and it is prudent to discuss the two most obvious factors: light and heat.

McLaren Vale, the Barossa and Clare Valleys are all warm regions, as indicated by the mean January temperature (MJT) in table 1. Furthermore, these regions also receive on average the most light of all Australian and New Zealand regions surveyed (table 1). In these warmer, more light-intense regions Sauvignon Blanc typicity is perceived as being delivered more by Strain A, which is characterised by wine containing higher levels of the non-varietal character isoamyl acetate (banana) and none of the varietal character 4MMP.

Langhorne Creek and the Limestone Coast share very similar values of both MJT and average sunshine hours per day (table 1). In these regions Sauvignon Blanc typicity was perceived as being strongly displayed by the wine made from ZYMAFLORE X5 + DYNASTART®, which correlates well with the aromatic data given that this wine displayed the highest levels of 4MMP, 3MH and 3MHA, the latter by a significant margin. In contrast to the warmer regions, Strain A was least or second-least perceived as showing Sauvignon Blanc typicity.

Marlborough is a region of particular interest to this study, given the strong global recognition that Sauvignon Blanc from this area receives. While it receives more light than Hawke's Bay, it is actually a significantly cooler wine region (table 1). Again, the greatest Sauvignon Blanc typicity was perceived in the ZYMAFLORE X5 + DYNASTART® wine, whilst that made from Strain A was deemed to be of low Sauvignon Blanc typicity. In Hawke's Bay, strong typicity was indicated for the ZYMAFLORE X5 + DYNASTART® wine, yet the second greatest typicity was indicated to be shown by the Strain A wine, indicating some preference on the part of the Hawke's Bay winemakers for the isoamyl acetate character, in common with the warmer regions of South Australia.

Since the winemakers from South Africa represented several regions in small numbers, no attempt was made to correlate their Sauvignon Blanc typicity data with regional climate characteristics.

Perception of the impact of DYNASTART

At each tasting the participants were asked to indicate the wine showing the greatest aromatic intensity and Sauvignon Blanc typicity between two masked wines, corresponding to those made from ZYMAFLORE X5 and ZYMAFLORE X5 rehydrated with DYNASTART®. No winemaker indicated that a difference could not be observed between the wines. Table 2 shows the relative percentage of winemakers in each region that indicated that the wine made using DYNASTART® was the more intense, varietally expressive wine. When all regions were combined, 4 out of 5 winemakers indicated that the wine made using DYNASTART® was superior in aromatic and varietal terms.

Interestingly, those regions where the fewest winemakers indicated that the wine made with DYNASTART® was superior (McLaren Vale and the Barossa Valley) were also two of the warmest regions surveyed, and in these regions the most preferred wine was that made using Strain A and Mix 1 respectively.

The Clare Valley was a clear exception, since all winemakers surveyed preferred the wine made using DYNASTART®. Given that the Clare Valley is known for the production of Riesling, another aromatic white variety, the clear preference of Clare Winemakers for the wine made using DYNASTART®, with its higher varietal and overall aromatic intensity, is perhaps not so surprising.

In the cooler regions of Langhorne Creek, the Limestone Coast and New Zealand the majority of winemakers indicated that the wine made using DYNASTART® was aromatically superior. South African winemakers likewise indicated a strong preference for the wine made with DYNASTART®.

In order to elucidate the exact manner in which DYNASTART® is able to provide enhanced aromatic expression in the yeast, LAFFORT has sponsored a doctoral research candidate at the AWRI, commencing 2008.

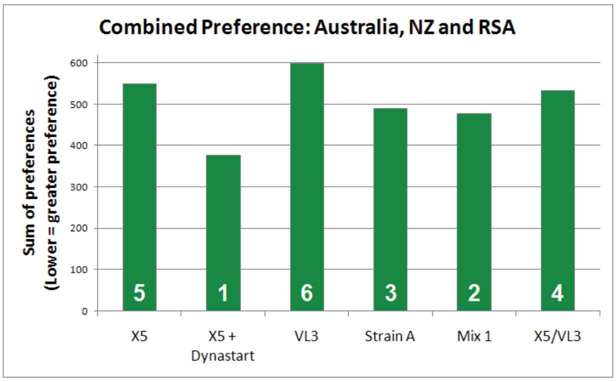


Figure 6: The combined preferences of all winemakers (n=143) surveyed, from Australia, New Zealand and South Africa. Columns indicate the sums of preferences, hence a lower score indicates higher preference.

Region	Sunshine hours per day	Mean January temperature (° C)	Most preferred wine
McLaren Vale	8.6	21.7	Strain A
Barossa Valley	8.8	21.4	Mix 1
Clare Valley	8.8	21.9	X5 + Dynastart
Langhorne Creek	8.3	19.9	X5 + Dynastart
Limestone Coast	8.0*	20.0*	X5 + Dynastart
Marlborough	7.8	17.7	X5 + Dynastart
Hawkes Bay	7.4	18.8	X5 + Dynastart

** Average of values for Coonawarra and Padthaway.*

Table 1: Approximate average total sunshine hours and mean January temperature (MJT) for the regions in which wine tastings were conducted (data sourced from Halliday, 1993).

Region	% Winemakers indicating greater aromatic intensity and Sauvignon typicity in the wine made using Dynastart®
McLaren Vale	63
Barossa Valley	77
Clare Valley	100
Langhorne Creek	83
Limestone Coast	86
Marlborough	79
Hawkes Bay	84
South Africa	82
Combined regional %:	**80**

Table 2: Percentage of winemakers who found greater Sauvignon Blanc typicity with greater intensity of aroma in the wine made using DYNASTART® as a rehydration nutrient with ZYMAFLORE X5 compared with ZYMAFLORE X5 alone.

SUMMARY

There are no absolute answers when it comes to winemaker interpretation of varietal characteristics and preference. Nevertheless, some clear conclusions can be drawn from this investigation:

- Winemakers in the warmer regions tend to favour yeast strains producing fermentation esters in Sauvignon Blanc, whilst those in the cooler regions tend to favour strains expressing varietal aromas.
- Over a 7 month period decreases in some wine aromatic characters can be expected, particularly for 4MMP, 3MHA, PEA and HA. To counter this, strains with high production of these compounds should be used.
- The use of DYNASTART® can dramatically increase aromatic intensity in terms of both varietal and non-varietal aromas, particularly for the varietal volatile thiol 3MHA (passionfruit), leading to significantly higher wine preference.
- The only yeast strain that was able to express all 3 varietal characters in Sauvignon Blanc (4MMP, 3MH and 3MHA) was ZYMAFLORE X5.

ACKNOWLEDGEMENTS

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Products discussed in this article are available in Australia through LAFFORT, phone 08 8260 7974 and in New Zealand from Oenological Resources, phone 0213 22290.

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