# Meeting consumer expectations through management in vineyard and winery

## the choice of yeast for fermentation offers great potential to adjust the aroma of Sauvignon Blanc wine

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### 1. INTRODUCTION

The wine industry needs to become more responsive to consumer expectations and less driven by production because, in global markets, it has become increasingly important to understand consumer preferences in relation to labelling, type of closure, bottle type and, importantly, wine quality and style of flavour and aroma (Figure 1; Pretorius and Høj 2005). For the industry to meet this change, consumer behaviours and preferences need to be studied in depth (Figure 2; Swiegers *et al.* 2005a,b; Francis and Newton 2005). Outcomes of these studies will help the wine industry to make informed choices on packaging and, importantly, on wine style. To take a hypothetical situation, it might be that a particular and lucrative market segment is increasingly interested in Sauvignon Blanc wines that display both 'green' and 'tropical' characters, with neither dominating the aroma. The question is: what needs to be done in vineyard and winery to achieve this pre-determined outcome? In this paper, we explore the possibilities of tailoring wines to meet market specifications and show how grape and wine research can greatly assist the industry in this pursuit. We have

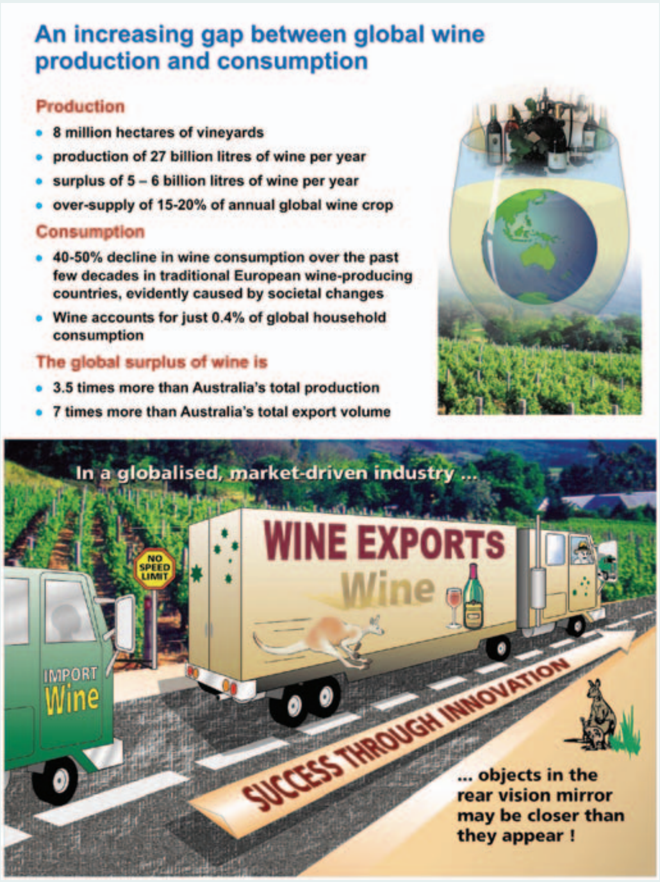


Figure 1. Wine producers are facing intensifying competition brought about by a widening gap between wine production and wine consumption; a shift of consumer preferences away from basic commodity wine to premium quality wine; and economic globalisation. In this era of 'globalisation', barriers to trade are increasingly being removed or eased, creating greater consumer choice, as well as new competitors in all markets. The process of transforming the wine industry from a production-orientated to a market-driven industry results in an increasing dependence on, amongst others, technological innovation. In fact, embracement of Australian wine by the world's consumers has not evolved through centuries of tradition but has been earned by our capacity to deliver exceptional value at various price points. Continuing along a pathway of success that is based on innovation and expertise, rather than tradition, naturally requires a strong R&D component. The Australian culture is all about innovation, driven by market preferences, fierce competitiveness and a keen sense of adventure. In the late 1900s, the Australian wine industry put the rest of the world 'on notice'. Hard work in vineyards, wineries and laboratories around Australia started paying off and wine quality reached new heights, exports grew at spectacular speed and consumers from Europe to North America could not get enough. Increasingly so, Australia's approach has been to listen to the consumers' desires and strive to meet those through innovation while respecting the cultural roots of wine. The Australian wine industry is now seen by many producers and consumers as a world leader when it comes to the production of quality wine at competitive prices. However, in the highly competitive globalised wine sector, it could be that 'objects in the rear vision mirror are closer than they appear'; therefore entertaining complacency is not an option. The road to success is always under construction and we as scientists are the construction workers building that road for the wine industry's journey towards the horizon rather than a destination. We need to help grow the global wine market by delivering benefit to wine producers and consumers through research and innovation.

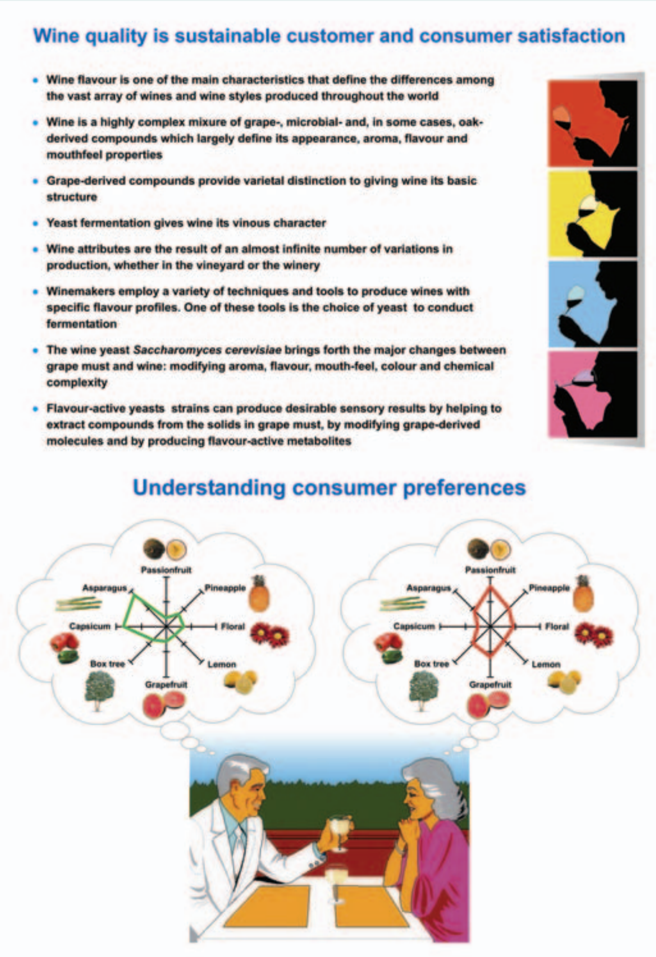


Figure 2. Rather than the 'commodity' wine buyer of yesteryear, the consumer today is quality-focussed, image-conscious and price-sensitive and this has led to a change in the rules of the marketplace to a degree where quality is defined as 'sustainable customer and consumer satisfaction'. For example, there is a diversity of consumer perceptions and preferences regarding tropical flavours versus herbaceous characters in Sauvignon Blanc wines in different markets in the world. The diversity in perceptions and preferences for certain flavours and wine styles between individuals and populations might be due to age, gender, cultural, ethnic and geographic factors. Discovering these preferences and why individuals are more receptive to different tastes and smells is an area that will enable winemakers to capture opportunities in a changing global marketplace.

focused our attention on Sauvignon Blanc as a model because this variety has been extensively studied both in the vineyard and in the winery. Models based on this variety can be applied to other grape varieties later if enough information and research outcomes are available to direct vineyard and winemaking practice.

*Vitis vinifera* L. cv. Sauvignon Blanc is a wine grape variety responsible for some of the world's most popular and aromatic dry white wines. Originally made famous by the wines of Sancerre and Pouilly in France, good Sauvignon Blanc wines today are made throughout the world, especially in New World regions such as Marlborough (New Zealand), which has gained a reputation as a producer of exceptionally good quality Sauvignon Blanc wines. In Australia, fine Sauvignon Blanc wines are produced particularly from cooler regions such as the Adelaide Hills (South Australia), Margaret River (Western Australia), Yarra Valley (Victoria) and Tasmania. Cooler climates appear to help preserve in some grape varieties some of the flavour compounds that impart varietal character.

On the palate, Sauvignon Blanc wines tend to be crisp and acidic and in most cases they are not exposed to oak. The common aroma descriptors of Sauvignon Blanc wines are capsicum, tomato leaf, asparagus, grapefruit, gooseberry and passionfruit. In general, these aroma characters can be classed as 'green' i.e. capsicum, tomato leaf and asparagus, and 'tropical' i.e. grapefruit, gooseberry and passionfruit.

## 2. THE 'GREEN' AROMA CHARACTERS: MODULATION IN THE VINEYARD

The 'green' characters are mostly the result of compounds called methoxypyrazines (Figure 3). These nitrogen-containing compounds are biosynthesised by plants as secondary products of amino acid metabolism and are, therefore, wine flavour compounds directly derived from the grape. The most common and important methoxypyrazines in Sauvignon Blanc wines are 3-isobutyl-2-methoxypyrazine (IBMP), imparting capsicum or asparagus-like aromas, and 3-isopropyl-2-methoxypyrazine (IPMP), imparting somewhat earthier aromas (Augustyn *et al.* 1982; Allen *et al.* 1991; Lacey *et al.* 1991). Taking into account the low detection threshold of methoxypyrazines (2 ng/L), it is clear why these compounds can have such pronounced effects on aroma. The IBMP concentrations analysed in a small survey of Sauvignon Blanc wines ranged from 5 to 40 ng/L from France, from 10 to 35 ng/L from New Zealand, and from approximately 2 to 15 ng/L from Australia (Lacey *et al.* 1991).

Methoxypyrazines in grapes are considered to be very sensitive to climatic conditions and sunlight. These conditions affect the methoxypyrazine levels to the extent that grapes grown in cooler climates commonly contain more methoxypyrazines than those grown in warmer climates. Therefore, Sauvignon Blanc wines from New Zealand will typically have a higher concentration of methoxypyrazines than wines from Australia (Lacey *et al.* 1991). However, in warmer climates, canopy management can prevent the loss of methoxypyrazines to some extent. Because methoxypyrazines are light sensitive, shaded bunches can contain more of these compounds than sun-exposed bunches. Canopy management is, therefore, a powerful tool viticulturists can use to manipulate the final concentration of methoxypyrazines in the wine (Marais *et al.* 1999).

## 3. THE 'TROPICAL' CHARACTERS: MODULATION IN THE VINEYARD AND WINERY

The 'tropical' characters in Sauvignon Blanc wines come primarily from volatile thiols in the wine, with contributions from fermentation-derived esters. The most important volatile thiols are 4-mercapto-4-methylpentan-2-one (4MMP), 3-mercaptohexan-1-ol (3MH) and 3-mercaptohexyl acetate (3MHA). 4MMP imparts box tree and passionfruit-like aromas, and 3MHA and 3MH impart passionfruit, grapefruit, gooseberry and guava type aromas to wine (Figure 3). These volatile thiols are extremely potent, having perception thresholds of 0.8 ng/L (4MMP), 60 ng/L (3MH) and 4 ng/L (3MHA) (Dubourdieu *et al.* 2000). In Sauvignon Blanc wines analysed from Bordeaux and Sancerre, the

4MMP concentration ranged from 4 to 24 ng/L, the 3MH concentration from 733 to 12,822 ng/L and the 3MHA concentration from 212 to 724 ng/L, indicating their important contribution to wine aroma (Dubourdieu *et al.* 2000). At excessive concentrations, these volatile thiols can impart strong, sweaty aromas reminiscent of cat's urine.

Volatile thiols in wine were originally identified after it had been observed that copper additions removed the characteristic 'tropical' aromas associated with Sauvignon Blanc. Therefore, because copper reacts with sulfur compounds to form insoluble odourless sulfides, it was concluded that the aroma compounds in Sauvignon Blanc were probably volatile thiols (SH functional group) (Darriet *et al.* 1995; Tominaga *et al.* 1995, 1998). This hypothesis was strengthened by adding a thiol-specific binding reagent called *p*-hydroxymercuribenzoate to Sauvignon Blanc wine, and finding that it removed the characteristic 'tropical' aromas. Furthermore, this effect (or reaction) could be reversed by adding another thiol, such as cysteine, in excess, thereby re-releasing the aroma-active thiols and restoring the original aromas (Dubourdieu *et al.* 2000).

Unlike methoxypyrazines, volatile thiols are almost non-existent in grape juice and develop only during fermentation. Therefore, it has been proposed that the wine yeast, *Saccharomyces cerevisiae*, is responsible for the formation of volatile thiols during wine fermentation. However, yeast does not biosynthesise 4MMP or 3MH *de novo*. Research has shown that 4MMP and 3MH exist in the grapes in the form of aroma-inactive, non-volatile, cysteine-bound conjugates, i.e. S-4-(4-methylpentan-2-one)-L-cysteine (Cys-4MMP) and S-3-(hexan-1-ol)-L-cysteine (Cys-3MH) (Darriet *et al.* 1995).

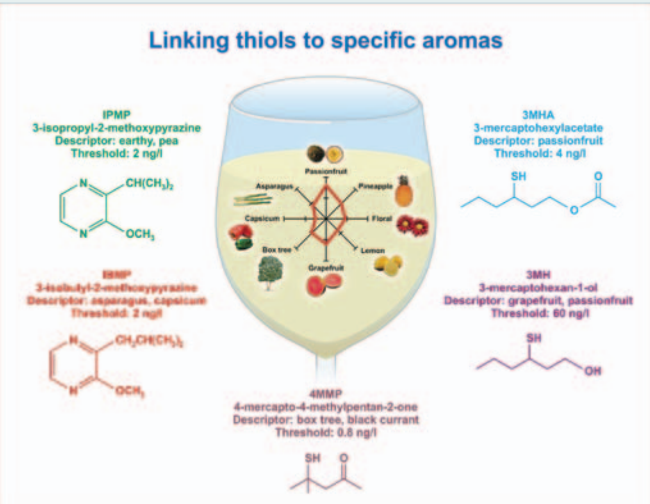


Figure 3. Important aroma compounds in Sauvignon Blanc wines. The common aroma descriptors of Sauvignon Blanc wines are capsicum, tomato leaf, asparagus, grapefruit, gooseberry and passionfruit. In general, these aroma characters can be classed as 'green' i.e. capsicum, tomato leaf and asparagus, and 'tropical' i.e. grapefruit, gooseberry and passionfruit. The 'green' characters are mostly the result of grape-derived methoxypyrazines, 3-isobutyl-2-methoxypyrazine (IBMP), and 3-isopropyl-2-methoxypyrazine (IPMP). The 'tropical' characters come primarily from volatile thiols in the wine, with contributions from fermentation-derived esters. The most important volatile thiols are 4-mercapto-4-methylpentan-2-one (4MMP), 3-mercaptohexan-1-ol (3MH) and 3-mercaptohexyl acetate (3MHA).

### *3.1 Volatile thiol modulation in the vineyard*

Research on the effects of viticultural practice, soil quality and environment on the levels of volatile thiol precursors produced by the vine is very limited. Only recently have the effects of water and nitrogen deficits on the levels of volatile thiol precursors produced by Sauvignon Blanc been investigated (Peyrot des Gachons *et al.* 2005). The concentrations of Cys-4MMP and Cys-3MH were monitored during fruit ripening under various water and nitrogen conditions imposed on the vine. The concentration of volatile thiols had earlier been found to be directly related to the concentration of the S-cysteine conjugate precursors in the grapes (Tominaga *et al.* 1998; Peyrot des Gachons *et al.* 2000). However, it was found that only a small portion of the volatile thiol precursors were released during fermentation, with a maximum of 4.2% of 3MH released from Cys-3MH (Peyrot des Gachons *et al.* 2000). The latter results indicated that stress caused by severe water deficit limited the amount of Cys-4MMP and Cys-3MH formed. However, moderate water deficit stress enhanced the amounts of Cys-4MMP and Cys-3MH that were synthesised (Peyrot des Gachons *et al.* 2005). Reduced levels of Cys-4MMP and Cys-3MH were also observed when the vine was low in nitrogen, but the role of nitrogen status could not be verified because of the potential impact of the varying amount of water available to the vine. However, it appeared that the concentration of the Cys-4MMP and Cys-3MH was optimal when the vine's nitrogen status was non-limiting but not in excess (Peyrot des Gachons *et al.* 2005).

It is clear from the work above that more needs to be done to understand what factors impact on the concentration of volatile thiol precursors formed in grapes in the vineyard. Factors such as shading, canopy density, sunlight exposure and time of harvest should also be investigated. Using information generated from this research, the viticulturist could potentially modulate the aroma potential of Sauvignon Blanc grapes to meet specifications required by the winery and ultimately the market.

### *3.2 Volatile thiol modulation in the winery*

After it had been shown that a carbon-sulfur lyase (C-S lyase) cell-free enzyme extract of the bacterium *Eubacterium limosum* could release 4MMP from its precursor, Cys-4MMP (Tominaga *et al.* 1995), an enzymatic mechanism for the release of thiols was suggested. This led to the hypothesis that the amplification of Sauvignon Blanc varietal aromas during fermentation occurs by the action of yeast carbon-sulfur lyases (Tominaga *et al.* 1998).

Researchers at the AWRI investigated how wine yeast strains were affected in their ability to release 4MMP from the cysteine conjugate when genes encoding putative yeast carbon-sulfur lyases were deleted. Results showed that release of 4MMP during fermentation was reduced when any one of four genes that express C-S lyases (or putative C-S lyases) was deleted in a laboratory strain of *Saccharomyces cerevisiae*. This indicated that release of 4MMP probably involves multiple enzymes. These findings were confirmed in a homozygous derivative of the commercial wine yeast, VL3 (Laffort Oenologie), showing that deletion of the identified carbon-sulfur lyase genes led to a decrease in the amount of 4MMP released during fermentation (Howell *et al.* 2005). Furthermore, researchers at the AWRI showed that 3MHA is produced from 3MH by the action of a yeast ester-forming alcohol acetyltransferase, encoded by the *ATFI* gene. This established the link between ester production and volatile thiol metabolism in yeast for the first time (Swiegers *et al.* 2005c).

It has been shown that as the concentration of chemically-synthesised Cys-3MH (added as a precursor to model ferments) decreased during fermentation, the concentration of 3MH increased. However, only a small fraction of added Cys-3MH was converted to 3MH during fermentation (1.6% at day six of fermentation). This indicated a limited ability of wine yeast to release thiols (Dubourdieu *et al.* 2000). In support of these data, it was shown in Cabernet Sauvignon and Merlot wines that only about 3.2% of 3MH was released from the total amount of Cys-3MH originally present in the must. Furthermore, the total amount of 3MH released depended on the amount of cysteine conjugate precursors present, with higher precursor concentrations in the grapes resulting in higher thiol concentration in the wine (Murat *et al.* 2001b). These results confirmed the limitations of wine yeast in releasing volatile thiols during wine fermentation. Therefore, there is a large, untapped source of aroma remaining in the wine after fermentation. In order to unlock some of this aroma concealed in the grape must, it will be necessary to develop flavour-active yeasts by targeting and modulating key aroma genes (Figure 4).

Research has shown that the amount of 4MMP released in wine ferments depends on the type of wine yeast strain used to conduct the fermentation (Dubourdieu *et al.* 2000; Howell *et al.* 2004, 2005). Therefore, the genetic and physiological characteristics of the wine yeast strain have a significant effect on the amount of volatile thiols released. It was shown that the VL3 yeast strain released more volatile thiols than strains VL1 (isolated from vineyards in France) and 522d (University of California Davis). Furthermore, *Saccharomyces bayanus* strains appeared to release more 4MMP than the VL3 strain (Murat *et al.* 2001a). Research at the AWRI has confirmed these findings by showing that different commercial wine strains have varying abilities to release 4MMP from the Cys-4MMP precursor in model ferments (Howell *et al.* 2004, unpublished work).

Based on the variation in the ability of yeast to release thiols, seven commercial wine yeast strains were selected for small-scale and large-scale production of Sauvignon Blanc wine in order to compare their ability to modulate thiol concentrations in wine conditions and assess the effect on wine aroma. Those selected were (in no particular order) VIN7 (Anchor Yeast), VIN13 (Anchor Yeast), NT116 (Anchor Yeast), VL3 (Laffort Oenologie), X5 (Laffort Oenologie), QA23 (Lallemand) and L2056 (Lallemand). The small-scale wines were made from homogenised Sauvignon Blanc juice sourced from the Adelaide Hills. The small-scale wines were made in triplicate in 20-litre volumes and no copper fining was applied because this had the potential to affect the concentrations of the volatile thiols.

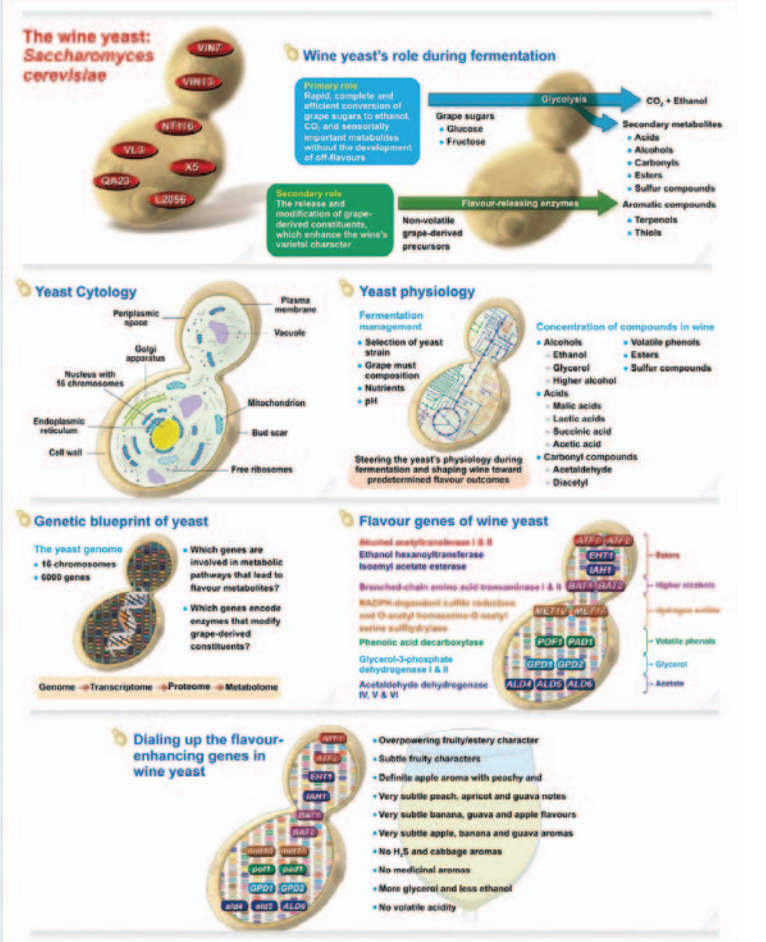


Figure 4. It is the defining characteristic of *flavour* that distinguishes the differences among the thousands of wines produced throughout the world. The flavour of wine varies according to the innumerable possible variations in its production, both in the realm of winemaking and in viticulture. One of the numerous tools that can assist winemakers in their endeavour to produce wines with specific flavour profiles, and to market specifications, is the choice of yeast for fermentation. *Saccharomyces cerevisiae*, universally known as the *wine yeast*, accounts for the major changes between grape must and wine, modifying the chemical, colour, mouth-feel and flavour complexity. Selected strains can produce desirable sensory properties by helping to extract compounds from solids present in grape must, by modifying grape-derived molecules and by producing flavour-active metabolites. For example, the characteristic fruity aromas of wine are caused mainly by yeast-derived acetate esters of higher alcohols and the $C_4$-$C_{10}$ ethyl esters, and in some cases, also by the release and conversion of grape-derived thiols.

The concentration of the important volatile thiols was measured (by Laffort Oenologie) in the small-scale wines (Figure 5). In these wines, VIN7 was by far the highest releaser of 4MMP followed by VIN13 and VL3 (Figure 5a). In the case of 3MH, VIN13 released the most followed by VIN7 and X5 (Figure 5b). However, it is important to take into account that the wines were made in small-scale (20-litre ferments) and that production on a commercial scale could have different results. Indeed, wines made with the same Adelaide Hills Sauvignon Blanc juice by a commercial winery in larger scale (5000-litre ferments) showed a slightly different pattern in some cases. In the case of 4MMP, VIN7 was again far above the rest but was followed by X5 and then VIN13, different to the small-scale ferments (data not shown). In the case of 3MH, VIN7 was the highest releaser followed by X5, VL3 and then VIN13 (data not shown). Due to the capacity constraints of the commercial winery, the large-scale ferments were not replicated and these data can, therefore, only be viewed as preliminary evidence for variation between small- and large-scale fermentations. However, a clear trend appears to exist for strains grouped as 'high thiol releasers' and 'low thiol releasers' regardless of the scale of ferment. This trend correlates with the results from model ferments in the laboratory. The AWRI will continue to perform winery trials with selected wine yeast in the coming vintages in order to confirm these trends.

Researchers at the AWRI investigated in the laboratory the ability of different commercial wine yeasts to convert 3MH to 3MHA in model ferments. Large variations in the ability of commercial wine yeasts to convert 3MH to 3MHA were observed (data not shown). In some cases, this did not correspond to the ability to release thiols (Swiegers *et al.* 2005c; unpublished work). In one example, a commercial yeast strain showed a high capacity to convert 3MH to 3MHA in model ferments spiked with 3MH. However, in the same model ferments this yeast did not appear to have the ability to release significant quantities of thiols.

The 3MHA concentration measured in the small-scale Sauvignon Blanc wines showed large variations (Figure 5c). On average, VIN7 produced the highest 3MHA concentration followed by QA23 and NT116. In order to establish the conversion capacities of the yeast involved in this trial, the ratio between 3MH released and 3MHA formed were determined. According to the 3MHA:3MH ratio determined for the small-scale wines, QA23 had the highest conversion capacity followed by NT116 and VL3 (Figure 6). The conversion capacities of the yeast strains used in commercial-scale fermentations showed the same pattern, i.e. QA23 and NT116 had much higher 3MHA:3MH ratios. However, VL3 did not have the third highest ratio and was on the same level as VIN7 and VIN13 (data not shown). The findings based on the commercial-scale fermentations cannot be considered as scientifically validated data because we were unable to conduct these large-scale fermentations in triplicate.

It has to be kept in mind that a high level of 3MHA in wine does not necessarily mean that the yeast has a high 3MH➛3MHA conversion capacity as it appears that 3MH must first be *released* before it can be *converted* to 3MHA. For example, because VIN7 is such a potent releaser of 3MH, this strain generates a large pool of this compound, which then becomes available for 3MHA production. Although VIN7 is not necessarily the most efficient 3MH➛3MHA converter, the net result is that wines fermented with VIN7 tend to contain high levels of 3MHA, simply because this yeast releases very high levels of 3MH from which 3MHA can be generated (Figure 5c, Figure 6). Based on our results, the two yeast strains with the lowest thiol-*release* capacity (Figure 5 a and b), had the highest 3MH➛3MHA *conversion* capacity (Figure 6). Therefore, the question arises whether we can push up the concentration of 3MHA even higher using a combination of high thiol-releasing and thiol-converting yeast strains. Given that 3MHA has a lower perception threshold than 3MH (therefore being more potent), this strategy might be desirable in order to increase the aroma impact of a wine. One possible solution would be to co-inoculate high 3MH to 3MHA converting yeast strains e.g. QA23 or NT116 with a yeast strain that has a high capacity to release 3MH e.g. VIN7, VIN13, VL3 or X5. Therefore, in theory and, given the compatibility of the yeast strains, the 'thiol-releasing' strain will release most of the 3MH whereas the 'thiol converting' strain will convert the available 3MH-3MHA. Recently, research at the AWRI has shown that the profile of flavour compounds present in wines made by mixed

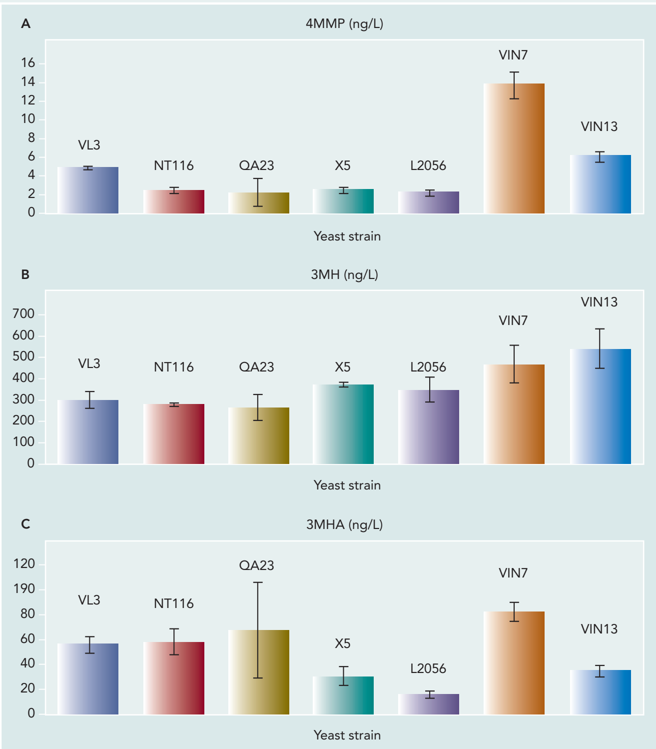


Figure 5. The ability of commercial wine yeast to (A) release 4MMP from the cysteine conjugate precursor, (B) release 3MH from the cysteine conjugate precursor and (C) convert 3MH to 3MHA in small-scale wine ferments. Small-scale (20L) wines were made in triplicate by Provisor using Sauvignon Blanc juice crushed from grapes sourced in the Adelaide Hills. Active dried commercial wine yeast were supplied by Anchor Yeast, Lallemand and Laffort Oenologie. Wines were fermented to dryness and alcohol concentration varied between 13.9% and 14.1%. Volatile thiol analyses were performed by SARCO Laboratories (Laffort Oenologie), France. The yeast population was not fingerprinted after fermentation.

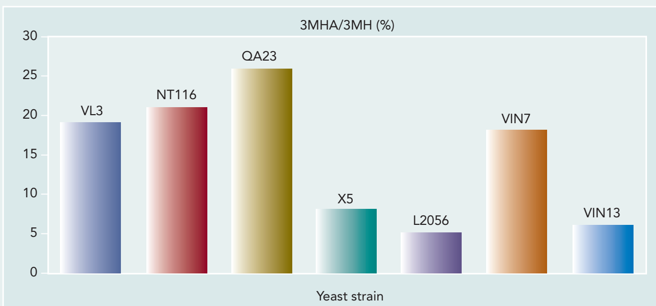


Figure 6. The ability of commercial wine yeast to convert 3MH to 3MHA in Adelaide Hills Sauvignon Blanc small-scale ferments. The 3MH and 3MHA concentrations were measured by SARCO Laboratories (Laffort Oenologie), France. The ratio between 3MHA concentration and 3MH concentration is an indication of the yeast's 'thiol-converting' capacity.

culture fermentation were different from those made with monoculture fermentations. Also, the same aroma profile of the mixed-culture fermented wine could not be achieved by the blending of two monoculture fermented wines (Howell *et al.* 2006). The metabolic interaction of the two yeast strains within the ferment can, therefore, explain the difference in the volatile chemical profile. In 2006, the AWRI intends to conduct small-scale and industry trials to test this hypothesis in Sauvignon Blanc wines, i.e. wines made with a mixed-culture consisting of a potent 'thiol-releasing' yeast strain and a 'thiol-converting' yeast strain would theoretically contain higher concentrations of the potent 3MHA than a blend of two wines; one made with a good 'thiol-releasing' yeast strain and the other with a good 'thiol-converting' yeast strain.

### *3.2.1 Effect of fermentation temperature on volatile thiol modulation*

There have been few publications on the effect of fermentation temperature on volatile thiol release by yeast. Work by Howell *et al.* (2004) suggested higher temperatures might increase 4MMP release from the precursor Cys-4MMP by commercial wine yeasts during model ferments at 18°C or 28°C. However, in this study, one commercial wine yeast showed no difference in volatile thiol release when fermented at 18°C or 28°C. Thus, it is unclear whether the temperature effect is related to yeast strain (Howell *et al.* 2004). In another study, Murat and Dumeau (2005) indicated that in Rosé wines, more 3MH is released at 20°C than at 13°C, as tested in four yeast strains. However, in both studies, the exact point (beginning, middle, end) of fermentation where the samples were taken was not specified.

In a recent study conducted at the AWRI, it was shown that higher fermentation temperatures can result in increased 4MMP release from Cys-4MMP by a commercial wine yeast in model ferments spiked with Cys-4MMP. However, in this experiment, the increased release was only observed at the beginning of fermentation. As the fermentation progressed, the cooler fermentation contained more 4MMP than the warmer ferments (Figure 7a). This phenomenon also appeared to be true for the conversion of 3MH to 3MHA. In the warmer ferments, more 3MHA was formed at the beginning of fermentation, but as the ferment progressed, the cooler ferment contained more 3MHA (Figure 7b). Interestingly, the 23°C ferment had the highest 4MMP concentration on the first day, with the 18°C ferment lowest and the 28°C ferment second lowest at this time point. This might reflect the optimal temperatures for volatile thiol release for the yeast strain used. Studying both 4MMP release and the conversion of 3MH to 3MHA in model systems, the results suggest that thiol release and thiol conversion are metabolic processes that probably occur during the early stage of wine fermentations. Therefore, it seems that most of the volatile thiols are produced by the yeast at the start of fermentation. Another possible reason for the bigger drop in 3MHA and 4MMP concentration in

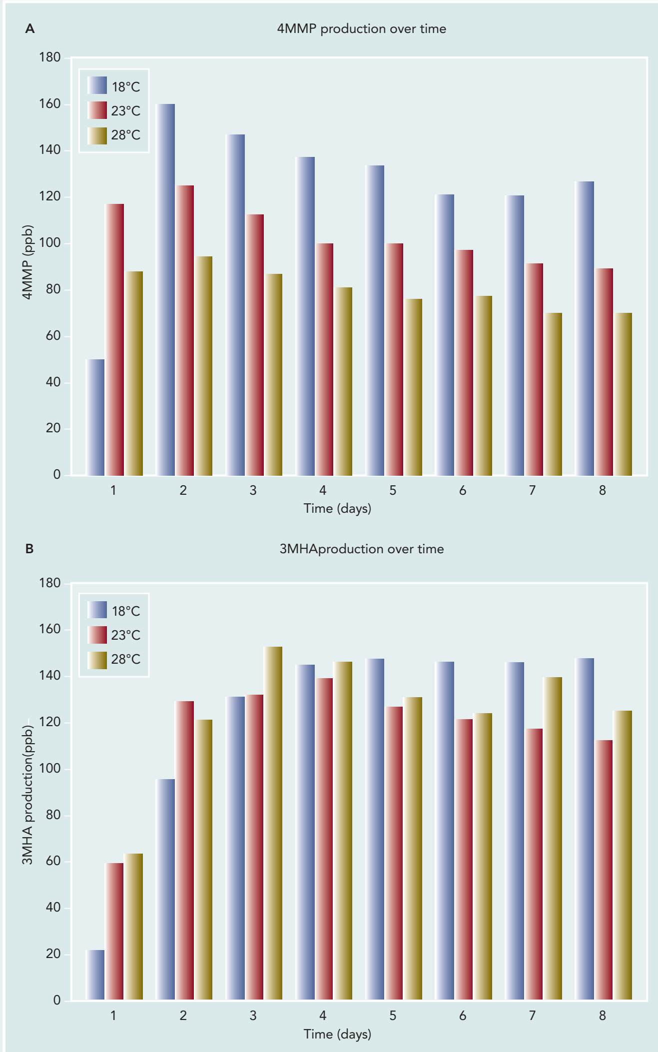


Figure 7. The effect of temperature on the ability of a commercial wine yeast to release 4MMP and convert 3MH to 3MHA. A commercial wine yeast was grown in Yeast-Peptone-Dextrose (YPD) medium overnight at 30°C. A Yeast Nitrogen Base (Difco) synthetic medium containing 80 g/L sugar was inoculated with 2mL of yeast preculture and fermented in 200mL volumes in fermentation flasks with airlocks for eight days. Fermentations conducted at 28°C and 23°C were completed between day four and day five. Fermentations conducted at 18°C were finished at day eight. The fermentation medium was spiked with 3.35 mg/L cys-4MMP and 0.72 mg/L of 3MH before inoculation.

the warmer ferments is that the volatiles are 'boiled off' due to the excessive vigour of the ferments. Therefore, it could be that, metabolically, the yeast is able to release and convert more thiols at warmer temperatures but that the excess thiols are then lost together with the fermentation gases. This is probably a reason why a temperature regime of starting fermentations warm and then cooling them down is employed by many wineries. It is not clear whether volatile thiols are still metabolically released or converted during the middle and later stages of fermentation and, if so, in what quantities. The results to date would suggest that this is not the case, but further experimental proof is needed.

Temperature can also play an important role in thiol modulation during skin contact. It has been shown that more Cys-3MH, which is mostly located in the skins of grapes, is extracted at 18°C compared to 10°C (Peyrot des Gachons *et al.* 2002). However, this did not have a big effect on the

4MMP concentration presumably because the precursor for this compound, Cys-4MMP, is present in the pulp. Therefore, it appears that the temperature during skin contact could have a profound effect on the amount of precursor released into the juice, and the temperature at which the grapes are pressed might similarly be important. Thus, a wine with a more complex volatile thiol repertoire could potentially be produced by applying the correct combination of fermentation temperature and thiol releasing/converting yeast strain. However, care should be taken not to allow too much skin contact time or too high temperatures because this could lead to excessive bitterness and astringency in Sauvignon Blanc wine. More research in this area will help to determine optimal skin contact times and temperatures and possibly also determine which yeast would best complement the enriched juice.

### *3.2.2 Effect of commercial wine yeast strains on the aroma profile*

As indicated before, research at the AWRI showed that different strains of commercial wine yeast have significant variation in their ability to release and convert volatile thiols. But what effect will this have on the perceived aroma profile? To answer this question, commercial yeast strains were selected based on their different abilities in volatile thiol modulation, and small-scale (20-litre ferments) Adelaide Hills Sauvignon Blanc wines were prepared. The volatile thiol concentrations of these wines were measured and large variations were observed as discussed above (Figure 5). In addition, eight commercial wineries were involved in trialing the selected yeast to produce Sauvignon Blanc wine during the 2005 vintage. The wineries trialed between three and seven of the different yeast strains in order to assess the impact of the yeast strain on the aroma and taste.

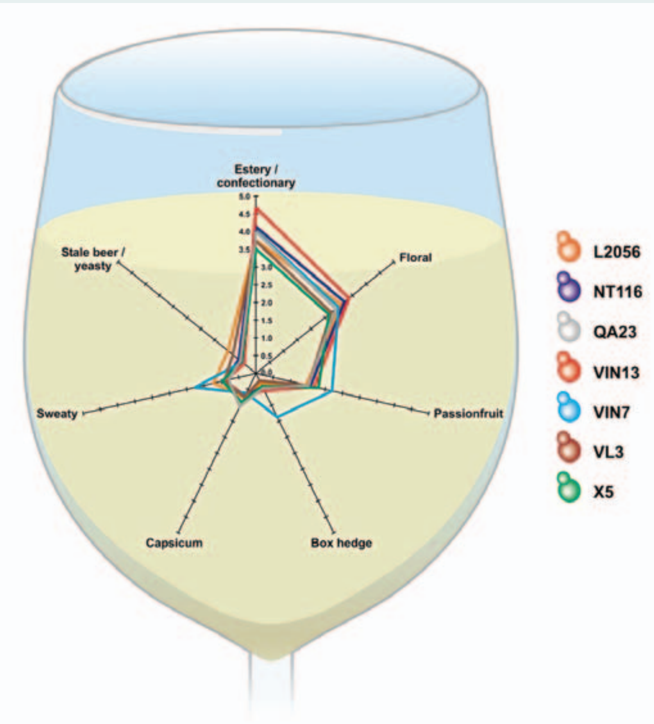


Figure 8. Mean intensity scores for the important aroma attributes rated by the trained sensory panel for each of the small-scale Adelaide Hills Sauvignon Blanc wines produced using the seven different wine yeast strains.

A formal sensory analysis was conducted on the small-scale wines. A trained AWRI sensory panel (12 assessors) assessed each wine in duplicate and rated the intensity of aroma and flavour attributes of the wines (made in triplicate for each yeast strain). Significant differences in the sensory profiles were observed. Figure 8 shows the results for some of the attributes that were most important in differentiating the strains. On average, VIN7 was found to produce wines with the highest intensity of passionfruit, sweaty and box hedge aromas. The aroma of the box hedge is reminiscent of cat's urine which is often associated with Sauvignon Blanc and high thiol concentrations. The high concentration of 4MMP in the wines made with VIN7 probably explains the box hedge aroma. It is not clear if the sweaty aroma produced by VIN7 is due to volatile thiols. The passionfruit aroma produced by VIN7 is probably due to the high amount of the three thiols produced. On average, VIN13 produced wines that were highest in both estery/confectionary and floral aroma attributes. The fact that VIN13 did not score as high as VIN7 in the passionfruit aroma, despite having the highest concentration of 3MH, could be due to the contribution to this aroma by 4MMP, or by masking by other fermentation volatiles such as esters, acids and alcohols. The concentration of these compounds is currently being determined. On average, the X5 strain produced wines which showed a relatively high intensity of passionfruit aroma and also appeared to expose the capsicum aroma of the wine to a slight degree. The QA23 strain also had a tendency to produce wines with an exposed capsicum aroma. From these data, it is clear that substantial differences in the aroma of the wines were detected. As the wines were made from the same juice and fermented under the same conditions (15°C), the impact of the yeast strains on the aroma of wine is clearly demonstrated. It is important to be aware when considering the results of this experiment that the performance of the yeast strains can vary from winery to winery and vintage to vintage.

To complement the formal sensory analysis of the small-scale wines, industry wine tastings were held where winemakers had the opportunity to taste all the wines (more than 40 small-scale and commercial-scale wines) produced during the yeast trials (Figure 9a). For the commercial wines prepared using the selected yeast strains, there was a range of preferences for yeast strains as noted by the winemakers participating in the tastings. For the small-scale wines, the winemakers, as a group, significantly preferred the wines made with the VIN7 strain (Figure 9b). The AWRI will continue to investigate the thiol-modulating ability of these and other yeast strains in the coming vintages as it is quite possible that different yeast strains might be better suited for certain regions, grapes and winery conditions.

As part of this study, the concentration of fermentation volatiles such as esters, acids and alcohols are currently being determined. The complete data set will be published as part of a more comprehensive research article in a peer-reviewed journal.

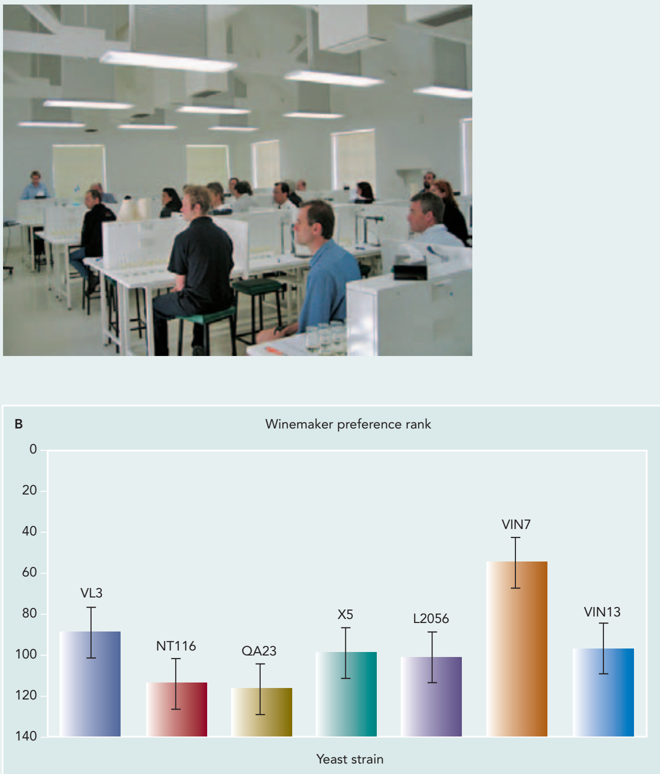


Figure 9. (A) A photograph taken during the Sauvignon Blanc industry sensory evaluation session held at The Australian Wine Research Institute in November 2005. (B) Preference rank sum values from the industry sensory evaluation for each of the small-scale Adelaide Hills Sauvignon Blanc wines produced using the seven different wine yeast strains. The lower the rank value, the more the wine was preferred (coded samples presented in a randomised order across 24 judges in a single session).

## 6. CONCLUSIONS

While the 'green' characters in Sauvignon Blanc wines can be manipulated through vineyard management, the 'tropical' characters appear to be largely dependent on the wine yeast strain used during fermentation. Although there can be variation in the amount of thiol precursors present in the grape, the major limiting factor is the ability of the selected yeast to release the thiols from the precursors. Research indicates that currently less than 5% of the precursors present are released (Tominaga *et al.* 1998; Dubourdieu *et al.* 2000; Peyrot des Gachons *et al.* 2000). Therefore, yeast selection appears to be one of the most promising tools in manipulating the passionfruit, grapefruit and gooseberry aromas in Sauvignon Blanc wines. A combination of environmental and viticultural influences and winemaking practices will determine the delicate balance of fruity, tropical aromas and the green, herbaceous aromas that define quality, New World Sauvignon Blanc styles, which consumers would prize highly.

In conclusion, this report does not imply that the yeast strains that produced the most preferred wines in this study would be the 'best' yeast for the production of all Sauvignon Blanc wines. Important variations in the flavour profile and style of Sauvignon Blanc wines might originate from the region in which the fruit was grown, or be dictated by the preferences of the wine producer and the winemaking methods adopted and, in some instances, those variations might be considered of greater importance than the differences conferred by the yeasts examined in this study. Rather, the significance of this work hinges on the fact that the choice of yeast strain for fermentation offers the potential to modulate aroma profiles in order to match predetermined specifications that are dictated by various consumer markets, enhance regional characteristics, or allow winemakers to more reliably produce wines in their desired style. So, depending on the desired style of Sauvignon Blanc wine required by the winemaker, different wine yeast strains can be selected to modulate specific thiol aromas that comprise the 'tropical' profile. For example, preference for box tree and passionfruit aromas would require a yeast that expresses greater release of 4MMP, while preference for passionfruit, grapefruit, gooseberry and guava aromas requires a yeast having the ability to release greater quantities of 3MH and/or convert 3MH into 3MHA. Therefore, this study offers prospects for the development of wine yeast starter strains with optimised thiol-producing capability that could assist winemakers in their effort to consistently produce wine to definable specifications and styles.

To deliver improved consumer preference information, flavour-active yeast strains, viticultural management and winemaking technologies to the Australian wine industry, future research at the AWRI will focus strongly on collaborative, multi-disciplinary research across the entire production chain from the vineyard through winemaking to the consumer (Figure 10).

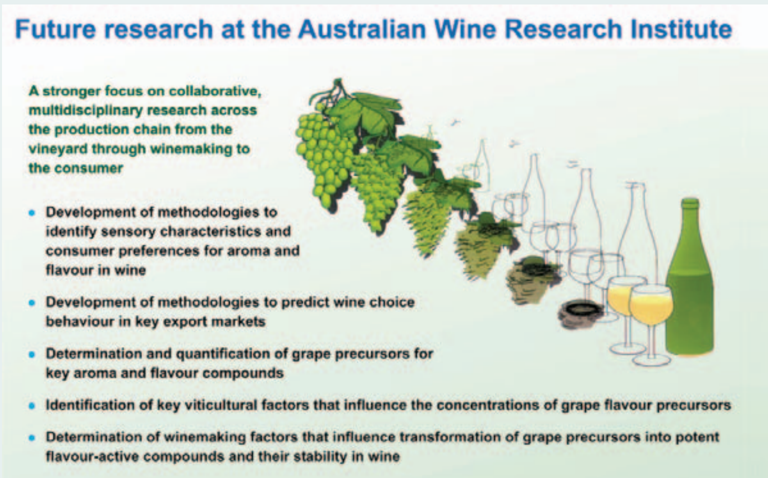


Figure 10. A diagrammatic representation depicting the need to meet consumer demands by generating and improving a responsive supply chain to deliver wines of appropriate quality to consumers in different world market segments. It is increasingly important to produce wine consistently to definable aroma and flavour specifications. With this comes the need to better integrate grape and wine research and to focus on the management of sensory features of wine that attract the consumer. Some of the key fundamental questions that relate to satisfying consumer preferences across the wine production chain that need to be answered are: (i) What are the grape-derived precursors to the impact flavour compounds in wine? (ii) How are these flavour-active compounds transformed during winemaking? (iii) How do viticultural and winemaking techniques influence the concentrations of these compounds in wine? (iv) How do these compounds influence wine flavour and aroma? (v) How do consumers respond in combination with other marketing cues?

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