

Harnessing AWRI's yeast and bacterial research to shape 'Next-Gen' Chardonnay Part 2: Influence of yeast, nutritional management and malolactic fermentation



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In Part 1 of this article - published in the January/February 2011 issue of the Wine & Viticulture Journal - the impact of 'wild' and non-conventional yeast on Chardonnay style was demonstrated. 'Wild' ferments were a hot topic at the 'Next-Gen Chardonnay' workshop (14th AWITC, July 2010) - adventurous winemakers finding their additional complexity consistent with stylistic goals in the development of 'Next-Gen' Chardonnay wines. In Part 2, we explore conventional options available to winemakers for modulation of Chardonnay aroma. Can commercially-available yeast, with appropriate nutritional management, be harnessed to generate aromas and flavours consistent with 'Next-Gen' Chardonnay styles? We also revisit malolactic fermentation (MLF). While associated strongly with classical styles of Chardonnay, MLF can be used to enhance complexity without imparting 'buttery' characters through careful application of appropriate bacterial strains.

COMMERCIALY-AVAILABLE YEAST

There are currently more than 100 wine yeast strains available commercially, predominantly made up by the yeast species *S. cerevisiae*¹. Recent studies have examined genetic diversity amongst some of these strains and found it to be relatively low compared with examples of the species from other environmental sources. Nonetheless, anecdotal evidence has existed for some time that yeast strains differ in their capacity to influence wine flavour, but there was little formal scientific information to confirm these observations.

An early study conducted at the AWRI investigated several *S. cerevisiae* strains (AWRI 796, AWRI 835, AWRI R2, EC1118, ICV D47 and Avise), which were believed by some winemakers to influence wine aroma in Chardonnay². The results demonstrated that wines made with each yeast were statistically distinguishable from at least two other wines by sensory difference testing, except for AWRI R2, which was not reliably distinguished from any of the other wines. These differences, however, dissipated with time such that after storage for 18 months at 4°C, only the wines made with AWRI 835 and ICV D47 could be distinguished from Avise. Although the aroma profiles could not be described reliably, the descriptors that gave the greatest variability among the wines were 'amyl acetate' ('banana'), 'confectionary', 'ethyl acetate' ('solvent'), 'lime', 'vanilla' and 'pear'; whereas 'honey', 'peach' and 'pineapple' showed little variability (Figure 1). The former aromas are associated to a greater extent with yeast metabolites formed through sugar fermentation, whereas the latter are derived from grape precursor compounds.

Recently, the effect of yeast strain on Chardonnay flavour was evaluated more comprehensively. A 2008 Margaret River Chardonnay juice was used in a small-lot winemaking trial under controlled conditions. Nine commercially-available active dry wine yeast (ADWY) were selected on the basis of their propensity for formation of aroma compounds, and their typical usage. For example, Anchor Alchemy I and NT1116 produce high concentrations of 'fruity' esters and are typically used in tank

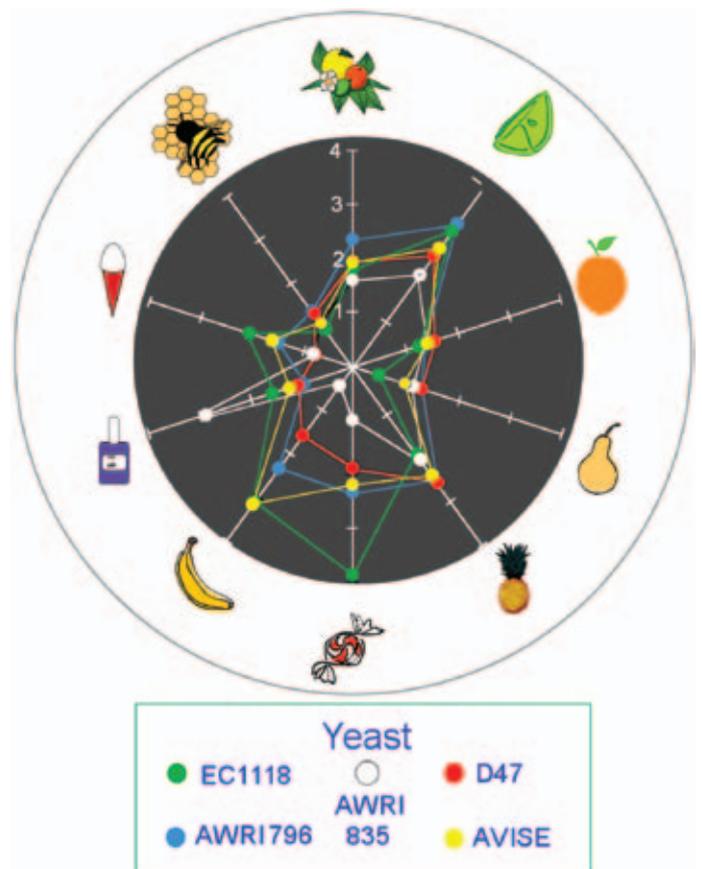


Figure 1. Aroma profiles of Chardonnay wines made with different strains of *Saccharomyces cerevisiae* (Jane *et al.* 1996²).

fermentations, whereas Lalvin D47 and Anchor VIN2000 exhibit flavour profiles suited for barrel fermentation.

An analysis of the sensory data is shown in Figure 2 [see page 16], where yeast treatments are separated on the basis of significantly different sensory attributes. The main separation of

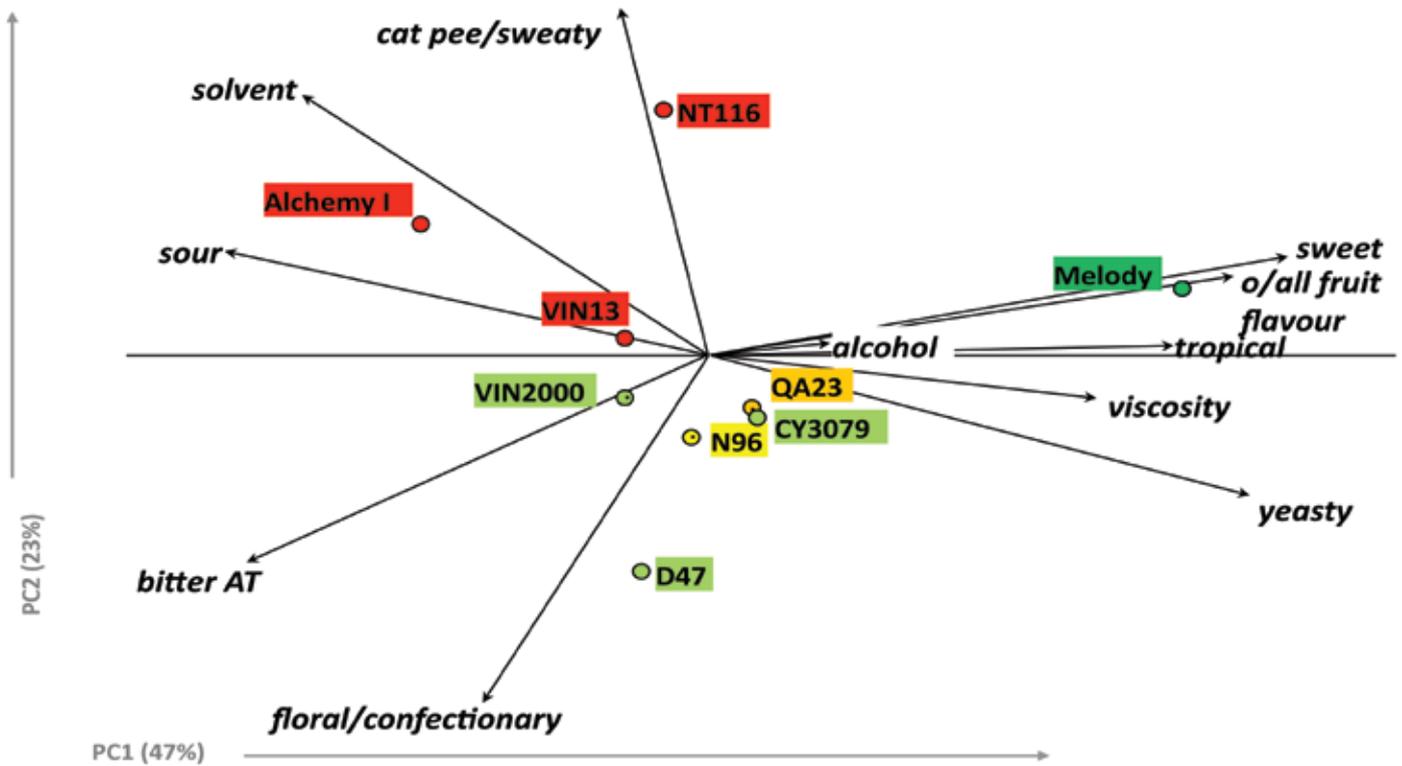


Figure 2. Principal Component Analysis biplot of mean sensory data for Chardonnay wines made with nine different active dry wine yeast products available commercially. Adapted from Curtin *et al.* 2009³.

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the wines along the main axis (Principal Component 1 [PC1]) was on the basis of differences in 'sweetness', 'overall fruit flavour', 'tropical fruit' and 'yeasty' to the right, and 'sourness', 'bitter aftertaste' and 'solvent' to the left. 'Solvent' scores were highly correlated with concentrations of acetate esters such as 3-methylbutyl acetate and ethyl acetate, but not volatile acidity.

Wine made with the Melody ADWY blend was rated the highest in 'tropical' and 'yeasty' aromas, 'overall fruit flavour', 'sweet' and 'viscosity'. All wines in this study were dry (<2g/L residual sugar). Nonetheless, those made with Melody were highest in residual sugar and this may have contributed to the perception of these attributes. On the other hand, 'wild' fermentations are known for their enhanced mouthfeel properties, and further examination of aroma compound production revealed that Melody was indeed a good approximation of a 'wild' fermentation – with highest concentrations of 2-methylpropanol, ethyl-2-methylpropanoate, ethyl decanoate and ethyl dodecanoate, and second highest concentration of 2-methylbutanoic acid in this study. These compounds were reported by Varela and colleagues⁴ to be characteristic of a range of Chardonnay wines made using 'wild' yeast. Interestingly, the attribute 'sour' was found to be significantly different, even though the total acidity of all the wines were very similar. Sourness was inversely correlated to sweetness, thus, it is possible that the slightly elevated residual sugar in wine made with Melody masked the perception of acidity.

The second axis (Principal Component 2 [PC2]) separated the wines based upon ratings for attributes 'cat urine'/'sweaty' and 'floral'/'confectionary'. Wine made with NT116 exhibited strongest 'cat urine'/'sweaty' aromas, while Alchemy and Melody yeasts were also rated highly in this attribute. Opposite to the 'cat urine'/'sweaty' aroma on PC2 is 'floral'/'confectionary' aroma, which was rated highest in the wine made with ICV D47. This wine was also rated highest in 'bitter aftertaste'.

'Cat urine'/'sweaty' aroma has been linked strongly to the polyfunctional thiol compound 3-mercaptohexyl acetate (3MHA), which contributes to the varietal character in Sauvignon Blanc wines⁵. In this particular Chardonnay juice, the yeast strain NT116 produced the highest concentration of this compound (Figure 3). Despite the related compound 3-mercapto hexan-1-ol (3MH) being detected in all wines at concentrations well above the perception threshold of 60ng/L (Figure 3), it did not exert as

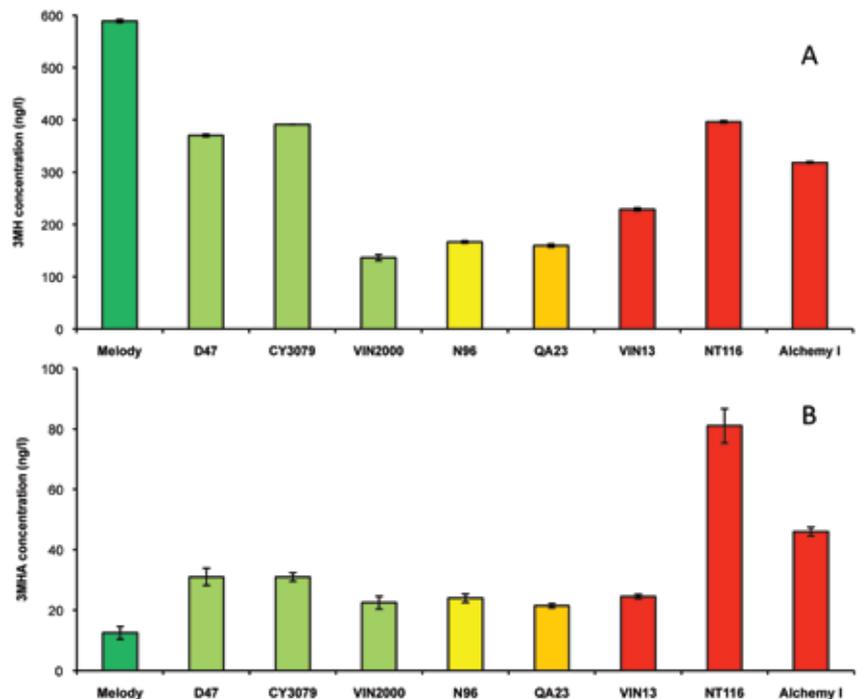


Figure 3. Results of volatile thiols analysis for 3MH (A) and 3MHA (B). Samples were analysed at the time of sensory analysis, from two representative fermentation replicates per treatment. Adapted from Curtin *et al.* 2009³.

clear an effect upon sensory profiles as that observed for 3MHA. While low concentrations of 3MH have been reported in Chardonnay⁶ previously, this was the first study to our knowledge where polyfunctional thiols have been shown to affect Chardonnay aroma. Research examining the parameters that affect the formation of polyfunctional thiols where Sauvignon Blanc has been used as a model system can now be turned to optimising Chardonnay flavour. The availability of methods recently developed to analyse precursors of the polyfunctional thiols⁷ may facilitate matching of yeast such as NT116 with low-precursor juices to boost 'tropical fruit' flavours, or the opposite where it is desired to minimise Sauvignon Blanc-like characters.

WERE THESE DIFFERENCES SUFFICIENT TO AFFECT CONSUMER PREFERENCES?

A significant recent trend in academic research and wine industry product development is the use of consumer preference data to confirm that alterations to the sensory properties of wine are sufficient to influence consumer liking. A clear case has been made that the choice of yeast inoculation strategy affects Sauvignon Blanc flavour, which in turn affects consumer preference⁸. Seven Chardonnay wines from the trial described above were selected for a central location consumer test.

Consumers were recruited for the study who matched the following selection criteria: regular white wine drinkers (drink white wine at least once per week, buy bottled wine \$10-\$20 from time to time); age 18-65; 50% males and 50% females; and living in Adelaide. A group of 101 consumers evaluated all the wines blind, with the wines presented one-by-one, in a randomised order in three-digit-coded wine glasses at approximately 10°C. Consumers rated each wine for overall liking on a nine-point hedonic scale, from 'dislike extremely' to 'like extremely'.

Figure 4 (page 18) shows the mean liking scores for wines made with different yeast strains. Cluster analysis identified three distinct segments in the population: consumers who liked the same wines are grouped together. The first segment consisted of 33% of the consumers who rated all wines with scores higher than 6, or 'liked moderately'. There was a significant difference ($p=0.05$) in the mean liking scores for the wines for the consumers in this segment. ICV D47 was the least preferred wine for this cluster with Melody being the most liked. It is important to acknowledge that some yeast strains in this trial, such as ICV D47, are typically used in barrel fermentation where secondary characters are more important and it is desirable to limit the formation of 'fruity' esters. Nonetheless, it is interesting to note that Melody, as an approximation of a 'wild' fermentation, would find usage in

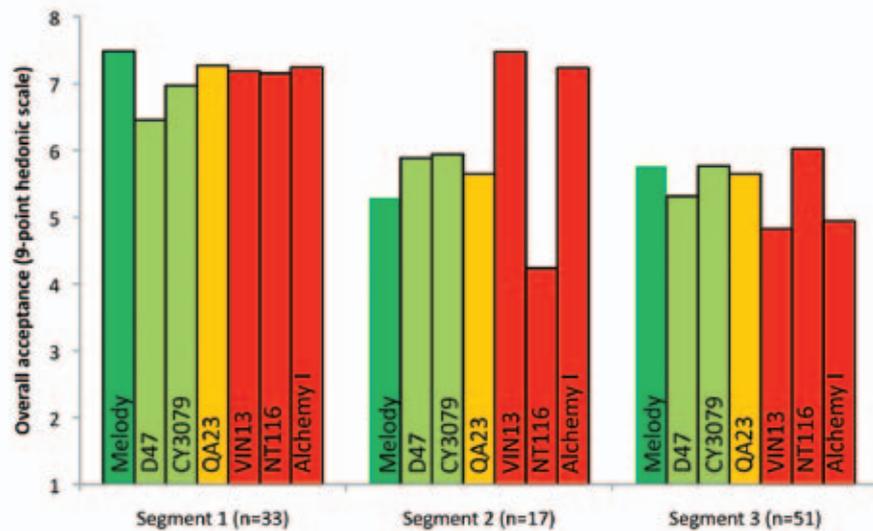


Figure 4. Results of central location test of consumer acceptance for Chardonnay wines made with nine different active dry wine yeast products available commercially. Adapted from Curtin *et al.* 2009³.

similar contexts to ICV D47 despite their apparent differences in chemical profile and sensory attributes.

There was a high correlation coefficient ($r=0.86$) between overall liking scores of Segment 1 and the total population, indicating that this segment's preferences were broadly representative of the total population. Segment 1 consumers were more experienced wine drinkers, being somewhat older as a group - 65% over 41 years old - and 80% had been drinking wine for more than 10 years. They also had a higher income than the average consumers, with more than 40% married with children, and a higher proportion of consumers had a post-graduate degree.

Segment 2 (17% of the consumers) and Segment 3 (50% of the consumers) did not show significant differences in the mean liking scores for the wines, although closer examination revealed an interesting trend. The most liked and disliked wines for these segments were those highest in ester concentrations and those rated highest in 'solvent'. The key differentiator was wine made with NT116, which combines the 'solvent' character with the 3MHA-driven 'cat urine'/sweaty' attribute. It would seem that Segment 2 consumers responded favourably to wine with high concentrations of esters except when this was combined with high concentrations of 3MHA, whereas Segment 3 consumers displayed the opposite tendency. This polarising effect of polyfunctional thiols upon consumer preferences was also observed by King and colleagues⁸, indicating the potential to target consumer segments through choices made at the time of inoculation.

IMPORTANCE OF NITROGEN MANAGEMENT

In addition to the choice of yeast strain, must nutrient content, and especially nitrogen can affect both basic wine composition and volatiles content. Diammonium phosphate (DAP) addition has been an integral part of fermentation management for several decades due to the fact that must nitrogen is highly variable across and within varieties, regions and vintages^{9,10}. The principal purpose of DAP addition is to supplement must nitrogen so as to improve fermentation kinetics, especially to reduce the risk of slow and stuck fermentation. DAP is also often added in response to H₂S formation. Despite the near universal use of DAP, surprisingly, the impact of DAP on wine flavour and style, apart from suppressing sulfidic off-flavours, has only become recognised recently^{11,12}.

The first comprehensive study of the effect of nitrogen supplementation of Chardonnay on wine composition and aroma compared ammonium nitrogen (inorganic N) in the form of chloride (a counter ion known to have little influence on yeast metabolism) and a mixture of amino acids and ammonium nitrogen (referred to as amino acid nitrogen; organic N), based on analysis of the juice¹³. The juice had a yeast assimilable nitrogen (YAN) content of 160mg/L and the supplements were added to increase YAN content to 320mg/L (moderate nitrogen) and 480mg/L (high nitrogen). The supplements increased yeast cell number and fermentation rate to a similar extent but had no important effect on residual sugar, ethanol and glycerol contents. Compared with the

control wine, titratable acidity was more affected by amino acid supplementation, whereas pH, malic acid and succinic acid were most affected by ammonium supplementation alone. Acidification of wine by DAP addition is well known¹⁴ and might represent a partial option for improving the balance of low acid wines.

Yeast volatiles were, to various extents, affected both by the amount and type of nitrogen (inorganic versus organic nitrogen). Acetate esters and medium chain fatty acid esters increased to a greater extent and higher alcohols decreased to a lesser extent in response to amino acid nitrogen supplementation when compared with ammonium nitrogen alone, whereas the latter greatly increased ethyl acetate and acetic acid concentrations.

Formal sensory descriptive analysis showed that both the amount and type of nitrogen supplementation influences wine aroma. The unsupplemented control wine was rated relatively low in aroma intensity and was low in 'floral'/'fruity' aroma descriptors but relatively high in less-desirable descriptors such as 'stale beer', 'cheese', 'sweat', 'wet cardboard', 'artificial grape', 'acetic' and 'nail polish remover' (Figure 5, page 20). The wines produced with moderate nitrogen supplementation showed better balances between desirable and less desirable aroma attributes, and were very similar to each other irrespective of supplementation type. On the other hand, the type of nitrogen source produced very strong differences in the high nitrogen wines. Amino acid supplementation produced wine with highest ratings for 'floral'/'fruity' attributes whereas the ammonium nitrogen supplemented wine had a strong 'acetic'/'solvent' character, which dominated the desirable 'floral'/'fruity' attributes. In conclusion, low-nitrogen fermentation is associated with a complex aromatic profile, which some consider undesirable, whereas nitrogen supplementation increased perceived aroma intensity and increased the desirable 'floral'/'fruity' attributes, but in the case of ammonium nitrogen, high addition produced an undesirable solvent character. Therefore, DAP use should be based on juice/must analysis so that excessive addition is avoided. The addition of pure amino acids is precluded according to Australian and New Zealand wine regulations and, additionally, is cost prohibitive. A variety of organic amino nitrogen sources are available commercially, which are based on inactivated yeast or their extracts, but little information on the flavour properties and addition amounts is available.

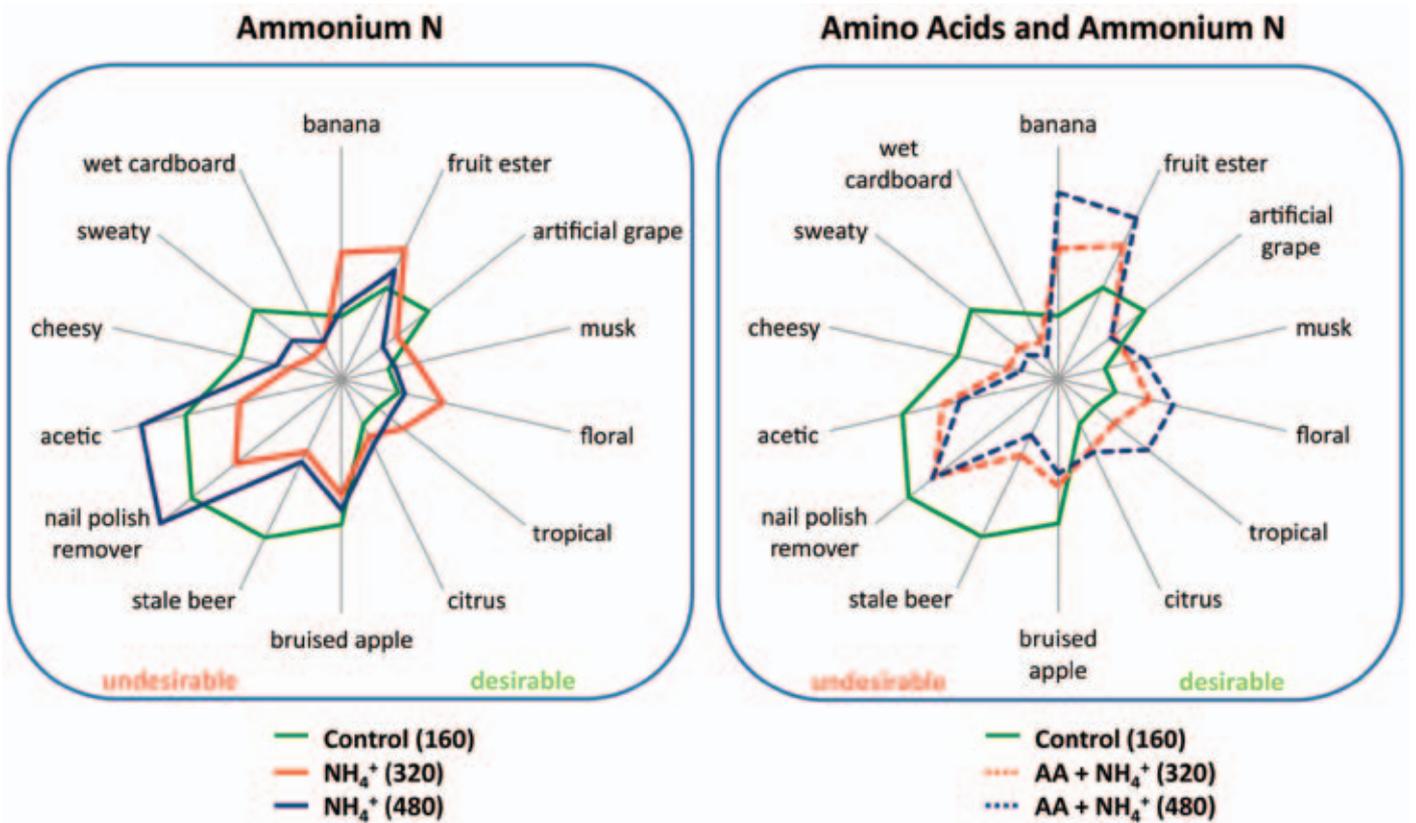


Figure 5. Aroma profiles of wines made from low YAN Chardonnay juice (Control – 160mg/L) supplemented with ammonium or a mixture of amino acids and ammonium to produce moderate (320mg/L) and high (480mg/L) YAN juices. Fermentations were made with AWRI 796 at 18°C¹³.

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The above-mentioned study was made with AWRI 796 (Maurivin) which, along with related yeast strains produced by different yeast manufacturers, has been widely used with Australian Chardonnay wines. Similar DAP studies conducted with Shiraz and with other yeast strains suggest that the sensory effects of DAP addition depend strongly on the strain of yeast¹⁵. For example, moderate (150mg/L YAN) but not high (300mg/L YAN) addition of DAP to a Shiraz must (100mg/L YAN), when fermented with D254 but not AWRI 1176, induced residual H₂S in the finished wine, which produced a sensorially evident 'reductive' character in the moderate YAN wine¹⁵. A survey of wine yeasts conducted in a low-YAN Chardonnay juice confirmed that some strains produce more H₂S when a moderate (150mg/L YAN) but not a high (300mg/L YAN) addition of DAP is made¹⁶. These early results suggest that the interaction of DAP, yeast and juice/must composition is complex and it is too early to generalise with respect to H₂S levels and associated 'reduction' character in wine. In addition, to assist with the management of H₂S production during winemaking, the AWRI has developed and commercialised (through Mauri Yeast Australia) three low-H₂S, PDM-based strains: Maurivin Advantage, Distinction and Platinum for use in problematic musts¹⁷.

In conclusion, low nitrogen fermentation is associated with a complex aromatic profile, which some consider undesirable while others consider to be 'funky' or 'feral' ferment in character. On the other hand, nitrogen supplementation increases perceived aroma intensity and increases the desirable 'floral'/'fruity' attributes but, in the case of ammonium nitrogen, high addition produced an undesirable solvent character. Therefore, DAP is a powerful tool for modifying the style of (low YAN) Chardonnay wine, ranging from complex to fruity to solvent/ester taint, whereas naturally moderate to high juice YAN might simply produce cleaner and less complex wines¹³. Increasing YAN of any juice or must beyond 350-400mg/L with DAP is unlikely to achieve any flavour benefit, and addition should always be based on juice/must analysis to ensure that an excessive addition is avoided, minimising risk of an 'ester taint' or excessive phosphate content in the wine.

JUDICIOUS APPLICATION OF MALOLACTIC FERMENTATION

During the heyday of Australian Chardonnay, MLF was not always an intentional production process¹⁸, often occurring in wines with insufficient natural acidity resulting in 'flabby' and unbalanced wines. In addition, the propensity for malolactic bacteria to influence aroma and flavour by various mechanisms was not appropriately harnessed. One of the most evident flavour changes that can occur during MLF is the development of a 'buttery' or 'butterscotch' character. Diacetyl has been attributed to being a major contributor to this 'buttery' character in wine^{19,20,21,22}, but can also contribute to 'yeasty', 'nutty', 'toasty' aromas at lower concentrations^{23,24}. A range of practical considerations for modulating diacetyl concentration through controlled MLF are detailed by Bartowsky and Henschke²⁵ and are summarised in Table 1 [page 22].

Other wine aroma and flavour components can also be influenced during MLF. Fermentation-derived volatile compounds, including ethyl esters, contribute to the fruity and floral notes in Chardonnay wines. Winemaking trials have shown that MLF is able to increase the concentration of these volatiles, translating into desirable sensory changes²⁶. Furthermore, glycosidase activity was found amongst numerous commercial starter cultures of *Oenococcus oeni*^{27,28}, and one strain was proven to release aglycons such as 3-hydroxydamascone, a-terpineol and vanillin purified from Chardonnay glycoside extract²⁹.

A current trend is the utilisation of partial MLF in Chardonnay, introducing complexity whilst retaining sufficient acidity. Co-inoculation of alcoholic and malolactic fermentations

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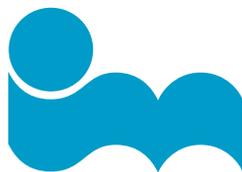
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Table 1. Winemaking practices and factors that can be used to modulate the diacetyl content of wine (adapted from Martineau *et al.* 1995²¹ and Bartowsky and Henschke 2004²⁵)

| Winemaking practice or factor | Effect on diacetyl concentration and/or sensory perception |
|---|---|
| Malolactic bacterial strain | <i>O. oeni</i> strains vary in production of diacetyl (information available from ML strain suppliers) |
| Rate of MLF as affected by: | |
| • Inoculation rate of malolactic bacteria | Lower inoculation rate (10 ⁴ vs 10 ⁶ cfu/ml) favours diacetyl production |
| • Temperature | 18°C versus 25°C may favour diacetyl production |
| • pH of wine | Lower pH may favour diacetyl production |
| Contact with active yeast culture (lees) | Yeast contact reduces diacetyl content of wine |
| Contact of wine with air during MLF | Oxygen favours production of diacetyl (conversion of α-acetolactate to diacetyl is promoted in the presence of air/oxygen) |
| Sulfur dioxide addition | <ul style="list-style-type: none"> • SO₂ binds diacetyl which renders it sensorily inactive (reversible reaction) • SO₂ addition inhibits yeast/bacteria activity (stabilises diacetyl content at time of addition) |



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in Chardonnay wines is another/ alternative winemaking practice that can be used to preserve 'fruity' characters whilst managing acidity through MLF²⁶.

CONCLUSIONS AND FUTURE PERSPECTIVES

Chardonnay wines succeeding in the wine show circuit over recent years epitomise a trend towards increased levels of natural acidity coupled with more subtle use of oak – 'Next-Gen' Chardonnay. If these wines are the style leaders around which a positive marketing campaign to lure consumers back to Chardonnay can be constructed, it is essential that wines consistent with these styles exist across market segments. Some of the techniques used to build complexity in super-premium wines are not readily transferrable to wines sold at lower price points, necessitating innovation.

Within this two-part report, we have outlined a concerted effort over recent years to provide a range of microbial tools and practices applicable to modulating Chardonnay aroma and flavour, whilst retaining control of the fermentation process. If 'complexity' is a key target, then harnessing yeast and bacterial diversity to deliver wine expressing different flavours and aromas can augment viticultural efforts to maximise fruit quality.

An important parameter that requires careful management during production of wine parcels for 'Next-Gen' Chardonnay is supplementation of nitrogen in the form of DAP. If some degree of 'funk', verging on 'struck-match-flint', is a characteristic of 'Next-Gen' Chardonnay, then pre-determined DAP additions may result in wines that are clean but one-dimensional. Careful consideration of yeast strain based upon nitrogen demand and initial nitrogen content would be required to avoid problem fermentations if strategies to minimise DAP addition were implemented. Fermentation in the presence of increased solids can improve yeast robustness and reduce the need for inorganic nitrogen, but careful trialling is necessary to avoid phenolic characters and the formation of 'reductive' off-flavours.

Current research at the AWRI is taking this direction, while other research in the areas of sulfur compound formation and fate throughout the winemaking process will augment the pursuit of complexity, that perhaps is best visualised as a tightrope walk with 'stuck' fermentation and excessive 'reductive' characters awaiting those who fall.

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