# The modulation of Sauvignon Blanc wine aroma through control of primary fermentation


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A thesis submitted for the degree of Doctor of Philosophy


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## Declaration

I declare that this thesis is a record of original work and contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution. To the best of my knowledge and belief, this thesis contains no material previously published or written by another person, except where due reference has been made in the text. The publications included in this thesis have not been previously submitted for the award of any degree at the University of Adelaide or other University.

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## Publications

With the exception of Chapters 1, 6 and 7, this thesis is a collection of manuscripts published in different journals. As such, the text and figures in these chapters are formatted differently, according to the requirements of the specified journals. Before each of these chapters is a Statement of Authorship, signed by all authors, listing individual contributions to the work.

The journals are ranked below in the order of impact factor in reference to their scientific significance (*Journal citation report* 2009, Thomson ISI).

| Journal title | Impact factor | ERA rating |
|---|---|---|
| *Food Chemistry* | 3.146 | A* |
| *Journal of Agricultural and Food Chemistry* | 2.469 | A |
| *Australian Journal of Grape and Wine Research* | 1.872 | B |
| *American Journal of Enology and Viticulture* | 1.171 | B |

The thesis is based on the following papers.

**Chapter 2.** King, E.S., Swiegers, J.H., Travis, B., Francis, I.L., Bastian, S.E.P. and Pretorius, I.S. (2008) Coinoculated fermentations using *Saccharomyces* yeasts affect the volatile composition and sensory properties of *Vitis vinifera* L. cv. Sauvignon Blanc wines. *Journal of Agricultural and Food Chemistry* 56**,** 10829-10837.

**Chapter 3.** King, E.S., Kievit, R.L., Curtin, C., Swiegers, J.H., Pretorius, I.S., Bastian, S.E.P. and Francis, I.L. (2010) The effect of multiple yeasts co-inoculations on Sauvignon Blanc wine aroma composition, sensory properties and consumer preference. *Food Chemistry* 122, 618-626.

**Chapter 4.** King, E., Francis, I.L., Swiegers, J.H. and Curtin, C. (*in press*) Yeast stain-derived sensory differences are retained in Sauvignon Blanc wines after extended bottle storage. *American Journal of Enology and Viticulture*.

**Chapter 5.** King, E.S., Osidacz, P., Curtin, C., Bastian, S.E.P. and Francis, I.L. (*in press*) Assessing desirable levels of sensory properties in Sauvignon Blanc wines – consumer preferences and contribution of key aroma compounds. *Australian Journal of Grape and Wine Research*.

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**Thesis summary**

There are a number of aroma compounds that are fundamental to the sensory properties of Sauvignon Blanc wines. Two such classes of compounds are volatile thiols and esters, both of which are modulated by yeast during alcoholic fermentation. Therefore, controlling fermentation using appropriate inoculated wine yeast is likely to be an effective means of enhancing wine aroma.

In an initial study, Sauvignon Blanc wines were made using different commercial *Saccharomyces* yeast strains, with two- and three-yeast co-inoculations, as well as single-strains, and equal blends of the single-strain wines after fermentation. The wines were analysed for volatile aroma compounds, and sensory descriptive analyses were performed approximately six months post-bottling. Differences in the chemical composition and sensory profiles were observed (which confirmed and elaborated on previous research). The co-inoculated yeast treatments generally had higher concentrations of volatile thiols and higher levels of esters, with higher ratings for 'tropical' and 'fruity' attributes, than their single-strain components and the blends of the single-strain components. The co-inoculated treatments generally fermented faster and issues with an 'acetic' flavour for one strain were eliminated when included in co-inoculation.

Some of the wines were stored, under screw cap closures, at 15°C for three years, and the chemical and sensory analyses were repeated. The results showed that some of the yeast-derived flavour differences in young Sauvignon Blanc wines were retained after extended bottle storage.

A subset of wines showing large sensory differences was subjected to consumer acceptance testing approximately six months after bottling. Differences in liking for the different yeast treatments were observed, with the largest group of consumers preferring the two-yeast co-inoculation with an intermediate sensory profile, while another group favoured the wine made using the three-yeast co-inoculation with highest ratings for the 'estery' and 'floral' aromas and highest concentrations of volatile thiols.

To further investigate this result, a study was conducted to identify which sensory attributes drive consumer preferences for Sauvignon Blanc wines, and furthermore, the volatile compounds and their levels responsible for these sensory attributes. Volatile thiols, esters and

methoxypyrazines were added to a neutral white wine at realistic levels to mimic those found in Sauvignon Blanc wines. A sensory descriptive analysis was conducted, and a subset of samples was evaluated by consumers for liking. All three classes of compounds were responsible for influencing consumer liking, with 'confectionary', 'cat urine/sweaty', 'cooked green vegetal' and 'fresh green' aromas identified as the strongest drivers of liking for different groups of consumers identified. Demographic information, and wine usage and attitudes of these white wine consumers were also used in a segmentation exercise to gain insights into consumer behaviour.

The results of this study demonstrate that the choice of yeast inoculum, using single or multiple yeasts, affects wine aroma composition and sensory properties even after an extended period of bottle age, and that there are sufficiently large differences to influence consumer preference. This study has also shown, for the first time, clear linking of Sauvignon Blanc aroma compounds, their associated sensory attributes and interactions, and effects on consumer preference. These findings highlight the importance of yeast strain selection, and give wine producers a clearer direction for tailoring white wine styles that can be targeted to specific consumer groups.

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# Chapter 1.

## Review of the literature

*Chapter headings*



### 1.1 Introduction

Differences in wine aroma and flavour are a primary reason that people seek out, enjoy and pay sometimes large amounts for particular wines. The aroma and flavour properties of wine express important regional, varietal and stylistic differences, and create interest and attractiveness of wines produced worldwide.

The perception of wine aroma and flavour by humans is the result of complex sensory and interpretation processes that makes it difficult, if not impossible, to predict wine aroma using instrumental measures (Lawless and Heymann 1998). Therefore, an objective measurement of wine aroma is used, namely the technique of sensory analysis, when investigating the perception of wine aroma and flavour by the human senses (Stone and Sidel 2004). In addition, measurement of wine aroma and flavour by sensory analytical means can be used to assess the quality and hedonic liking of wines, as judged by the end-users of wine, consumers.

Wine aroma and flavour are dependent upon complex interactions of hundreds of volatile and non-volatile compounds in wine. These compounds originate from the grape berry, winemaking practices, in particular primary fermentation, and the maturation process (Ribéreau-Gayon et al. 2006). Primary fermentation is the conversion of grape sugars and other grape-derived compounds into ethanol, carbon dioxide and hundreds of compounds, many of which contribute to wine aroma. During this process, yeasts are also able to modify some grape-derived compounds that can impact wine aroma, including volatile thiols (Dubourdieu et al. 2006, Murat et al. 2001b). Primary fermentation is therefore, a favourable stage of the winemaking process to modulate wine aroma. Different yeasts can conduct primary fermentation, in particular specific strains of the wine yeast *Saccharomyces cerevisiae*, which have been shown to differ in their production of wine aroma compounds (Fleet 2008). Fermentation strategies, such as choice of yeast strain, can be used to control primary fermentation and potentially enhance wine aroma profiles.

This review firstly focuses on the chemical aspects of wine aroma related to Sauvignon Blanc wines, particularly three important classes of volatile compounds; methoxypyrazines, volatile thiols and esters. It then explores the modulation of some of these compounds using yeast strategies. Finally, the review examines the measurement of wine aroma using sensory analysis, specifically descriptive analysis and consumer acceptance testing.

## 1.2 Wine aroma compounds

Wine aroma composition is complex, with interactions among volatile compounds that can lead to masking or suppressing effects, generally for compounds above their aroma detection thresholds*. Sub-threshold compounds can also have possible additive effects (Francis and Newton 2005).

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* The aroma detection threshold is the lowest concentration at which a compound can be perceived by sensory means and provides a guide to the potency of volatile compounds. There are limitations regarding threshold measures, including the media used, the number of assessors and their familiarity with the compound. The threshold value is the concentration at which half the assessors detect the compound, and thus, half of the assessors (and supposedly a percentage of the population) can detect the compound below the reported threshold concentration. On the other hand, for some compounds, such as the norisoprenoid β-ionone, a percentage of the population is unable to perceive the compound even at high concentrations, referred to as specific anosmia Plotto, A., Barnes, K.W. and Goodner, K.L. (2006) Specific anosmia observed for β-Ionone, but not for α-Ionone: Significance for flavor research. *Journal of Food Science* 71, S401-S406..

There are also interactions with non-volatile compounds that alter aroma and flavour, but these aspects are not discussed. These many interactions give rise to different sensory properties in wine, which are characterised using various sensory descriptors. This review gives a brief description of each of the classes of volatile compounds in wine and their general sensory properties, but does not discuss their interactions in wine.

Of the hundreds of volatile compounds that exist in wine, some are formed in the grape berry, while others are produced by yeasts or bacteria during fermentation or spoilage and through chemical reactions during storage in oak barrels and/or glass bottles.

*1.2.1 Grape-derived aroma compounds*

Grape-derived aroma compounds are produced in the grape berry during development and generally contribute varietal differences to wines. The factors that influence wine aroma compound formation for grape-derived compounds include nutrient levels in the soil, water availability, climatic conditions, sunlight exposure and the balance of vegetative and fruit growth (Marais et al. 1999, Reynolds and Vanden Heuvel 2009). Most grape-derived aroma compounds are formed in the skin or exocarp of the grape berry and include methoxypyrazines, volatile thiols, terpenes and norisoprenoids (Figure 1).

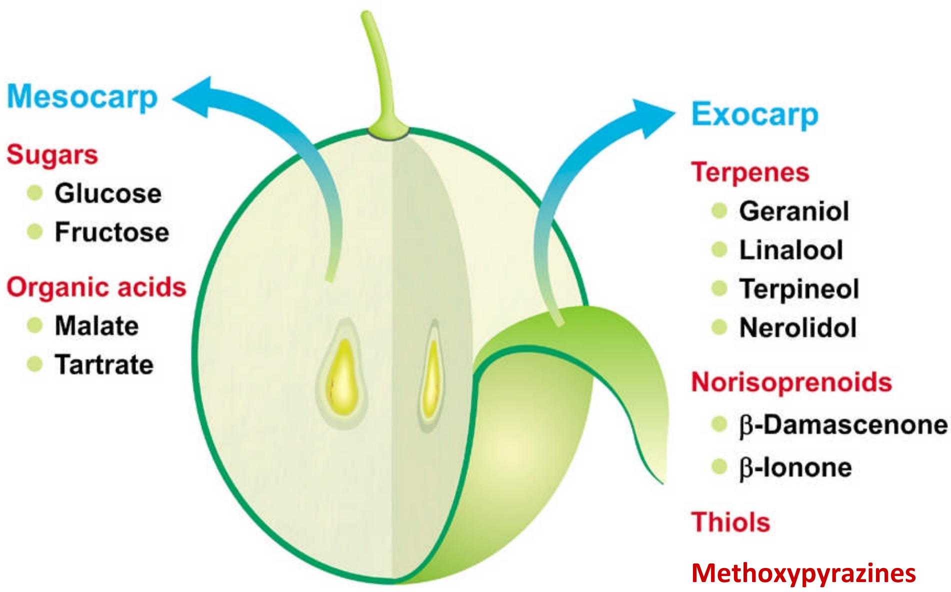


**Figure 1.** Schematic diagram of some grape-derived compounds and their location in the grape berry. Modified from Pretorius (2006).

*1.2.1.1 Methoxypyrazines*

Methoxypyrazines have extremely low aroma detection thresholds (in the parts per trillion range) and generally impart 'green' characteristics to wine (Allen and Lacey 1999). Many of the Bordeaux varieties contain methoxypyrazines, including Sauvignon Blanc and Cabernet Sauvignon. The three main methoxypyrazine compounds in wine are 2-isobutyl-3-methoxypyrazine (IBMP), its structural isomer 2-*sec*-butyl-3-methoxypyrazine (SBMP), and 2-isopropyl-3-methoxypyrazine (IPMP). IBMP is the predominant methoxypyrazine compound and has aromas reminiscent of 'green capsicum' ('bell pepper'), 'green bean' and 'herbaceous' (Allen et al. 1991, Harris et al. 1987, Lacey et al. 1991). IPMP and SBMP occur at lower levels in wine and have aromas of 'earthy', 'asparagus' and 'vegetal' (Allen and Lacey 1999). At high levels, these same aroma descriptors are generally considered undesirable and overpowering in wine, and are associated with unripe characteristics (Ferreira et al. 2007, Rapp 1998, Roujou de Boubée et al. 2000).

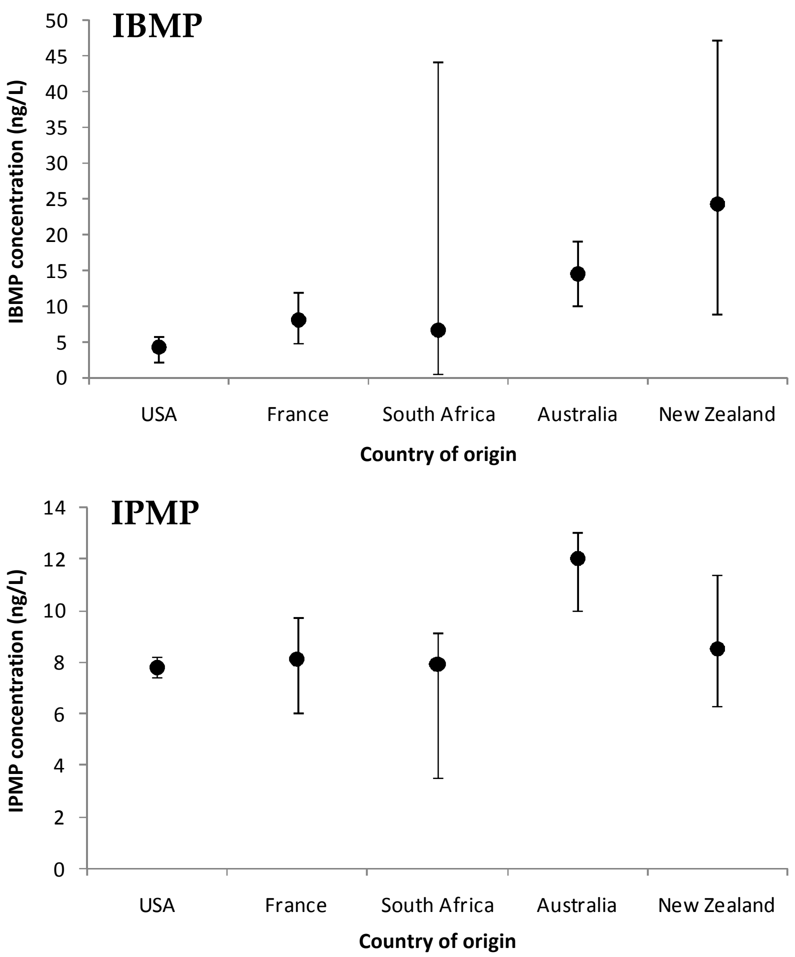


**Figure 2.** Concentrations of IBMP and IPMP in Sauvignon Blanc wines made in the USA (United States of America), France, South Africa, Australia and New Zealand. Closed circles represent mean values, and the bars represent the range of concentrations. Adapted from Alberts et al. (2009) (n=575), Lacey et al. (1991) (n=22) and Lund et al. (2009) (n=52).

A range of concentrations of IBMP and IPMP found in Sauvignon Blanc wines from different countries is shown in Figure 2. The highest levels of IBMP occurred in Sauvignon Banc wines made in New Zealand, while the lowest levels were found in Sauvignon Blanc wines from the USA, France and some from South Africa. The concentrations of IPMP were highest in Australian Sauvignon Blanc wines.

Methoxypyrazine concentrations in the grape berry have been shown to increase prior to veraison (grape berry colour development) and decrease markedly with ripening (Lacey et al. 1991). This decrease in methoxypyrazine concentration after veraison was thought to be caused by sunlight exposure degrading methoxypyrazines (Allen and Lacey 1999, Hashizume and Samuta 1999), but this has since been refuted (Sala et al. 2004). It has since been demonstrated that sunlight exposure during berry development can reduce the accumulation of methoxypyrazines pre-veraison (Ryona et al. 2008). Cooler seasonal temperatures have also been associated with higher methoxypyrazine concentrations (Falcao et al. 2007, Lacey et al. 1991, Marais et al. 1999).

A number of viticultural techniques also modify methoxypyrazine concentrations, including vine training (Sala et al. 2004), irrigation and planting density (Sala et al. 2005), leaf removal (Arnold and Bledsoe 1990) and yield manipulation (Chapman et al. 2004a, 2004b). As methoxypyrazines are present in the skins of grape berries, skin contact increases the methoxypyrazine content in juice and wine (Maggu et al. 2007, Marais 1998, Roujou de Boubée et al. 2002), and conversely, clarification and settling results in decreased concentrations of methoxypyrazines (Roujou de Boubée et al. 2002). IBMP is also resistant to oxidation in wine (Marais 1998).

IPMP can also originate from non-grape sources and negatively contribute to juice and wine flavour, known as ‘ladybug off-flavour’ or *Harmonia axyridis* (*HA*) taint. This occurs when Multicoloured Asian Lady Beetles become incorporated with grapes during processing, giving atypical off-aromas of ‘peanut’, ‘bell pepper’ and ‘asparagus’ in white and red wines (Pickering et al. 2005). IPMP can also be synthesised by some wine yeast (Pickering et al. 2008) and bacteria not associated with wine (Cheng et al. 2002, Emde et al. 1992, Gallois et al. 1988, Schulz et al. 2004).

*1.2.1.2 Volatile thiol compounds*

The volatile thiol compounds 4-mercapto-4-methylpentan-2-one (4MMP) and 3-mercaptohexan-1-ol (3MH) exist in the grape berry bound to the amino acids, *L*-cysteine and glutathione (Subileau et al. 2008a). Another volatile thiol, 3-mercaptohexyl acetate (3MHA) is the product of 3MH esterification by yeasts (Swiegers et al. 2009), and is discussed in this section. The characteristic aromas of these three volatile thiol compounds differ in reported literature, a summary of which is presented in Table 1. The common descriptors for the volatile thiol compounds include 'box tree' ('boxwood', 'box hedge'), 'grapefruit' and 'passionfruit' (Table 1). The descriptors substantially differ, however, depending on the concentration of these compounds in wine. At high concentrations, these volatile thiols have been associated with 'sweaty', 'cat urine' ('cat pee') or 'sulfur-like' aromas (Dubourdieu et al. 2006, Howell et al. 2004). Therefore, the sensory properties conferred by these compounds may be perceived as either positive or negative, depending upon the concentration in wine and/or the sensitivity and expectations of the individual. There is also ambiguity among the aroma terms used for volatile thiol compounds by individual researchers. For instance, 'box hedge', 'box tree' or 'boxwood' are often synonymous with the sensory descriptors 'cat urine' or 'cat pee', and at other times, the term seems to imply a 'green' aspect.

**Table 1.** Summary of the reported sensory descriptors and aroma detection thresholds of three volatile thiol compounds, as described in the literature reviewed.

| Volatile thiol compounds | Reported sensory descriptors | Reported aroma detection thresholds | Reference |
|---|---|---|---|
| 4-Mercapto-4-methylpentan-2-one (4MMP) | 'Box tree', 'blackcurrant' | 0.1 ng/L (aq)<br>3 ng/L (white wine) | Darriet et al. (1995) |
| | | 0.6 ng/L (9%w/w†) | Guth (1997b) |
| | 'Box tree', 'broom' | 0.8 ng/L (12%w/w†) | Tominaga et al. (1998b) |
| | 'Box tree', 'broom' | 0.1 ng/L (aq‡) | Tominaga et al. (2000a) |
| | 'Cat's urine' (at high concentrations) | | Dubourdieu et al. (2006) |
| 3-Mercaptohexan-1-ol (3MH) | 'Grapefruit' | 60 ng/L (12%w/w) | Tominaga et al. (1998c) |
| | 'Grapefruit', 'passionfruit' | 17 ng/L (aq) | Tominaga et al. (2000a) |
| *R*-3MH | 'Grapefruit', 'citrus peels' | 50 ng/L (12%w/w) | Tominaga et al. (2006) |
| *S*-3MH | 'Passionfruit' | 60 ng/L (12%w/w) | Tominaga et al. (2006) |
| 3-Mercaptohexyl acetate (3MHA) | 'Grapefruit', 'passionfruit', 'box tree' | 2 – 4 ng/L | Tominaga et al. (1996) |
| | 'Box tree', 'passionfruit' | 4.2 ng/L (12%w/w) | Tominaga et al. (1998b) |
| | 'Box tree', 'passionfruit' | 2.3 ng/L (aq) | Tominaga et al. (2000a) |
| *R*-3MHA | 'Passionfruit' | 9 ng/L (12%w/w) | Tominaga et al. (2006) |
| *S*-3MHA | 'Boxwood' | 2.5 ng/L (12%w/w) | Tominaga et al. (2006) |

† Hydroalcoholic model solution (%w/w ethanol)
‡ Aqueous (water)

**Table 2.** A list of grape varieties containing volatile thiol compounds and the relevant references.

| Grape variety | Reference |
|---|---|
| Cabernet Franc | Blanchard et al. (2004) |
| Cabernet Sauvignon | Blanchard et al. (2004), Bouchilloux et al. (1998), Darriet et al. (2001), Murat et al. (2001a), Tominaga et al. (2004) |
| Chardonnay | Curtin (2008), Tominaga et al. (2003) |
| Chenin Blanc | Du Plessis and Augustyn (1981) |
| Colombard | Du Plessis and Augustyn (1981), Tominaga et al. (2000a, 2004) |
| Gewürztraminer | Tominaga et al. (2000a, 2004) |
| Grenache | Ferreira et al. (2002b) |
| Gros Manseng | Tominaga et al. (2004) |
| Koshu | Kobayashi et al. (2010) |
| Maccabeo | Escudero et al. (2004) |
| Melon B. | Roland et al. (2010) |
| Merlot | Blanchard et al. (2004), Bouchilloux et al. (1998), Darriet et al. (2001), Murat et al. (2001a), Tominaga et al. (2004) |
| Muscadet | Schneider et al. (2003) |
| Muscat | Tominaga et al. (2000a, 2004) |
| Petit Arvine | Fretz et al. (2005), Luisier et al. (2008) |
| Petit Manseng | Tominaga et al. (2000a, 2004) |
| Pinot Blanc | Tominaga et al. (2000a) |
| Pinot Gris | Tominaga et al. (2000a) |
| Riesling | Tominaga et al. (2000a, 2004) |
| Sauvignon Blanc | Darriet et al. (1995), Dubourdieu et al. (2006), Lund et al. (2009), Murat et al. (2001b), Swiegers et al. (2009), Tominaga et al. (1998a, 1998c) |
| Scheurebe | Guth (1997a, 1997b) |
| Semillon | Bailly et al. (2009), Tominaga et al. (2000a, 2004) |
| Sylvaner | Tominaga et al. (2000a) |
| Tokay | Tominaga et al. (2004) |

Stereochemistry plays an important role in the sensory characteristics of volatile thiols. The two enantiomers of 3MH and 3MHA, *R* and *S*, have different aroma detection thresholds and aroma descriptors, shown in Table 1. Tominaga et al. (2006) found that *R*- and *S*-3MH were uniformly distributed in white wines (racemic), whereas, the *R*- and *S*-3MHA enantiomeric ratio was approximately 30:70 in white wines.

Volatile thiols have now been identified in a number of grape varieties in varying concentrations, shown in Table 2. Due to their low aroma detection thresholds (Table 1), these volatile thiols have generally been shown to exist in wine at perceptible levels. A range of concentrations of volatile thiols found in Australian, South African, French and New Zealand wines is shown in Figure 3. Wines from Australia and South Africa generally had relatively low concentrations of all three volatile thiols, followed by France. Much higher concentrations of volatile thiols were found in wines from New Zealand.

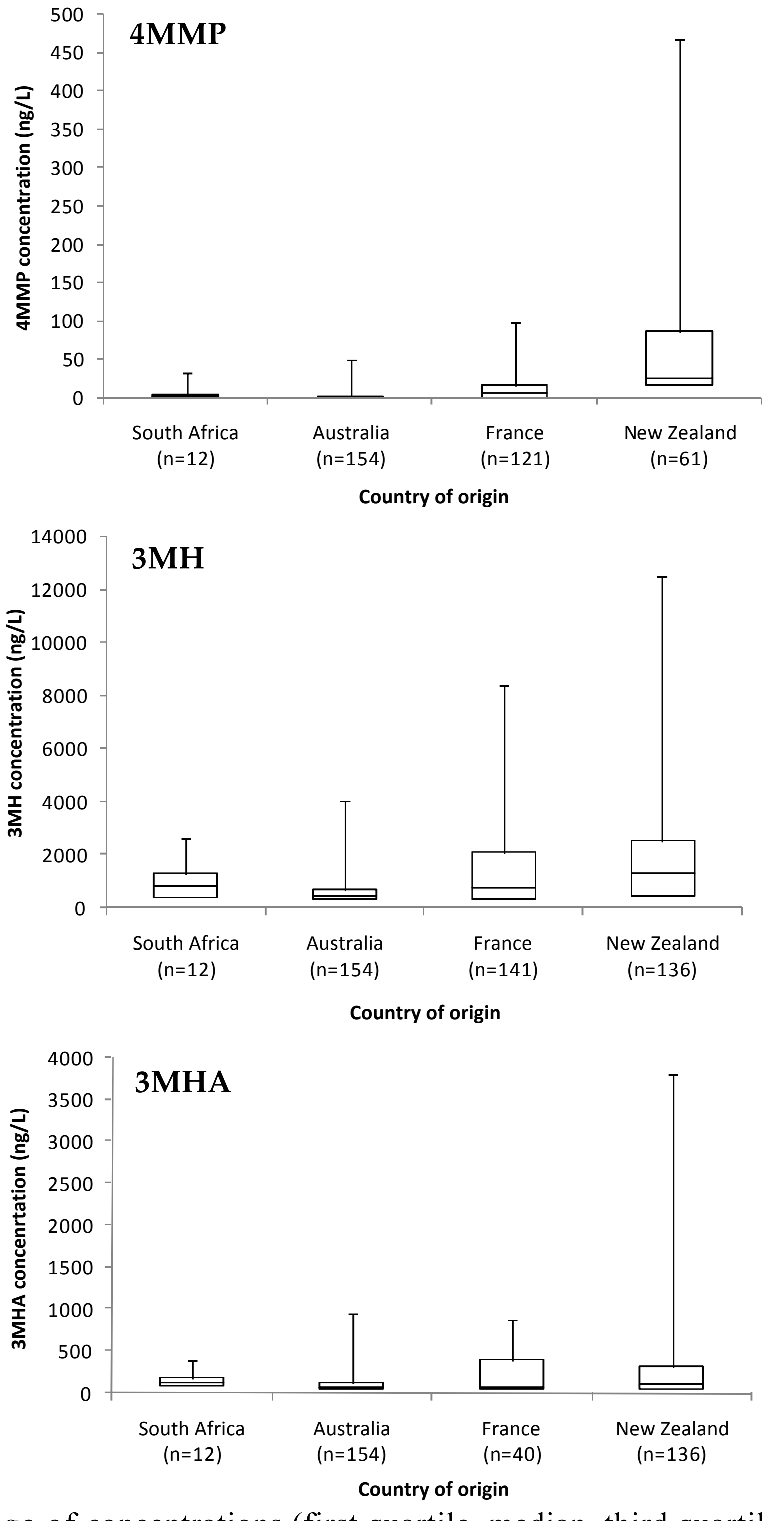


**Figure 3.** A range of concentrations (first quartile, median, third quartile, maximum) of volatile thiols 4MMP, 3MH and 3MHA in wines from South Africa, Australia, France and New Zealand. Numbers in parentheses are number of samples per country for each compound. Values obtained from published and unpublished data (Anfang et al. 2009, Benkwitz et al. 2007, Blanchard et al. 2004, Bowyer et al. 2008, Brajkovich et al. 2005, Curtin 2008, Dubourdieu et al. 2006, Ellett et al. 2007, Guth 1997b, Herbst et al. 2007, Kilmartin et al. 2008, King et al. 2010, King et al. 2008, Lee et al. 2008, Lund et al. 2009, Marullo et al. 2006, Masneuf-Pomarède et al. 2006, Masneuf et al. 2002, Mateo-Vivaracho et al. 2007, Mateo-Vivaracho et al. 2006, Murat et al. 2001b, Schneider et al. 2003, Subileau et al. 2008b, Swiegers et al. 2009, Tominaga et al. 2000a, Van Der Westhuizen 2006). The grape varieties included are shown in Table 2.

Two flavourless amino acid conjugates of 4MMP and 3MH have been identified to date in the grape berry: the cysteine conjugates S-4-(4-methylpentan-2-one)-*L*-cysteine and S-(*R*/*S*)-3-(hexan-1-ol)-*L*-cysteine (Peyrot Des Gachons et al. 2000, Tominaga et al. 1998c), and the glutathione conjugates S-4-(4-methylpentan-2-one)-glutathione (Fedrizzi et al. 2009) and S-(*R*/*S*)-3-(hexan-1-ol)-glutathione (Peyrot Des Gachons et al. 2002). Additionally, an alternative biogenetic pathway from (*E*)-hex-2-enal leading to 3MH formation was demonstrated, although this contributed only about 10% of the total 3MH produced (Schneider et al. 2006). It is also likely that cysteinyl-glycine conjugates of 4MMP and 3MH exist in grape berries as an intermediate breakdown product of the glutathione conjugates. Subileau et al. (2008a) found that neither (*E*)-hex-2-enal nor the cysteine 3MH conjugate were responsible for the majority of free 3MH detected in wine, and inferred that as much as 50% of total 3MH in wine may be derived from the glutathione precursor.

It is during primary fermentation that the sensory effects of these volatile thiol compounds become detectable in wine, through the action of yeasts. It has been shown that amino acid and peptide transporters are at least in part responsible for the conjugated precursors of volatile thiols entering the yeast cell. In particular Gap1p, a general amino acid permease was found to transport the cysteinylated thiol precursors (Subileau et al. 2008b) and the main glutathione transporter protein, Opt1p (Hgt1pk) was implicated in the uptake of glutathione thiol precursors by yeasts (Subileau et al. 2008a).

It has been proposed that yeast enzymatically cleave the amino acid molecules from the conjugated precursors, which releases the thiols into their volatile (aroma-active) states in wine. The putative effect of carbon-sulfur lyase enzymes was confirmed (Howell et al. 2005, Swiegers et al. 2007, Tominaga et al. 1995, Wakabayashi et al. 2004) and several candidate genes were implicated in studies with deletion mutants (Howell et al. 2005, Thibon et al. 2008). A putative cystathionine beta-lyase encoded by the *IRC7* gene, was strongly implicated in release of 4MMP, while release of 3MH may involve multiple enzymes (Howell et al. 2005, Thibon et al. 2008). Swiegers et al. (2009) demonstrated that an ester-forming alcohol acetyltransferase enzyme, encoded by the *ATF1* gene, is responsible for the production of 3MHA through esterification of 3MH. The degree of volatile thiol release and modulation by yeasts varies according to the concentration of conjugated precursors in the grape berry and extracted in the must, and the yeast species and strains used to undergo fermentation. This is discussed further in later sections.

Alternatively, the *S*-cysteine 3MH conjugate can be transformed to 3MH by anaerobic salival bacteria (Starkenmann et al. 2008). This research demonstrated that within 20-30 seconds of exposure to cysteine conjugates, 3MH was produced in the mouth, exhibiting a 'sulfur-like' aroma that persisted for up to three minutes. Thus, it is possible that the release of volatile thiols from their conjugated precursors in the mouth may contribute a proportion of the sensory perception of volatile thiol compounds in wine. A study by Itobe et al. (2009) also demonstrated a compositional change of 4MMP to the methylthiol ester 4-methylthio-4-methylpentan-2-one in the mouth. This compound has an aroma detection threshold twenty thousand times higher than 4MMP (Darriet et al. 1995). This result demonstrates that these in-mouth changes can also reduce the sensory perception of the volatile thiol compounds.

*1.2.1.3 Other grape-derived aroma compounds*

Like volatile thiols, many other aroma compounds present in the grape berry can be found bound to metabolites, which renders the aroma compounds non-volatile and thus, flavourless. Terpenes and norisoprenoids exist in the grape berry in both free and bound forms. Their bound forms are conjugated to sugar molecules, called glycoconjugates and are hydrolysed by acid-catalysed reactions and glycosidase enzymes (Francis et al. 1996, Ribéreau-Gayon et al. 2006). Glycosidase enzymes can be produced by the grapes themselves, as well as yeasts and bacteria.

Monoterpenes can contribute 'citrus', 'rose-like' and 'floral' aromas to wine and are generally important to aromatic grape varieties, such as Gewürztraminer, Muscat and Riesling (Ribéreau-Gayon et al. 2006). While monoterpenes have also been implicated as contributing to Sauvignon Blanc wine aroma (Ribéreau-Gayon et al. 2006), they are not discussed in any great detail in this chapter. The related sesquiterpenes are present in grape berries in large numbers, and were not thought to be important to wine aroma until recently, when rotundone was discovered. This compound is responsible for the 'spicy' and 'black pepper' notes in Shiraz wines (Pollnitz et al. 2008).

Norisoprenoids can give rise to aromas of 'violets' or 'fruit' in wine, and are present in most grape varieties at varying concentrations (Aldave et al. 1993, Kotseridis et al. 1998). β-Damascenone is particularly important to many varieties, contributing 'quince' and 'cooked apple' aromas, and is also able to enhance 'fruity' aromas of other compounds (Pineau et al. 2007). Another norisoprenoid, *trans*-1-(2,3,6-trimethylphenyl)buta-1,3-diene (TPB), may contribute aromas of 'green' and 'cut grass' to wines at low concentrations (Cox et al. 2005), including Sauvignon Blanc.

Hexanol is another grape-derived aroma compound that can give rise to 'green' characteristics in varieties, such as Sauvignon Blanc. It is thought to contribute to a lesser extent than methoxypyrazines, as it is mostly present below its aroma detection threshold (Berna et al. 2009).

There are also compounds that can enter the grape berry externally and affect wine aroma, whether from taint compounds or *terroir* (vineyard location). These include 'smoke' taint compounds, such as guaiacol (Sheppard et al. 2009) and 1,8-cineole (eucalyptol), which contributes an 'eucalyptus-like' aroma to wines (Saliba et al. 2009).

### *1.2.2 Yeast- and bacteria-derived aroma compounds*

Aroma compounds are produced by yeast and bacteria during fermentation and also microbial spoilage. The fermentation factors that influence wine aroma include solids level, fermentation vessel, type and amount of yeast and bacteria strain(s) used to conduct fermentation, fermentation temperature, nutrient addition and mixing techniques (Girard et al. 1997, Marais 1998, Swiegers et al. 2005, Ugliano et al. 2008).

There are many more volatile compounds in wine than grape juice, as yeasts and bacteria produce hundreds of volatile compounds during fermentation that contribute to wine aroma. These include esters, alcohols, volatile fatty acids, carbonyl compounds and sulfur-containing compounds. The metabolic pathways of some of these compounds by yeast and bacteria are shown in Figures 4 and 5. For a more comprehensive review of wine aroma compounds produced by yeasts and bacteria, see Swiegers et al. (2005).

As previously discussed, yeasts are also able to modify grape-derived compounds. Some yeasts and bacteria contain glycosidase enzymes that can release glycoconjugates of monoterpenes and norisoprenoids into their aroma-active forms during fermentation. Similarly, yeast carbon-sulfur lyase enzymes can release amino acid conjugates of volatile thiol compounds. It is also noteworthy that the composition of the grape juice plays an important role in the aroma compounds produced by yeasts and bacteria during fermentation, such as amino acid content, and concentrations of vitamins, metal ions, non-volatile acids and sugars.

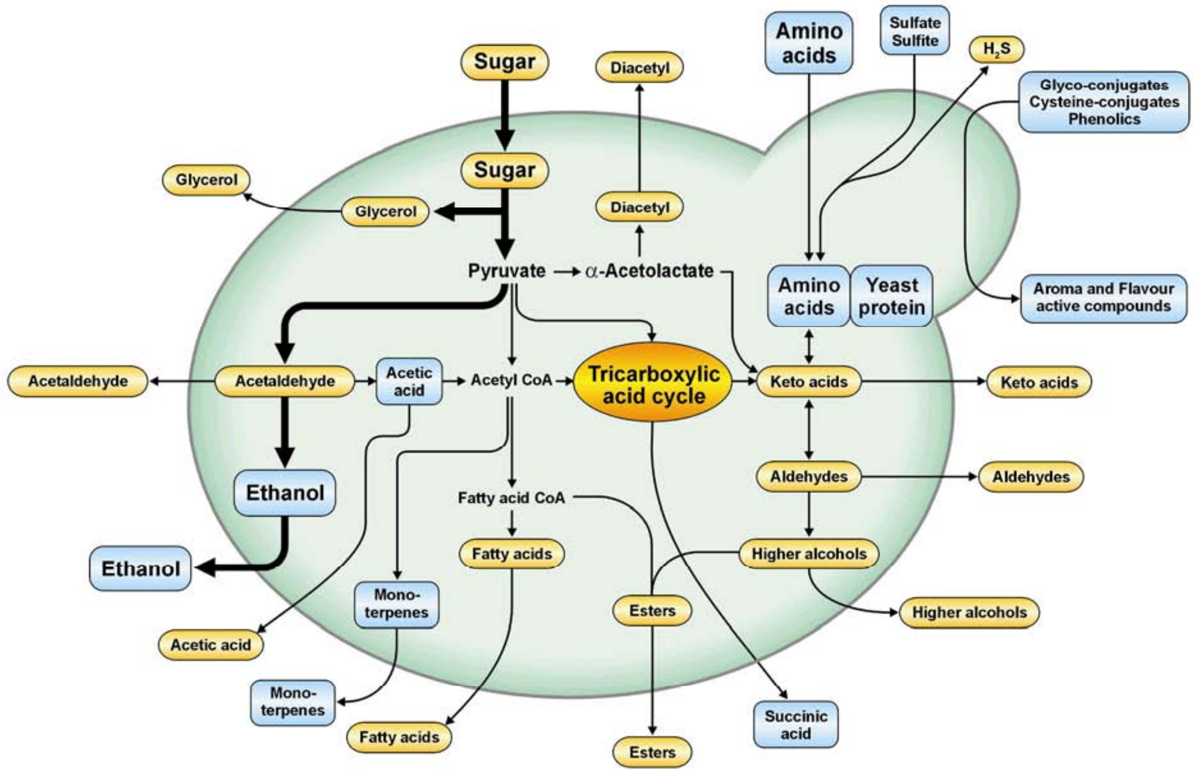


**Figure 4.** Schematic representation of the metabolic pathways of volatile and non-volatile compound production by yeasts during primary fermentation via sugar, amino acid and sulfur metabolism. Taken from Swiegers et al. (2005).

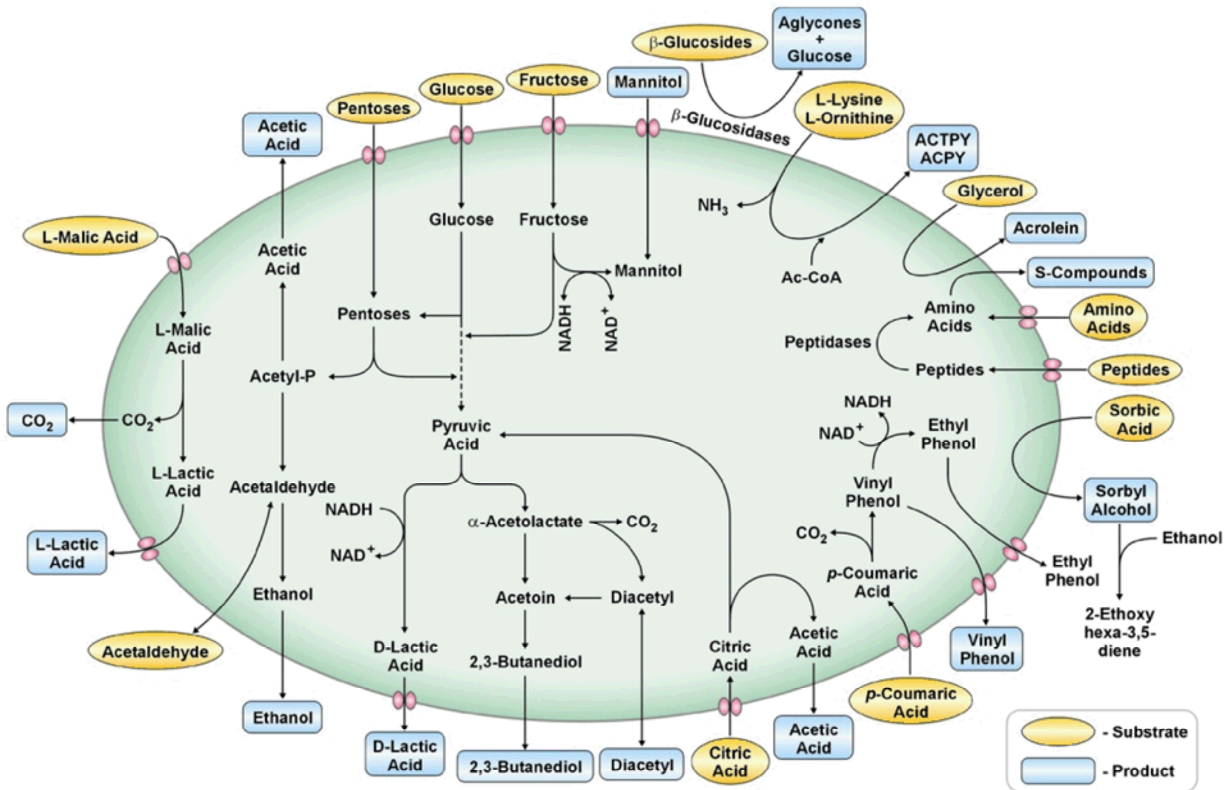


**Figure 5.** Schematic representation of the biosynthesis and modulation of wine aroma compounds by malolactic bacteria. Taken from Swiegers et al. (2005).

*1.2.2.1 Esters*

Esters represent the largest and most important group of aroma compounds produced during fermentation. They are present in all wines and are considered to significantly influence wine aroma and quality by contributing 'fruity' characteristics of wine (Etievant 1991). The two main types of esters produced are acetate esters and fatty acid ethyl esters. There are also a number of organic acid ethyl esters in wine. Examples of each type of ester, their sensory descriptors and aroma detection thresholds are provided in Table 3.

Ester formation can occur either by chemical reactions, which are slow and contribute little to wines, or, much more importantly, via microbial intracellular enzymatic reactions during fermentation (Mason and Dufour 2000). Acetate ester synthesis occurs between higher alcohol compounds and acetic acid in the form of acetyl-CoA or acyl-CoA. Ethyl ester synthesis occurs between ethanol and either activated short-chain fatty acids or lipids (for fatty acid ethyl esters) or organic acids (for organic acid ethyl esters) (Fleet 2003, Mason and Dufour 2000, Swiegers et al. 2005). The main enzymes responsible for catalysing ester synthesis are alcohol acetyltransferases (AATases), which are also responsible for 3MHA formation by yeasts (Swiegers et al. 2009). AATase I (or Atf1p), encoded by *ATF1*, has been shown to significantly contribute to acetate and fatty acid ethyl ester formation, in a series of gene manipulation experiments (Mason and Dufour 2000). AATase II (or Atf2p), encoded by *ATF2*, has been shown to affect the concentration of acetate esters to a lesser extent than Atf1p (Mason and Dufour 2000). Another gene in the alcohol acetyltransferase family, Lg-AATase I (or Lg-ATF1p), is homologous to Atf1p and found in lager yeast. It has also been identified in acetate ester production (Lilly et al. 2000).

An additional alcohol acetyltransferase, ethanol hexanoyl transferase, encoded by the gene *EHT1* is responsible for fatty acid ethyl ester synthesis, particularly ethyl hexanoate and ethyl octanoate (Lilly et al. 2006). There are also enzymes responsible for degradation of ester compounds, called esterases, such as those encoded by *IAH1*, which hydrolyse isoamyl acetate (Lilly et al. 2006). The balance between ester synthesis and hydrolysis by yeast enzymes is important for the net rate of ester accumulation in wine. See Sumby et al. (2010) for a more detailed review of esters in wine.

**Table 3.** A selected list of yeast- and bacteria-derived aroma compounds produced during fermentation, their sensory descriptors, adapted from Siebert et al. (2005) and Moio and Etievant (1995), and aroma detection thresholds, references shown in superscript letters, modified from King et al. (2008).

| Aroma compound | Sensory descriptors | Aroma detection threshold (mg/L) |
|---|---|---|
| *Acetate esters* | | |
| Ethyl acetate | 'Fruity', 'nail polish remover' (at high concentrations) | 7.5 [a] |
| 2-Methylpropyl acetate | 'Banana', 'fruity' | 1.6 [d1] |
| 2-Methylbutyl acetate | 'Banana', 'fruity' | - |
| 3-Methylbutyl acetate | 'Banana' | 0.030 [a] |
| Hexyl acetate | 'Sweet', 'perfume' | 2.4 [d2] |
| 2-Phenylethyl acetate | 'Flowery' | 0.25 [a] |
| *Fatty acid ethyl esters* | | |
| Ethyl propanoate | 'Fruity' | 1.8 [d2] |
| Ethyl-2-methylpropanoate | 'Fruity' | 0.015 [a] |
| Ethyl butanoate | 'Acid fruit' | 0.020 [a] |
| Ethyl-2-methylbutanoate | 'Sweet fruit' | 0.001 [a] |
| Ethyl-3-methylbutanoate | 'Berry' | 0.003 [a] |
| Ethyl hexanoate | 'Green apple' | 0.005 [a] |
| Ethyl octanoate | 'Sweet soap' | 0.002 [a] |
| Ethyl decanoate | 'Pleasant', 'soap' | 0.20 [b] |
| Ethyl dodecanoate | 'Soapy', 'estery' | 0.64 [c] |
| *Organic acid ethyl esters* | | |
| Ethyl lactate | 'Strawberries' | 14 [c] |
| Ethyl cinnamate | 'Sweet', 'cherry', 'plum', 'cinnamon' | 0.001 [a] |
| Ethyl 2,3-dihydrocinnamate | 'Fruity', 'sweet', 'cinnamon' | 0.002 [b] |
| Ethyl anthranilate | 'Sweet', 'fruity' | - |
| Methyl anthranilate | 'Fruit', 'grape', 'peach' | 0.011 [f] |
| *Higher alcohols* | | |
| 2-Methylpropanol | 'Fusel', 'spiritous' | 40 [a] |
| Butanol | 'Fusel', 'spiritous' | 150 [d2] |
| 2-Methylbutanol | 'Nail polish' | 75 [c] |
| 3-Methylbutanol | 'Harsh', 'nail polish' | 30 [a] |
| Hexanol | 'Green grass' | 8.0 [a] |
| 2-Phenylethanol | 'Roses' | 10 [a] |
| *Volatile fatty acids* | | |
| Acetic acid | 'Vinegar' | 720 [e] |
| Propanoic acid | 'Vinegar' | 20 [c] |
| 2-Methylpropanoic acid | 'Cheese', 'rancid' | 2.3 [b] |
| Butanoic acid | 'Cheese', 'rancid' | 0.17 [b] |
| 2-Methylbutanoic acid | 'Cheese', 'sweaty' | 3.0 [a] |
| 3-Methylbutanoic acid | 'Blue cheese' | 0.033 [b] |
| Hexanoic acid | 'Cheese', 'sweaty' | 0.42 [b] |
| Octanoic acid | 'Rancid', 'harsh' | 0.50 [b] |
| Decanoic acid | 'Fatty' | 1.0 [b] |

[a] Guth (1997a) in 10%w/w water/ethanol solution
[b] Ferreria et al. (2000) in 11%w/w synthetic wine
[c] Salo (1970) in 9.5%w/w water/ethanol solution
[d] Etievant (1991) in [1] beer or [2] white wine
[e] Ribéreau-Gayon et al. (2006) in white wine
[f] Escudero et al. (2004) in 10%w/w water/ethanol solution

*1.2.2.2 Alcohols*

Alcohols are produced by yeasts and bacteria during fermentation. Ethanol is the main product of grape sugar conversion by yeasts. Ethanol is capable of suppressing ‘fruitiness’ in wines, by masking the perception of esters (Escudero et al. 2007, Ferreira et al. 2007) and probably not through a change in volatility (Guth and Sies 2001). Excessive levels of ethanol can also result in an unpleasant ‘hotness’ and can contribute ‘sweetness’, while enhancing ‘bitterness’ (Noble 1994). Glycerol is also produced by yeasts and, although odourless, also has a slightly ‘sweet’ taste (Noble and Bursick 1984). Higher alcohols or fusel alcohols are produced by yeast, and probably contribute ‘fruit’ characteristics at optimal levels. Excessive concentrations of higher alcohols results in a strong ‘pungent’ smell and taste (Nykänen 1986). A number of higher alcohol compounds are listed in Table 3, along with their sensory descriptors and aroma detection thresholds.

*1.2.2.3 Volatile fatty acids*

Free or saturated volatile fatty acids generally contribute negative characteristics to wine, but are rarely above their aroma detection thresholds (Table 3). Acetic acid makes up about 90% of the volatile fatty acids produced by yeasts and bacteria. Acetic acid is associated with volatile acidity, the ‘vinegar-like’ or ‘acetic’ characteristics considered a fault in most wines when present at excessive levels (Ribéreau-Gayon et al. 2006).

*1.2.2.4 Carbonyl compounds*

The most abundant carbonyl compound in wine is acetaldehyde, which contributes aromas reminiscent of ‘bruised apple’ and ‘nutty’. Another important carbonyl compound is diacetyl or 2,3-butanedione, mainly produced by wine bacteria during malolactic fermentation. Diacetyl has aromas of ‘butter’ or ‘butterscotch’, which can be considered undesirable depending on the wine style (Bartowsky et al. 2002, Bartowsky and Henschke 2004).

*1.2.2.5 Sulfur-containing compounds*

Sulfur-containing aroma compounds, produced by both yeasts and bacteria, generally have low aroma detection thresholds and contribute negative characteristics to wine (Pripis-Nicolau et al. 2004, Rauhut 1993). The grape-derived ‘tropical’ volatile thiols seem to be the one exception to this, as previously discussed. Of the sulfur-containing off-flavours, there are sulfides, such as hydrogen sulfide ($H_2S$) and dimethyl sulfide (DMS), and thiols, including methyl mercaptan and ethyl mercaptan. Hydrogen sulfide is produced by yeasts during fermentation when nitrogen sources are depleted and has an unpleasant aroma of ‘rotten egg’ (Rauhut 1993). Dimethyl sulfide contributes ‘asparagus’, ‘corn’ and ‘molasses’ aromas to wine (Rauhut 1993), but can be beneficial to wine aroma at low concentrations. Methyl mercaptan or methanethiol has aromas reminiscent of ‘cooked cabbage’, and ethyl mercaptan or ethanethiol is described as ‘rubber’ and ‘onion’ (Pripis-Nicolau et al. 2004, Vermeulen and Collin 2003).

#### *1.2.2.6 Off-flavour compounds*

Yeasts and bacteria can also produce off-flavour compounds in wine as a result of microbial spoilage. Generally the microorganisms responsible are not those that conduct fermentation. Although acetic acid can be produced by wine yeasts, excessive concentrations in wine are the result of spoilage by acetic acid bacteria (Bartowsky and Henschke 2008, Bartowsky et al. 2003). Volatile phenol compounds impart aromas of ‘barnyard-like’, ‘leathery’ and ‘medicinal’, and are produced by *Brettanomyces/Dekkera* spp. yeast, and to a lesser extent certain *Lactobacillus* bacteria (Chatonnet et al. 1995). *Brettanomyces* yeast, *Lactobacillus* bacteria and the wine bacteria *Oenococcus oeni* can also produce nitrogen heterocyclic compounds that contribute aromas reminiscent of ‘mouse urine’ (Costello and Henschke 2002, Heresztyn 1986).

### *1.2.3 Aroma compounds from maturation processes*

During maturation processes, wine aroma is influenced by factors such as the storage vessel, including size, age and toasting or charring level of oak barrels, lees contact, addition of fining agents, oxygen contact, chemical equilibrium (acid-ethanol balance) and length of storage time. The wine aroma compounds derived from grape berries, and yeast and bacteria are generally modified during the aging process via chemical reactions (Aldave et al. 1993, Marais 1986, Rapp and Marais 1993). Moreover, new wine aroma compounds are formed, and all of these contribute to the bottle-aging bouquet of wines (Du Toit et al. 2006, Singleton 2000). A schematic representation of these processes is shown in Figure 6.

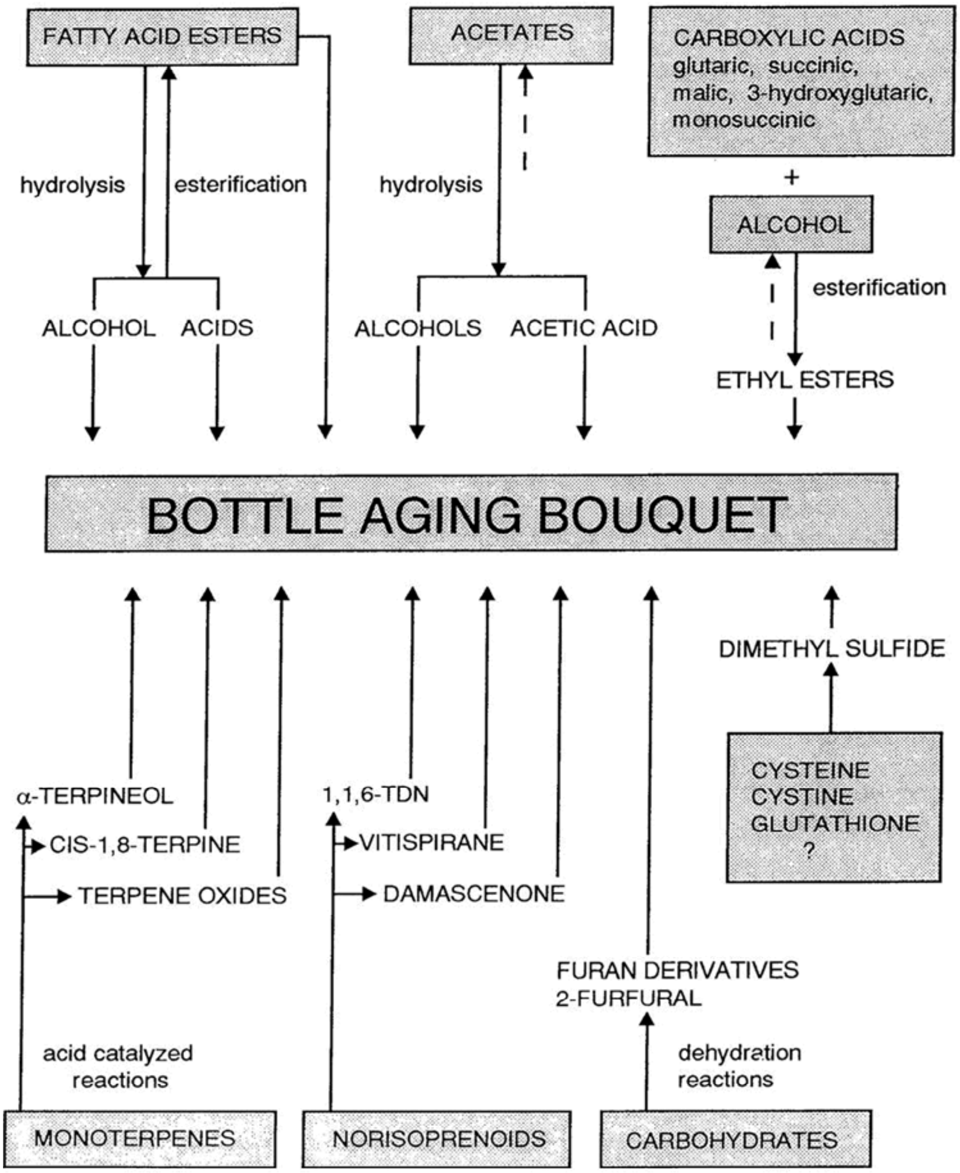


**Figure 6.** Schematic diagram of chemical reactions that occur during wine aging. Taken from Aldave et al. (1993).

*1.2.3.1 Grape-derived aroma compounds*

A number of the previously mentioned grape-derived compounds are important in the maturation process of wine. Most monoterpenes decrease in concentration over time due to acid-catalysed reactions, however, some increase in concentration and may contribute to the aging bouquet of wine (Figure 6) (Aldave et al. 1993, Rapp 1998). The concentrations of norisoprenoids have also been shown to increase in aged wines, including β-damascenone, TPB and 1,1,6-trimethyl-1,2-dihyrdonapthalene (TDN). TDN is formed during wine aging and causes distinctive 'kerosene' and 'petrol-like' aromas in aged wines, particularly Riesling, that at high levels is considered an off-flavour (Winterhalter 1991). Similarly, at high concentrations TPB can contribute undesirable aromas of 'fly spray' and 'chemical' (Cox et al. 2005) to aged Riesling wines and possibly other white varieties, such as Sauvignon Blanc. Methoxypyrazines are known to be particularly stable in wine (Falcao et al. 2007, Marais 1998), although Blake et al. (2009) indicated that methoxypyrazine concentrations decreased as a result of tetrapak cartons (bag-in-box).

The volatile thiols 4MMP and 3MH are reported to be relatively stable in wine, with Culleré et al. (2004) and Tominaga et al. (2000a) indicating their presence in aged red and white wines. 3MHA is thought to be present mainly in young wines (Mateo-Vivaracho et al. 2006, Tominaga et al. 2000a, 2006), as it can hydrolyse to release 3MH (Brajkovich et al. 2005), particularly in high acid environments (Kilmartin et al. 2010) and at high storage temperatures (Makhotkina et al. 2010). There are discrepancies in the literature, however, regarding the stability of 3MHA. Brajkovich et al. (2005) measured 3MHA above its aroma detection threshold in two-year old Sauvignon Blanc wines, and Bouchilloux et al. (1998) and Culleré et al. (2004) detected 3MHA in aged red wines. It was suggested that the volatile thiols 3MH and 3MHA influenced the sensory properties of two year old Sauvignon Blanc wines (Brajkovich et al. 2005). The discrepancies in the literature might be due to different matrices, vintage and climate variability, winemaking and bottling conditions (such as amount of antioxidants added and oxygen ingress), packaging and closure type, and storage conditions.

*1.2.3.2 Yeast-derived aroma compounds*

Extensive research has shown that acetate esters decrease in concentration during wine aging through chemical hydrolysis, shown in Figure 6 (Aldave et al. 1993, Blake et al. 2009, D'Auria et al. 2009, Díaz-Maroto et al. 2005, Lilly et al. 2000, Marais 1986, Moio et al. 2004, Rapp 1998). This leads to a loss of 'fruit' flavours in aged wines (Marais 1986, Rapp 1998). The stability of fatty acid ethyl esters differs depending on the structure of the fatty acid carbon chain. Straight-chain fatty acid ethyl esters decrease in concentration over time (Díaz-Maroto et al. 2005), whereas, branched-chain fatty acid ethyl esters are stable and their concentration can increase during wine aging (Díaz-Maroto et al. 2005, Rapp and Marais 1993). Higher alcohols are also relatively stable in wine (Aldave et al. 1993, Blake et al. 2009, Marais 1986, Rapp and Marais 1993). Volatile fatty acid stability is not uniform, with some compounds reportedly increasing, while others remain stable or decrease in wines over time (Blake et al. 2009, Chaves et al. 2007, Lilly et al. 2000, Loscos et al. 2010, Pereira et al. 2010, Rapp and Marais 1993). This may be in part due to the chemical hydrolysis of some fatty acid ethyl esters, which can result in the formation of acid compounds (Figure 6).

### *1.2.3.3 Oak-derived aroma compounds*

Storage of wine in oak barrels also results in modified aroma profiles, mostly due to the extraction of aroma compounds from the wood into the wine. *Cis*- and *trans*-oak lactones are the most important oak-derived aroma compounds. They impart aromas of 'vanilla' and 'coconut-like' aromas to wine (Jarauta et al. 2005, Pollnitz et al. 2000). Vanillin, an aromatic aldehyde, also contributes 'vanilla' aromas to wine (Jarauta et al. 2005). Other aldehydes extracted from oak include 2-furfural and 4-methylfurfural, which have aromas of 'sweet', 'butterscotch' and 'woody' (Campo et al. 2008, Du Toit et al. 2006). 2-Furanmethanethiol has an aroma reminiscent of 'roasted coffee' (Blanchard et al. 2001, Bouchilloux et al. 1998, Tominaga et al. 2000b).

Guaiacol and 4-methylguaiacol impart 'smoky' aromas to wine, and are indicative of the level of toasting or charring of oak barrels (Jarauta et al. 2005). Benzylmercaptan or benzenemethanethiol is also thought to be contribute 'smoky' aromas in aged wines, including Sauvignon Blanc (Tominaga et al. 2003), although the mechanisms of formation of this compound have yet to be determined. β-Ionone and 30 other norisoprenoid compounds were found by Sefton et al. (1990) to be present in oak barrels, from the degradation of carotenoids in wood.

### *1.2.3.4 Off-flavour compounds*

There are also a number of off-flavour compounds that can develop during the maturation and aging of wines. Volatile phenol compounds can increase in wines to varying extents during maturation in oak barrels, due to the presence of *Brettanomyces* spoilage yeasts in the wood (Jarauta et al. 2005, Pollnitz et al. 2000). Other off-flavour compounds include an atypical aging flavour and cork off-flavour. Cork off-flavour, such as 'mouldy' and 'cork' aromas in wine are caused by a number of compounds as a result of faulty packaging products. These include 2,4,6-trichloroanisol (TCA), 2,4,6-tribromoanisole (TBA), geosmin and 1-octen-3-one (Howland et al. 1997).

### *1.2.3.5 Wine oxidation*

In addition to chemical aging of wines, oxidative degradation or oxidation of wine compounds can occur, mainly due to oxidative reactions promoted by oxygen itself. This results in profound alterations in wine composition (Ferreira et al. 2002a, 2003a). The presence of oxidation-spoiled aromas of white wines such as 'honey-like', 'farm-feed', 'hay', ‘woody-like’ and ‘boiled potato’ has been attributed to acetaldehyde, phenylacetaldehyde, TDN and 3-(methylthio)propionaldehyde (methional) (Ferreira et al. 2002a, 2003a). 4,5-Dimethyl-3-hydroxy-2-(5)-furanone (sotolon) smells like ‘caramel’, ‘curry’ and ‘spicy’ (Bailly et al. 2006, Campo et al. 2008) and is thought to be responsible for premature-aging flavours in white wines through ascorbic acid degradation (Pons et al. 2010).

## 1.3 Yeast modulation of wine aroma

Primary, or alcoholic fermentation can occur naturally or spontaneously, and involves the succession of indigenous or non-*Saccharomyces* yeasts from the vineyard and winery. Some of the species involved include the genera *Dekkera*, *Candida*, *Hanseniaspora*, *Hansenula*, *Pichia*, *Rhodotorula*, *Schizosaccharomyces*, *Torulaspora*, *Williopsia* and *Zygosaccharomyces*. See Jolly et al. (2006) for a more detailed list of non-*Saccharomyces* yeasts. These yeasts generally have a low tolerance for alcohol, sulfur dioxide and acid conditions, and thus, can exhibit growth during the first few days of wine fermentation before dying off and giving way to dominant growth by a more ethanol-tolerant wine yeast strain *S. cerevisiae* (Fleet 1990). Non-*Saccharomyces* yeasts, naturally present in all primary fermentations to a greater or lesser extent, are metabolically-active and have been shown to contribute to wine aroma and impact on wine quality (Ciani and Maccarelli 1998, Heard 1999, Jolly et al. 2006, Romano et al. 2003). However, spontaneous fermentations have often been seen as a source of microbial spoilage in wines, as well as being unpredictable and potentially problematic. Thus, the addition of precultured, selected yeast strains, known as inoculation, generally of *S. cerevisiae*, is a widely used means of controlling primary fermentation.

### *1.3.1 The effect of* Saccharomyces *yeast on wine composition and aroma*

Many studies have demonstrated the capacity of *Saccharomyces* yeasts to modulate wine aroma compounds, including different species, such as *Saccharomyces bayanus* (Antonelli et al. 1999, Domizio et al. 2007, Eglinton et al. 2000, Estévez et al. 2004, Favale et al. 2007, Hayasaka et al. 2007, Ranieri et al. 1998), *Saccharomyces paradoxus* (Majdak et al. 2002, Orlić et al. 2007), *Saccharomyces uvarum* (Favale et al. 2007, Tosi et al. 2009) and species hybrids (Gangl et al. 2009, Gonzalez et al. 2007, Lopandic et al. 2007, Masneuf et al. 2002, Ranieri et al. 1998). Strains of *S. cerevisiae* have also been shown to vary in their production of esters and other yeast-derived compounds during fermentation (Antonelli et al. 1999, Callejon et al. 2010, Erasmus et al. 2004, Estévez et al. 2004, Farthing et al. 2007, Marais

2001, Mauriello et al. 2009, Miller et al. 2007, Moio et al. 2004, Molina et al. 2009, Nikolaou et al. 2006, Nurgel et al. 2002, Patel and Shibamoto 2002, 2003, Regodón Mateos et al. 2006, Swiegers et al. 2009, Vilanova and Sieiro 2006, Wondra and Berovic 2001). They can also differ in their abilities to produce grape-derived glycoconjugates (Loscos et al. 2009, Ugliano et al. 2006) and volatile thiol compounds (Anfang et al. 2009, Dubourdieu et al. 2006, Howell et al. 2004, Murat et al. 2001a, Swiegers et al. 2009).

Until quite recently, it was not certain that there were substantial flavour differences between wines made using different yeast strains (Thorngate III 1999). It was well known that yeast strains differed in production of yeast-derived volatiles, however, there was minimal sensory data to demonstrate that these chemical differences were important. Some studies indicated differences in the sensory properties of wines made using different *S. cerevisiae* yeast strains (Callejon et al. 2010, Egli et al. 1998, Erasmus et al. 2004, Estévez et al. 2004, Farthing et al. 2007, Fischer 2000, Molina et al. 2009, Nikolaou et al. 2006, Reynolds et al. 2001, Vilanova et al. 2005, Wondra and Berovic 2001). Many of these studies, however, used insufficient experimental designs and questionable methodology. Some studies did not account for fermentation replicates, used small fermentation volumes, limited yeast treatments (ie. only two yeast strains) and model media or less important grape varieties. The sensory analyses conducted were generally informal, using small numbers of assessors with little or no training, and lack of clarity as to whether the tests were replicated, and whether the treatments were randomised and tested blind (without information).

As a prelude to the work reported in this thesis, a recent investigation by Swiegers et al. (2009) clearly showed that different yeast strains used to ferment Sauvignon Blanc wines result in modified sensory profiles. Swiegers et al. (2009) also studied the preference of wine experts for the different wines, showing that the yeast strain used to conduct wine fermentation had a significant effect on expert preference.

The differences in chemical and sensory profiles of wines made using different yeast strains have been generally measured within six months of bottling. It is commonly thought that any flavour differences, if present, are short lived in wine. Given that yeasts are able to modify some compounds that are reportedly stable in wine over time, yeasts might play an important role in the sensory properties of aged wines.

The studies that have investigated yeast strain effects over time generally used extended yeast lees contact, where yeast autolysis of different strains leads to varying levels of released amino acids (Martinez-Rodriguez et al. 2001) and aroma compounds. Yeast autolysis of different strains has been shown to affect the colour and aging potential of one year old Sherry wines (Lopez-Toledano et al. 2006), the chemical composition of nine month old white wines (Loscos et al. 2009), the sensory properties of Vinsanto wines after 18 months of barrel aging (Domizio et al. 2007) and the chemical composition, sensory profiles and aging potential of Champagne wines over three years (Leroy et al. 1990). However, no information is available about whether yeast-derived flavour differences in young wines are retained after a period of bottle age.

### *1.3.2 Co-inoculated fermentations*

There are a number of approaches used in selecting new wine yeast strains to modify wine aroma and flavour, including selection of natural variants, mutagenesis, hybridisation and genetic modification. See Table 4 for more details. Such approaches can be time consuming and costly. An alternative to selecting new wine yeast strains is the use of co-inoculated fermentations. A co-inoculated fermentation, mixed culture or multistarter, is the simultaneous inoculation of a mixture of microbial cultures to conduct wine fermentation. It can involve the use of multiple yeasts, multiple bacteria or yeast and bacteria together. For the purpose of this review, co-inoculated fermentation refers to the use of multiple yeast strains conducting primary fermentation in wine. From a cost perspective, co-inoculated fermentations are inexpensive, as they use existing, preselected microorganisms. Moreover, they are commercially-attractive, as there are no developmental and validation hurdles. Co-inoculations of multiple yeasts can potentially be used in order to manipulate the volatile composition and sensory profiles of wines.

**Table 4.** Methods for developing new wine yeast strains. Modified from Pretorius and Bauer (2002).

| Method | Applications in the wine industry | Wine research examples |
|---|---|---|
| Screening natural isolates - from vineyard and winery populations | A simple and direct method, provides no dramatic improvements | Lopandic et al. (2007)<br>Lopes et al. (2007)<br>Tosi et al. (2009) |
| Mutagenesis - the application of mutagens to induce genetic mutations | Has limited strain development | Subileau et al. (2008b)<br>Urrestarazu et al. (1998) |
| Hybridisation - intra-species sexual and non-sexual mating (spore-cell mating, rare mating, cytoduction and spheroplast fusion) | A non-targeted approach, can be time-consuming | Masneuf et al. (2002)<br>Romano et al. (1985)<br>Shinohara et al. (1994) |
| Transformation or genetic modification - precisely change specific characteristics | Currently not a viable option due to legal regulations and consumer resistance | Swiegers et al. (2007)<br>Thibon et al. (2007) |

There are numerous studies that have investigated the co-inoculation of non-*Saccharomyces* yeast with *S. cerevisiae*, in comparison with single-strain inoculations (monocultures) of the respective yeasts. This technique has the benefit of controlling primary fermentation with *S. cerevisiae*, while modifying the wine aroma using non-*Saccharomyces* yeasts. For details on non-*Saccharomyces* and *S. cerevisiae* co-inoculations and an extensive list of studies using this fermentation strategy, see Ciani et al. (2010), Fleet (2008) and Jolly et al. (2003). A recent study by Anfang et al. (2009) demonstrated that co-inoculations of non-*Saccharomyces* and *S. cerevisiae* yeasts resulted in enhanced levels of volatile thiol compounds in Sauvignon Blanc wines.

Fewer studies have investigated the effect of co-inoculating multiple *Saccharomyces* yeasts during fermentation, which eliminates the problematic issues associated with the inclusion of non-*Saccharomyces* yeasts. Favale et al. (2007) studied co-inoculations of *S. bayanus* and *S. uvarum* in synthetic media and determined that there were no differences in the volatile composition compared with single-strain inoculations. In contrast, co-inoculation of two *S. cerevisiae* strains resulted in modified wine aroma compounds (Grossmann et al. 1996, Howell et al. 2006, Nikolaou et al. 2006) and there were indications of differences in sensory profiles (Grossmann et al. 1996, Nikolaou et al. 2006). A similar result was suggested for three-yeast co-inoculations of *S. cerevisiae* strains (Nikolaou et al. 2006).

Interactions between yeast strains in co-inoculated fermentations might be the cause of these distinct chemical profiles, brought about by the sharing of metabolic intermediates (Favale et al. 2007, Grossmann et al. 1996, Howell et al. 2006). Evidence for this hypothesis was provided by the work of Cheraiti et al. (2005), who found that the redox status of co-inoculations differed from that of the single-strain inoculations, thereby indicating that the interactions between compatible yeast strains involved the diffusion of metabolite(s) within the co-inoculated fermentations. A comparison of the volatile aroma composition of co-inoculated wines and a blend of the single-strain wines after fermentation by Howell et al. (2006) provided support for this conclusion, as the volatile aroma composition of the co-inoculated fermentations could not be replicated by the blended wines. However, the fundamental mechanisms of metabolic yeast interactions remain unknown.

Due to the influence on the volatile aroma profile of wines, co-inoculated fermentations have the potential to assist the wine industry by providing novel products to increase diversity of wine styles. There remains limited information, however, on the co-inoculation of two or more *Saccharomyces* strains, with as yet no information available on the effect of yeast interactions on grape-derived aroma compounds, in particular volatile thiols. More research is also required using rigorous, formal sensory analyses to determine the sensory properties of co-inoculated wines.

### 1.4 Sensory analyses of wine aroma

Measures of wine aroma can include chemical analysis as an indirect measure, or directly through sensory analysis. Combining the two sets of data is usually the most informative. Many of the studies previously reported in this review have used chemical analyses to measure the effects of yeasts on levels of presumed aroma compounds, with only inference made to the resulting effect on wine aroma. The results of chemical analyses using current methods can be used to predict the sensory profile of wine. However, due to the interactive effects of wine aroma compounds and the complex human perception of wine aroma, these models are rarely accurate, although interest in modelling continues (Aznar et al. 2003, Ferreira et al. 2003b).

There are a small number of potent aroma compounds in wine, labelled 'impact' compounds, that can effectively transmit their aromas in wine without the need for support from other compounds (Ferreira et al. 2000, Guth 1997a). These impact compounds, when chemically measured, can provide reasonable predictions of certain wine sensory properties. Examples of impact compounds include volatile thiols, dimethyl sulfide, diacetyl and rotundone, although minimum evidence is currently available to confirm their impact status. Multivariate statistical methods are often used to determine the relationship between sensory data and chemical measures of aroma compounds, with the ultimate aim to understand how differences in sensory properties among a range of samples are produced by variations in chemical composition (Lee and Noble 2006).

Sensory science is a separate and distinct discipline "used to evoke, measure, analyse and interpret reactions to those characteristics of foods and materials as they are perceived by the senses of sight, smell, taste, touch and hearing" (Stone and Sidel 2004). It provides an objective* measure of the sensory properties of products using reliable and validated data. The fundamentals of sensory analysis include good experimental design, careful selection of assessors, standard procedures and methodology, controlled testing conditions, replication of

* Describing data that are "capable of being verified by independent observation or measurement, and therefore, not dependent on the report of a single individual." Lawless, H.T. and Heymann, H. (1998) Sensory evaluation of food. Principles and practices. Chapman & Hall, New York, USA..

tests and treatments, appropriate statistical analyses and conservative interpretation of results (Lawless and Heymann 1998).

**Table 5.** Categories of tests, types of methods used and wine research examples of sensory analyses, modified from Stone and Sidel (2004).

| Category | Test type | Wine research examples |
|---|---|---|
| Discriminative | Difference: paired comparison, duo trio, triangle | Differences between yeast treatments (Farthing et al. 2007) |
| Descriptive | Descriptive analysis: QDA® †, FPA®‡, SDA®§ | Varietal sensory profiling (Lund et al. 2009); characterising profiles of yeast treatments (Swiegers et al. 2009) |
| Affective | Acceptance – preference: nine-point hedonic | Consumer testing (Lattey et al. 2010, Lund et al. 2009) |

† Quantitative descriptive analysis® (Tragon, Palo Alto, CA)
‡ Flavour profile analysis (A.D. Little Company, Boston, MA)
§ Spectrum descriptive analysis (Sensory Spectrum, East Hanover, NJ)

Sensory analysis is a relatively new science to the wine industry. This is because rigorous, formal sensory analysis is generally time consuming and expensive, and requires extensive training by organisers. It also involves complex and varied statistical analyses, and requires an understanding of human psychology and physiology, as well as knowledge of the products being tested (Howe 2000). A summary of commonly used sensory tests and wine research examples is shown in Table 5. This review focuses on sensory descriptive analysis and consumer acceptance testing.

### *1.4.1 Sensory descriptive analysis*

Sensory descriptive analysis is an objective description of a wine in terms of its perceived sensory attributes using the most precise terminology possible (Gawel 1997, Lawless and Heymann 1998, Stone and Sidel 2004).

There are two main approaches to descriptive analyses. The consensus training method was originally introduced as Quantitative Descriptive Analysis (QDA®) (Stone et al. 1974), although many variations now exist. It is where panellists generate attributes and come to an agreement regarding their terminology and reference standards during training sessions, with minimal influence of the panel leader (Meilgaard et al. 1999). Samples are then assessed by panellists individually and statistical analyses used to determine treatment differences. The other approach is ballot training method and uses Spectrum Descriptive Analysis (SDA®) or a variation thereof. This method uses set attributes or 'lexicons' and 'absolute' intensity standards created by experts (Meilgaard et al. 1999). There are further alternative descriptive analyses, such as Flavour Profile Analysis (FPA®), where a small number of panellists generate attributes, and after assessing the samples, they discuss the results and arrive at a 'consensus' profile of each sample, under the instruction of the panel leader (Meilgaard et al. 1999).

The consensus training method can result in one-sidedness, where the panel's opinion is dominated by senior members or dominating personalities, and the panel can develop erroneous terms due to lack of guidance (Meilgaard et al. 1999). However, the results from the consensus training method may be more realistic for the products tested, than when the terms are at least partially created by others in the ballot training method. The ballot training method also limits psychological freedom, as panellists are not allowed to score intensity ratings as they like, although this in itself can lead to inconsistencies of results for the consensus training method (Meilgaard et al. 1999). For SDA® in particular, the results can be argued to be absolute, whereas for QDA®, the results are relative, due to the scaling differences (Lawless and Heymann 1998).

When selecting panellists, there is a screening and training process that involves determining their ability to detect and recognise differences in characteristics, and their ability to describe those characteristics both verbally and using scaling methods (Meilgaard et al. 1999). The source of panellists can greatly alter the results of sensory tests. Expert or technical panellists, persons with a high degree of knowledge of the products being tested, generally have assumptions regarding the product, which may bias the outcome of the test, however, experts generally have a good ability to discriminate specific aroma and flavour differences. Non-technical or consumer panellists, persons with a low degree of knowledge, on the other hand, have no background information regarding the products and thus, no assumed knowledge, and they generally use non-technical language, which is useful when translating or disseminating results. Both types of panellists are useful depending on the overall outcome of the tests. For example, a non-technical panel may be more appropriate for the QDA® approach, particularly when relating the descriptive analysis results to consumer acceptance testing (Lawless and Heymann 1998).

Some of the other issues regarding sensory descriptive analysis include the number of panellists used, the method and length of training, replication of samples, randomisation and blinding of the samples, the number of samples tested in each session, the duration of the sessions and time between sessions (Meilgaard et al. 1999).

In the wine industry, sensory descriptive analysis can be used for product maintenance, product improvement or optimisation, development of new products, category review and process changes, due to new technologies or raw ingredient substitution (Meilgaard et al. 1999). The results of sensory descriptive analysis can be related to the samples using principal component analysis (PCA), and also related to chemical (instrumental) data of the samples using partial least squares regression (PLSR) (Lee and Noble 2006, Noble and Ebeler 2002). It is highly useful in identifying the sensory effect that a specific processing treatment has on wine, such as the yeast strain used to conduct fermentation (Swiegers et al. 2009). It can also be used in wine research to identify varietal or stylistic wine sensory profiles. For example, Lund et al. (2009) characterised the sensory attributes of a set of New Zealand Sauvignon Blanc wines. The results of sensory descriptive analyses can also help to determine which sensory attributes are important to consumer preference (Lawless and Heymann 1998).

### *1.4.2 Consumer acceptance testing*

Consumer acceptance testing, referred to as preference, hedonic or affective testing, is the measurement of liking or preference by consumers for a product (Stone and Sidel 2004). Consumer sensory tests determine a set of products' relative sensory acceptability to a group of consumers and also can be used to assess market potential. This form of testing commonly uses a 9-point hedonic scale, originally developed in the 1950s (Peryam and Pilgrim 1957), with a centred neutral category and scale points chosen to represent equal psychological intervals (Lawless and Heymann 1998), an example of which is shown in Figure 7. Other scales may also be used in consumer acceptance tests.

To date, consumer acceptance testing in the wine industry have mainly focussed on varietal and wine style preferences of wine consumers. These include Chardonnay (Lattey et al. 2004, Lesschaeve et al. 2002, Yegge and Noble 2000), Riesling (Lattey et al. 2004), Sauvignon Blanc (Lund et al. 2009), Semillon (Blackman et al. 2010), Godello (Vilanova 2006), white wine blends (Lesschaeve and Findlay 2004), Cabernet Sauvignon (Lattey et al. 2010, Robichaud et al. 2007), Shiraz (Lattey et al. 2010), Merlot (Lesschaeve 2003), Tannat (Varela and Gámbaro 2006) and red wine (Frøst and Noble 2002).

Most of the above studies conducted a sensory descriptive analysis, using trained panellists to quantify sensory attributes of wines selected to encompass wide sensory variation, and measured consumer liking using hedonic scales. The results of the consumer testing were then related to the sensory descriptive analysis data, generally using multivariate approaches, such as preference map analysis, to determine the sensory attributes driving consumer preference, and in some cases, also the aroma compounds responsible for those sensory attributes. For instance, volatile thiols and methoxypyrazines were identified as key aroma compounds driving consumer preference for Sauvignon Blanc wines (Lund et al. 2009).

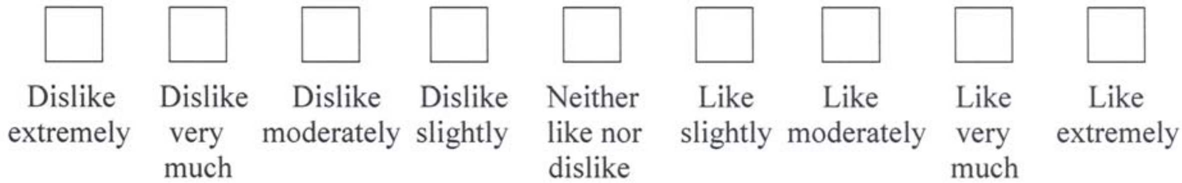


**Figure 7.** An example of a categorical 9-point hedonic scale used in consumer acceptance testing to assess consumers' liking and disliking for products.

Preference map analysis is a multivariate approach using a variation of principal component analysis, where the consumer acceptance results are overlayed with the products and also with the descriptive analysis data (Noble and Ebeler 2002). It provides valuable information about consumers' acceptance in a visual format that attempts to define what consumers want or like based on product characteristics (Lawless and Heymann 1998).

It was concluded from the preference studies listed above, as expected, that consumer preference is significantly affected by wine aroma and flavour. In some cases, sub-groups of consumers were also identified with diverse preferences for different sensory attributes using cluster analysis. This is because, when dealing with consumers, not everyone has the same preferences. There are a diverse range of consumer behaviours, and as such, the population cannot be treated as a single entity (Kotler and Keller 2009). Instead, consumers are segmented using cluster analysis, based on various criteria, such as preference scores for wines, so that consumers with similar behaviours are grouped together (Meilgaard et al. 1999).

A small number of studies have investigated the effects of processing treatments on consumer liking, including oak contact (Hersleth et al. 2003, Lockshin and Rhodus 1993), dealcoholisation (removal of alcohol) (Meillon et al. 2010), acidification (acid addition) (Fischer 2000), malolactic fermentation and chapitalisation (sugar addition) (Hersleth et al. 2003), as well as the allergenic properties of processing aids (Rolland et al. 2006) and the effects of specific off-flavour compounds in wine, such as cork off-flavour (Prescott et al. 2004), *Brettanomyces* off-flavour (Curtin et al. 2007) and eucalyptol (Saliba et al. 2009). No studies have currently investigated whether the flavour differences brought about by yeast strains are sufficiently large to influence consumer preference.

Other factors, apart from intrinsic sensory quality, also play an important role in consumer purchase behaviour, such as brand, price, region of origin and awards (Lockshin et al. 2006), closure type (Marin and Durham 2007, Marin et al. 2007), wine label information (Thomas and Pickering 2003), place of wine purchase (Martínez et al. 2006), context of tasting (Hersleth et al. 2003) and shape of the wine glass (Fischer 2000, Hummel et al. 2003). Although these studies are investigating consumers, they are not forms of consumer sensory testing; instead, they can be considered marketing research studies. Consumer sensory testing generally does not take into account extrinsic or external factors (Lawless and Heymann 1998), as wines are tasted blind and a direct measure of the response to sensory differences alone can be established. The results of consumer sensory tests may, however, need to be compared to marketing research studies, for identification and characterisation of a group of consumers (Lawless and Heymann 1998).

Additional information obtained from consumer sensory tests on consumers' demographics, and wine usage and attitudes can sometimes be related to taste preferences, while other times it is used in market segmentation exercises to gain insights into consumer behaviours. This information can then be used by persons in the wine value chain to target products and marketing communications for increased retail success and competitive advantage (Johnson and Bastian 2007). Segmentation criteria used to subdivide wine consumers include age (Thach and Olsen 2006), nationality (Guo and Meng 2008, Lockshin et al. 2001), differing attitudes or mind-sets (Hughson et al. 2004), wine-related lifestyles (Bruwer and Li 2007, Johnson and Bruwer 2003), wine involvement (Dodd et al. 1996, Hollebeek et al. 2007, 2006, 2001, Lockshin et al. 1997) and wine knowledge (Frøst and Noble 2002, Johnson and Bastian 2007).

Some limitations regarding consumer sensory testing include: often limited responses to recruitment advertising; limited availability or commitment of participants; the requirement of standardised environments and uniform conditions across all testing times; consumer fatigue after a relatively small number of samples; increased blood alcohol levels in participants sampling alcoholic beverages; ethics approval for all tests, particularly the gathering and reporting of consumers' personal information; boredom of participants during forced rest periods or while completing lengthy questionnaires; incorrect use of scales or ambiguity of questions; computer-illiterate participants; computer/software malfunctions during testing times, and unaccounted for variation in human responses.

The measurement of wine aroma and flavour is, overall, a measure of wine quality. The definition of quality is dependent upon the endpoint of the product. For most wines, the endpoint is consumer enjoyment. Measuring the chemical composition and analysing the sensory properties of a wine to determine wine aroma and flavour is not always a clear indication of the quality rating from a consumer perspective. Therefore, an extra facet of wine aroma measurement is to determine the acceptability of the product to consumers, which currently, is a small part of wine research. Wine producers can use information from sensory tests to target certain wines styles to meet the expectations of the consumer subgroups (Lambrechts and Pretorius 2000, Lattey et al. 2010, Pretorius and Bauer 2002, Swiegers et al. 2009).

### 1.5 Summary of research aims

It has been shown that the type of yeast strain used to conduct primary fermentation has an effect on wine chemical profiles. There is also some evidence that yeast strains influence the resulting wine aromas and flavours (Swiegers et al. 2009), however, more evidence is required to confirm these findings. Wine aroma and flavour are important to consumer liking. Volatile thiols and their sensory influence have been indicated as drivers for consumer preference of Sauvigion Blanc wines (Lund et al. 2009). Considering that yeast can modify the concentration of volatile thiol compounds, choice of yeast strain may cause sufficient sensory differences to affect consumer preference. However, no studies have investigated and confirmed this link. Further to this, anecdotal evidence suggests that the effects of yeast strains might be short-lived in wine. Given that yeasts produce aroma compounds that are reportedly stable in wine, it is hypothesised that yeast-derived flavour differences will be retained after a period of bottle age.

This research project aims to investigate the effect of co-inoculating wine yeast strains on the chemical and sensory profiles of Sauvignon Blanc wines. Based on the literature reviewed in this chapter, it is expected that significantly different wine aroma profiles will be achieved using co-inoculated fermentations, due to metabolic interactions occurring between the yeast strains. Sauvignon Blanc wines made using different yeast strains will also be analysed after a period of bottle age, to determine whether yeast strain differences remain in aged white wines.

Following this, it will be determined whether the yeast strains used to ferment Sauvignon Blanc wines have an effect on the preference of regular wine-drinking consumers. The results of this project will provide a better understanding of the sensory attributes and aroma compounds responsible for driving consumer preference of Sauvignon Blanc wines. From the evidence presented, it is expected that yeast strains will have an effect on consumer preference, through changes in volatile thiol compounds and wine aroma.

The yeast strains used in this project will be commercial *Saccharomyces* strains available as active-dried yeast, and experiments will be carried out using industrial winemaking techniques. Therefore, the results from the co-inoculations used in this project will be highly applicable to, and may be extrapolated for use in the wine industry, as a viable fermentation strategy to alter wine styles at little or no extra cost. These investigations will highlight the importance of yeast strain selection as a tool for the wine industry, to modulate wine styles and target specific consumer markets.

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# Chapter 2.

## Coinoculated fermentations using *Saccharomyces* yeasts affect the volatile aroma composition and sensory properties of *Vitis Vinifera* L. cv. Sauvignon Blanc wines

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## Chapter 3.

# The effect of multiple yeasts co-inoculations on Sauvignon Blanc wine aroma composition, sensory properties and consumer preference

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# The effect of multiple yeasts co-inoculations on Sauvignon Blanc wine aroma composition, sensory properties and consumer preference

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ABSTRACT

Consumer acceptance testing has been only recently applied in wine research, to assess wine sensory attributes that affect hedonic liking. The aim of this study was to investigate the effect of *Saccharomyces* yeast co-inoculations on wine volatile composition and sensory profiles, and to determine if differences were sufficient enough to affect consumer acceptance. Fermentations were conducted using two- and three-yeast co-inoculations, and single strains. Yeast inocula differed substantially in volatile thiols and other flavour compounds, and in their sensory properties. Wines from four yeast inocula which showed large sensory differences were subjected to consumer testing by 120 consumers, with differences in overall liking found. Four clusters of consumers were identified, with one group strongly preferring the two-yeast co-inoculated wine with an intermediate sensory profile, while another group favoured the wine made using the three-yeast co-inoculation. This study has demonstrated that the yeast inoculum used to conduct fermentation affects consumer acceptance.



## 1. Introduction

Wine aroma and flavour is derived from hundreds of volatile chemical compounds arising from the grape berry, and from winemaking and aging processes. Human perception of wine aroma can be measured using formal sensory evaluation practices, designed to detect differences between wines, quantify sensory attributes, and test the preference or liking of wines by consumers (Stone & Sidel, 2004). Consumer sensory tests determine a set of products' relative sensory acceptability to a group of consumers.

Other factors apart from sensory quality play an important role in consumer purchase behaviour, such as brand, pricing, packaging and promotion. However, when wines are tasted blind without influence of extrinsic factors, a direct measure of the response to sensory differences alone can be established.

To date, wine consumer research has mainly focussed on assessing the effect of sensory attribute differences on preferences of wine consumers for certain varieties and categories of commercial wines. These include Chardonnay (Lattey, Smyth, D'Costa, Leibich, & Francis, 2004; Yegge & Noble, 2000), Riesling (Fischer, 2000; Lattey, Smyth, D'Costa, Leibich, & Francis, 2004), Sauvignon Blanc (Lund et al., 2009), Godello (Vilanova, 2006), Cabernet Sauvignon and Shiraz (Lattey, Bramley, & Francis, 2010) and Merlot (Lesschaeve, 2003).

All of these studies firstly used trained panellists to quantify sensory attributes of wines selected to encompass wide sensory variation and then measured consumer liking using a hedonic scale. The investigations were able to conclude that consumer preference, whether of the entire sample tested or of sub-groups, is significantly affected by variations in particular sensory attributes.

There has been little research linking viticultural or winemaking processes to changes in sensory attributes and consumer liking. One study by Hersleth, Mevik, Naes, and Guinard (2003) measured consumer preference of Chardonnay wines made with or without malolactic fermentation, oak contact and sugar addition. Studies have also investigated the effect upon consumer preference of specific aroma compounds in wine, in particular taint compounds, such as cork taint (Prescott, Norris, Kunst, & Kim, 2004).

Lund et al. (2009) used chemical analyses to identify key aroma compounds in Sauvignon Blanc potentially responsible for driving consumer preference. It was found that two volatile thiol com-

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pounds, 3-mercaptohexan-1-ol (3MH) and 3-mercaptohexyl acetate (3MHA), and 3-isobutyl-2-methoxypyrazine (IBMP) were at relatively high concentrations in Marlborough Sauvignon Blanc wines compared to wines from other regions studied, and that these wines were most preferred by the consumers tested.

The volatile thiol 4-mercapto-4-methylpentan-2-one (4MMP) is also important to Sauvignon Blanc wine aroma (Tominaga, Baltenweck-Guyot, Peyrot des Gachons, & Dubourdieu, 2000), as are numerous fermentation-derived ester compounds, such as 3-methylbutyl acetate and ethyl hexanoate (Swiegers et al., 2009). Volatile thiols 4MMP, 3MH and 3MHA are highly potent aroma compounds with very low sensory detection thresholds. At wine-like concentrations they have sensory characteristics reminiscent of grapefruit, passionfruit, tropical fruit, box hedge and cat urine. The sensory properties conferred by these compounds may be perceived as either positive or negative, depending upon the concentration in wine and/or the sensitivity and expectations of the individual.

4MMP and 3MH are grape-derived compounds, released from cysteine and glutathione conjugates by yeast enzymes during fermentation (Subileau, Schneider, Salmon, & Degryse, 2008), while 3MHA is produced by yeast metabolism through the esterification of 3MH during fermentation (Swiegers et al., 2009). Recent research has shown that wine yeast strains of *Saccharomyces cerevisiae* and *Saccharomyces sensu-stricto* interspecific hybrids differ in their ability to produce volatile thiol compounds (Anfang, Brajkovich, & Goddard, 2009; King et al., 2008; Masneuf, Murat, Naumov, Tominaga, & Dubourdieu, 2002; Swiegers et al., 2009), which results in modified sensory profiles (King et al., 2008; Swiegers et al., 2009). Differences in Sauvignon Blanc flavour due to yeast strain have also been shown to affect winemaker preference (Swiegers et al., 2009), with the strain giving rise to the highest levels of 4MMP and 'box hedge' aroma being most preferred by the group tested.

Co-inoculations of two wine yeast strains used to conduct fermentation have been shown to result in modified volatile thiol concentrations and large sensory differences (King et al., 2008). A recent study showed a similar effect on thiols using a wine yeast strain with a non-*Saccharomyces* co-inoculation (Anfang et al., 2009). There have been some informal sensory studies of co-inoculated fermentations that indicated differences in wine preferences (Grossmann, Linsenmeyer, Muno, & Rapp, 1996) and in sensory properties (Nikolaou, Soufleros, Bouloumpasi, & Tzanetakis, 2006). The question remains as to whether the wine flavour changes brought about by yeast strains are sufficient to affect wine quality, as perceived by consumers.

The aim of this study was to develop an understanding of the response of consumers to Sauvignon Blanc wines fermented using different yeast inocula. Wines made from single strains, as well as co-inoculations containing two and three *Saccharomyces* yeast strains in differing proportions were investigated. The wines were analysed for their chemical composition and sensory profiles, and consumer acceptance testing was conducted on a subset of wines.

## 2. Materials and methods

### 2.1. Juice and winemaking

Clarified 2007 Adelaide Hills (Australia) Sauvignon Blanc juice was fermented using four commercial wine yeast strains in single-strain fermentations, labelled Treatments *A*, *B*, *C* and *D*, and co-inoculated fermentations of two-yeast strains, labelled Treatment *AD* and three-yeast strains in two different proportions, labelled Treatments *ABC* and *Abc* (Table 1) – the lettering of the co-inoculations indicates the approximate relative proportion of single-strain components. Also included in the study was a hybrid yeast, which was constructed from two commercial wine yeast strains A and B, labelled Treatment *A/B*. Details of this construction will be presented elsewhere. Initial must analysis results were as follows: total soluble solids 19.3 °Brix, pH 3.44, titratable acidity 5.9 g/L, free sulphur dioxide 26 mg/L and total sulphur dioxide 98 mg/L.

The winemaking procedures used were as described in King et al. (2008). Briefly, they involved triplicate fermentations in 20 L stainless steel vessels at approximately 15 °C. Sulphur dioxide additions were made as needed to maintain approximately 30 mg/L of free sulphur dioxide. In an attempt to mimic commercial fermentations, the grape juice was sulfited and unfiltered, fermentations were conducted at relatively low temperatures and conventional winemaking practices were used. In total, 24 wines were generated in the study, involving three fermentation replicates of eight yeast inocula (Table 1).

### 2.2. Verification of strain implantation

Yeast samples were collected during the racking process after all fermentations were complete, stored at 4 °C for no longer than one week and plated onto YPD medium according to King et al. (2008). The identity of individual colonies was determined by polymerase chain reaction (PCR)-based transposon and internal transcribed spacer (ITS) methods, as detailed below. For Treatments *B*, *C*, *D* and *A/B*, 33 isolates were identified using transposon PCR, as described in Ness, Lavallée, Dubourdieu, Aigle, and Dulau (1993). Isolates from Treatments *A*, *AD*, *ABC* and *Abc* were identified using ITS-region PCR restriction fragment length polymorphism (RFLP), as detailed by Bradbury et al. (2005).

Yeast isolates obtained from the wine fermentations were compared to reference strains sourced from frozen glycerol stocks (−80 °C) of the culture collection of The Australian Wine Research Institute (AWRI) (Adelaide, Australia).

### 2.3. Chemical analyses

Standard chemical analysis was conducted on all wines after bottling as previously described (King et al., 2008). Twenty-five fermentation-derived volatile aroma compounds (six acetate esters, nine fatty acid ethyl esters, six higher alcohols and four volatile acids) and two volatile thiol compounds were measured in all wines three months after bottling using the methods of Siebert et al. (2005) and Tominaga, Murat, and Dubourdieu (1998), respectively.

### 2.4. Sensory analyses

All wines were subjected to a sensory descriptive analysis three months after bottling. Eleven assessors were recruited (five female), all of whom were AWRI staff. Nine assessors had previously participated in recent Sauvignon Blanc wine descriptive analysis studies. Assessors completed five training sessions: two 60 min discussion sessions to generate attributes and three practice rating sessions in isolated booths, prior to formally rating the wines. The assessors rated two appearance attributes, 13 aroma attributes and eight palate attributes (Table 2). The intensity of each aroma and palate attribute was rated using an unstructured 15 cm line scale with indented anchor points of 'low' to 'high' placed at 10% and 90% respectively. A computerised data collection system was used (FIZZ, Version 2.1, Biosystemes, France).

The samples were assessed under sodium lighting in isolated, temperature-controlled (22–24 °C), ventilated tasting booths. Assessors were presented with six wines per session, in coded, covered ISO tasting glasses. The wines were presented in an incom-

**Table 1**
Details of the treatment codes of yeast inocula used in the study.

| Treatment code | Yeast strain(s) | Inoculum type | Inoculation procedure |
|---|---|---|---|
| *A* | Strain A, *Saccharomyces cerevisiae/ Saccharomyces kudriavzevii* | Single strain | Recommended rate as rehydrated yeast |
| *B* | Strain B, *S. cerevisiae* | Single strain | Recommended rate as rehydrated yeast |
| *C* | Strain C, *S. cerevisiae* | Single strain | Recommended rate as rehydrated yeast |
| *D* | Strain D, *S. cerevisiae* | Single strain | Recommended rate as rehydrated yeast |
| *A/B* | Hybridised yeast of strains A and B | Single strain | Recommended population rate as cultured yeast |
| *AD* | Strains A and D | Co-inoculation | Equal proportions of strains A and D; amount to recommended rate as rehydrated yeast |
| *ABC* | Strains A, B and C | Co-inoculation | Equal proportions of strains A, B and C; amount to recommended rate as rehydrated yeast |
| *Abc* | Strains A, B and C | Co-inoculation | Higher proportion of strain A than strains B and C; amount to recommended rate as rehydrated yeast |

**Table 2**
Appearance, aroma, taste and mouthfeel attributes rated by the sensory panellists, the reference standard compositions for aroma attributes, and synonyms for appearance, taste and mouthfeel attributes, where applicable.

| Attributes | Reference standard compositions[a] or synonyms |
|---|---|
| *Overall colour intensity* | Light–dark |
| *Grey hue* | – |
| *Estery* | 50 μL 'estery' mix stock solution[b] |
| *Floral* | 50 μL *cis*-rose oxide |
| *Fresh citrus* | 5 mL lemon cordial (Bickfords) & ½ tspn[c] fresh lemon zest, ½ tspn fresh lime zest & ½ tspn fresh grapefruit zest |
| *Apple/pear* | 1 ½ $cm^2$ piece of canned pear (Goulbourn Valley) & 1 ½ $cm^2$ piece of fresh apple |
| *Pineapple* | ½ $cm^2$ piece canned pineapple (Golden Circle) & 5 mL canned pineapple juice (Golden Circle) |
| *Passionfruit* | ½ tspn fresh passionfruit zest and three fresh passionfruit seeds |
| *Box hedge* | Four fresh box tree leaves, crushed – no base wine |
| *Fresh green* | ½ $cm^2$ piece fresh green capsicum & ½ fresh green bean |
| *Grassy* | ½ doz blades fresh-cut grass – no base wine |
| *Cooked citrus* | 5 mL lime cordial (Bickfords) |
| *Cooked vegetal* | 1 tspn canned asparagus brine (Black and Gold) & 1 tspn canned green bean brine (Edgell) |
| *Sweaty* | 50 μL hexanoic acid |
| *Bruised apple* | 50 μL acetaldehyde |
| *Overall fruit flavour* | Tropical, stone fruit, citrus |
| *Floral flavour* | Perfume, talc, rose |
| *Overall green flavour* | Herbal, grassy, asparagus |
| *Acidity* | Sourness |
| *Viscosity* | Roundness, thickness |
| *Bitterness* | – |
| *Hotness* | Warmth |
| *Length* | Flavour persistence |

[a] In 30 mL Chenin Blanc, 2007, 2 L bag in box wine (11% alc/vol) per glass, unless otherwise specified.
[b] Estery mix contains: 0.5 mg isobutyl acetate, 0.09 mg ethyl butyrate, 0.2 mg ethyl hexanoate, 0.2 mg ethyl octanoate and 100 ml redistilled ethanol.
[c] Tspn: teaspoon.

plete block design, 3 × 3 balanced lattice, such that only one fermentation replicate of each yeast inoculum was presented in any one session, and that no two yeast inocula were presented together more than once. Each sample was presented at a constant volume (30 mL) and temperature. The practice rating sessions were held under the same conditions as the subsequent formal sessions, except that each assessor was presented with the same sample presentation order in the former. Triplicate fermentation replicates of nine yeast inocula were assessed in duplicate, however, only eight yeast inocula, those detailed in Table 1 will be discussed further. Samples were assessed in nine formal sessions held over a three week period.

Consumer acceptance testing was carried out on four yeast inocula, Treatments *A*, *D*, *AD* and *ABC*. The fermentation replicates from the yeast inocula were randomly selected, such that each replicate was presented an equal number of times to an equal number of consumers. Consumers were approached in three retail liquor stores across the Adelaide (Australia) metropolitan area, one national chain and two independent stores, and asked to participate in testing. Selection and exclusion criteria were as follows: age between 18 and 65 years; regular white wine consumers (defined as drinking white wine at least once per week); no professional wine education; in good health; had not consumed alcohol or drugs in the past two hours; and had no ethical or medical reasons for not consuming alcohol. Testing occurred in the retail liquor stores immediately following recruitment. A portable table with side and back vertical dividers, which could fit three assessors seated at any one time, was used to provide an isolated, standardised environment. No specific information about the grape variety or inocula was given to consumers prior to undertaking the study. In total, 120 consumers participated in the testing (67 females). The Human Research Ethics Committee of The University of Adelaide (Adelaide, Australia) approved the methods of testing and data collection employed in this study.

The wines were presented monadically, in a randomised, balanced order. Wines (30 mL) were served chilled at approximately 14 °C, in ISO tasting glasses with randomly assigned three-digit codes. Consumers were asked to rate their liking for each wine on a hedonic nine-point category scale from 'dislike extremely' to 'like extremely'. Paper score-sheets were used for data collection. Fruit and chocolate were provided as a token payment for completing the testing session.

Information was also collected on participants' personal details, and wine usage and attitudes. There was a reasonably equal distribution among the participants in the study for gender and age (Table 3). There was a slight bias in the subjects tested towards higher levels of education achieved, possibly due to the socioeconomic areas that the testing was conducted in.

### 2.5. Data analysis

Statistical software (JMP 5.1, SAS Institute, Cary, NC) was used for analysing all data. Sensory descriptive data for each attribute were analysed using an analysis of variance (ANOVA) testing for the effects of treatment, fermentation replicate nested within treatment, as well as presentation replicate and assessor. Assessor performance was assessed using FIZZ and Senstools (OP&P, Utrecht, Netherlands). Hierarchical cluster analysis was performed for the consumer data using Ward's method on the standardised data and a series of one-way ANOVAs were used for testing the effects of treatment and consumer cluster. A chi-squared test was performed on all demographic data for each cluster. A one-way ANOVA was used to analyse all chemical data and odour activity values were calculated as concentration divided by aroma detection threshold (taken from King et al. (2008)). The Unscrambler software (Version 9.5, CAMO Inc., Norway) was used for relating the standardised chemical data as *x*-variables (predictor variables) and sensory data as *y*-variables by partial least squares regression (PLSR). The optimal number of components for the models was determined by inspection of residual variance explained by each principal component (PC).

## 3. Results and discussion

### 3.1. Winemaking parameters and verification of strain implantation

All wine fermentations were completed successfully to less than 1 g/L total soluble solids. The rates of sugar consumption varied among the yeast inocula (data not shown). The yeast inocula with the fastest rate of sugar consumption was Treatment *C*, which finished fermentation within 10 days, while Treatment *A* was the slowest to complete fermentation, 50 days after inoculation. All other yeast inocula had intermediate fermentation rates. The slow fermentation rates were attributed to the cool temperatures and small-scale 20 L volumes of the ferments.

The co-inoculations completed fermentation faster than some of the single-strain components (data not shown), also observed in King et al. (2008). The hybrid yeast Treatment *A*/*B* had a fermentation rate intermediate to its parental strains. Similar results have been reported for progeny formed from the mating of two wine yeast strains (Ranieri, Giudici, & Zambonelli, 1998).

Standard chemical analyses of the wines showed that all measurements were similar across inocula and within acceptable levels. The pH values ranged from 3.46 to 3.48, while titratable acidity was between 5.4 and 5.8 g/L with concentrations highest in Treatment *AD* and lowest in Treatments *B* and *ABC*. There was a negligible difference in alcohol concentrations across the yeast inocula (11.8–11.9% v/v).

Interestingly, the volatile acidity concentrations showed greater differences among the yeast inocula, although concentrations measured in all the wines were far below the level generally considered to be detrimental to wine flavour. Treatments *A* and *AD* contained the highest concentrations of volatile acidity of 0.33 g/L and 0.32 g/L acetic acid, respectively, while Treatments *B* and *C* contained the lowest concentrations of 0.17 g/L and 0.16 g/L acetic acid, respectively. It is unlikely that these differences have caused a negative vinegar-like effect on the aroma and flavour of the wines.

Molecular techniques were used to confirm implantation of all inoculated strain(s) at the end of fermentation. With the exception of Treatments *A* and *A*/*B*, all inoculated yeast strains in the single-strain fermentations were present at high proportions (>90%) at the end of fermentation (data not shown).

For Treatment *A*, strain A represented, on average, 46% of the total yeast isolated, and for Treatment *A*/*B*, hybrid strain A/B represented, on average, 25% of total yeast isolated. At completion of the co-inoculated fermentations, strain A was not detected in Treatment *AD*, was found in low proportions (<10%) for Treatment *ABC* and was present at moderate levels (31%) for Treatment *Abc* (data not shown). Due to often inconsistent banding patterns of strains B and C in the molecular techniques used, these strains were not able to be distinguished from one another in Treatments *ABC* and *Abc*. It is reasonable to expect that strain A and hybrid strain A/B were present in high numbers at the time of inoculation (or at the desired proportions for the co-inoculated fermentations for strain A) and were thus involved in flavour production during the key stages of fermentation (Fraile, Garrido, & Ancin 2000; Howell, Cozzolino, Bartowsky, Fleet, & Henschke, 2006).

### 3.2. Volatile thiol concentrations

Volatile thiols are known key impact odourants of the grape variety Sauvignon Blanc and are influenced by yeast during fermentation (Tominaga et al., 2000). For this study, the single-strain components of the co-inoculated yeast combinations were selected based upon their previously demonstrated capacity to enhance thiol formation during fermentation. The concentrations of 3MH and 3MHA were measured at the completion of fermentation, and are shown in Fig. 1.

All wines contained concentrations of 3MH and 3MHA above their aroma detection thresholds of 60 ng/L and 4.2 ng/L, respectively (Tominaga et al., 2000). Accordingly, it is assumed that the volatile thiols would contribute to sensory differences among the yeast inocula. Treatments *A*, *B*, *C*, *D* and *A*/*B* had low to intermediate concentrations of both 3MH and 3MHA (Fig. 1). Treatment *AD* had an intermediate 3MH concentration and a relatively high 3MHA concentration. Treatment *ABC* contained a higher concentration of 3MH than Treatment *Abc*, although both three-yeast

**Table 3**
Demographic data for the 120 participants of consumer acceptance testing.

| Demograpic data | Percentage |
|---|---|
| *Gender* | |
| Male | 55.8 |
| Female | 44.2 |
| *Age (years)* | |
| 18–25 | 15.8 |
| 26–35 | 25.0 |
| 36–45 | 14.2 |
| 46–55 | 21.7 |
| 56–65 | 22.5 |
| *Highest level of education achieved* | |
| High school certificate | 15.8 |
| Technical certificate/apprenticeship/diploma | 14.2 |
| Bachelor's degree | 34.2 |
| Masters/doctorate degree | 32.5 |
| *Personal income (pre-tax AUD)*[a] | |
| Less than $25,000 | 13.6 |
| $25,000–$49,999 | 25.4 |
| $50,000–$74,999 | 27.1 |
| $75,000–$99,999 | 9.3 |
| $100,000 plus | 16.1 |

[a] Percentage does not total 100% due to extra category for 'Prefer not to answer' (data not shown).

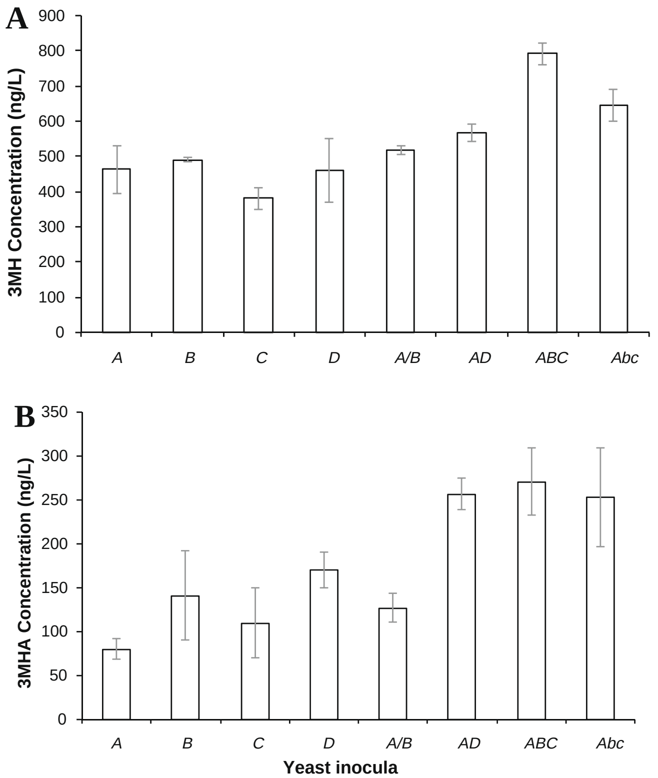


**Fig. 1.** Concentrations of volatile thiols averaged across fermentation replicates for all yeast inocula. (A) 3-mercaptohexan-1-ol (3MH) and (B) 3-mercaptohexyl acetate (3MHA). Error bars show ± one standard error of the mean ($n = 3$). Treatments *A–D*, single-strain fermentations of *Saccharomyces cerevisiae* or *S. cerevisiae* / *S. kudriavzevii*, strains A, B, C or D; Treatment *A/B*, single-strain fermentation of hybridised yeast strains A and B; Treatment *AD*, co-inoculation of two strains, A and D, and Treatments *ABC* & *Abc*, co-inoculations of three strains, A, B and C in differing proportions.

co-inoculations contained higher concentrations of 3MH and 3MHA than other yeast inocula.

The volatile thiol results in Fig. 1 support previous findings that wine yeast strains differ in their ability to modulate volatile thiol compounds (Anfang et al., 2009; King et al., 2008; Masneuf et al., 2002; Swiegers et al., 2009). No substantial differences in volatile thiol production by the hybrid strain Treatment *A/B* to its parental strains were observed, contrary to results by Masneuf et al. (2002). Co-inoculations were also found to modify the formation of volatile thiols differently to that of the single strains, as previously reported (Anfang et al., 2009; King et al., 2008).

The volatile thiol levels measured in this study are lower than those reported elsewhere (Anfang et al., 2009; Masneuf et al., 2002). These differences are likely due to the level of thiol precursors in the juice used and may reflect a general trend of lower volatile thiols levels in Australian Sauvignon Blanc wines, as determined in the fairly small number of Australian wines studied in a recent investigation, compared to those in New Zealand and French Sauvignon Blanc wines (Lund et al., 2009).

### 3.3. Sensory profiles

From the results of the sensory descriptive analysis, 12 of the 23 attributes were significantly different among the yeast inocula ($p < 0.07$): *overall colour intensity*, *estery*, *floral*, *fresh citrus*, *passionfruit*, *box hedge*, *fresh green*, *cooked citrus*, *cooked vegetal*, *bruised apple*, *floral flavour*, *overall green flavour* and *viscosity*. The average ratings of these significantly different attributes for the eight yeast inocula are shown in Table 4.

From an inspection of Table 4, it can be seen that Treatment *A* gave wines rated highest in *cooked citrus*, *cooked vegetal*, *bruised apple* and lowest in *estery*, *floral*, *fresh citrus, passionfruit*, *box hedge* and *fresh green*. Conversely, Treatment *Abc* had the highest ratings for *floral*, *fresh citrus*, *passionfruit*, *fresh green* and *overall green flavour*. Treatment *ABC* was also relatively high in these five attributes and both three-yeast co-inoculation treatments were also rated highly for *estery* and low in *overall colour intensity*, *cooked citrus*, *cooked vegetal* and *bruised apple*. Treatment *D* was intermediate in most attributes but rated highly in *fresh citrus*. The other yeast inocula were intermediate in ratings of all attributes, with Treatments *C*, *A/B* and *AD* being rated slightly higher in *box hedge*.

There were found to be significant differences among fermentation replicates for *overall colour intensity*, *passionfruit* and *box hedge*. For *overall colour intensity*, Treatment *C* replicate 3 was rated lower than the other replicates and Treatment *A/B* replicate 3 was higher than the other fermentation replicates. It was noteworthy that for *passionfruit*, there was a large difference among Treatment *C* replicates, with a mean value of 2.6 for replicate 3 compared to mean values of 1.5 and 1.6 for replicates 1 and 2, respectively. Furthermore Treatment *AD* replicate 1 was also somewhat higher in *passionfruit* rating compared to the other replicates. Three-yeast inocula had differences in their fermentation replicates for the ratings of *box hedge* aroma. These were Treatment *B* replicate 1, Treatment *C* replicate 2 and Treatment *A/B* replicate 3, all of which were rated significantly lower than the other two replicates.

The wines made using single-strain *Saccharomyces* yeasts differ in their sensory profiles (Table 4), consistent with previous findings by King et al. (2008), Molina et al. (2009) and Swiegers et al. (2009). The hybrid yeast, Treatment *A/B* had an intermediate sensory profile to its parental strains Treatments *A* and *B*. This is an important result, as, to our knowledge, no studies have reported the effect of hybrid yeast on sensory properties compared to their parental strains.

The sensory profiles of the co-inoculated wines in Table 4 were different to that of the single-strain components, verifying that *Saccharomyces* yeast co-inoculations result in modified sensory profiles (King et al., 2008; Nikolaou et al., 2006) and that the single-strain yeasts within the co-inoculated fermentations have contributed significantly to the aroma profiles.

Treatment *A* was shown to be relatively high in the undesirable aroma attribute *bruised apple*, as well as *cooked citrus* and *cooked vegetal* (Fig. 2), and also had slower fermentation rates than other yeast inocula (data not shown). These aroma attributes can be observed in winemaking when ferments are delayed or incomplete, particularly relating to longer exposure of oxygen. Considering this result, analyses were conducted excluding Treatment *A*. All sensory attributes were significant ($p < 0.1$) except *fresh green*, *box hedge*, *cooked citrus* and *bruised apple*. This result indicates that there were substantial differences among strains for the fresh fruit aromas, providing strong evidence that the differences in sensory properties among the wines were due to strain effects instead of oxidative influences.

### 3.4. Relationships of chemical and sensory data

In order to assess the relationships between the chemical composition data and the sensory data, a partial least squares regression (PLSR) was conducted. Two components were considered optimal and a relatively high 73% of the sensory variance was explained by the model. The PLSR biplot (Fig. 2) shows the volatile chemical compounds and how they relate to the significant aroma attributes from the sensory descriptive analysis. The fermentation-derived compounds included in the PLSR were those with odour activity values (OAV) above one, and several compounds below reported aroma detection thresholds that improved the predictive

**Table 4**
Mean data ($n$ = 11 assessors × 3 fermentation replicates × 2 presentation replicates) and least significant difference (LSD) values for sensory attributes that differed significantly ($p$ < 0.07) across the eight yeast inocula.

| Treatment | Overall colour intensity | Estery | Floral | Fresh citrus | Passion-fruit | Box hedge | Fresh green | Cooked citrus | Cooked vegetal | Bruised apple | Floral flavour | Overall green flavour | Viscosity |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *A* | 3.57 | 3.01 | 2.24 | 1.74 | 1.59 | 0.44 | 1.07 | 1.94 | 0.98 | 0.99 | 1.14 | 2.29 | 2.21 |
| *B* | 3.38 | 3.69 | 2.58 | 2.23 | 1.59 | 0.72 | 1.50 | 1.51 | 0.55 | 0.50 | 0.95 | 2.26 | 1.95 |
| *C* | 3.38 | 3.42 | 2.64 | 1.98 | 1.88 | 0.77 | 1.44 | 1.32 | 0.55 | 0.63 | 0.81 | 2.31 | 1.96 |
| *D* | 3.18 | 3.43 | 2.44 | 2.36 | 2.03 | 0.55 | 1.33 | 1.29 | 0.59 | 0.65 | 1.14 | 2.28 | 2.17 |
| *A/B* | 3.39 | 3.30 | 2.56 | 1.97 | 1.80 | 0.75 | 1.54 | 1.60 | 0.80 | 0.58 | 1.10 | 2.47 | 2.22 |
| *AD* | 3.24 | 3.02 | 2.42 | 1.88 | 1.87 | 0.81 | 1.64 | 1.53 | 0.69 | 0.65 | 1.00 | 2.33 | 2.13 |
| *ABC* | 3.13 | 3.96 | 2.63 | 2.20 | 1.91 | 0.67 | 1.61 | 1.14 | 0.36 | 0.60 | 0.99 | 2.43 | 2.05 |
| *Abc* | 3.10 | 3.76 | 2.99 | 2.36 | 2.13 | 0.56 | 1.81 | 1.15 | 0.54 | 0.57 | 1.03 | 2.66 | 1.97 |
| LSD | 0.18 | 0.35 | 0.36 | 0.40 | 0.32 | 0.26 | 0.38 | 0.43 | 0.29 | 0.28 | 0.18 | 0.26 | 0.22 |

Treatments *A–D*. Single-strain fermentations of *Saccharomyces cerevisiae* or *S. cerevisiae/S. kudriavzevii*, strains A, B, C or D.
Treatment *A/B*. Single-strain fermentation of hybridised yeast strains A and B.
Treatment *AD*. Co-inoculation of two strains, A and D.
Treatments *ABC* & *Abc*. Co-inoculations of three strains, A, B and C in differing proportions.

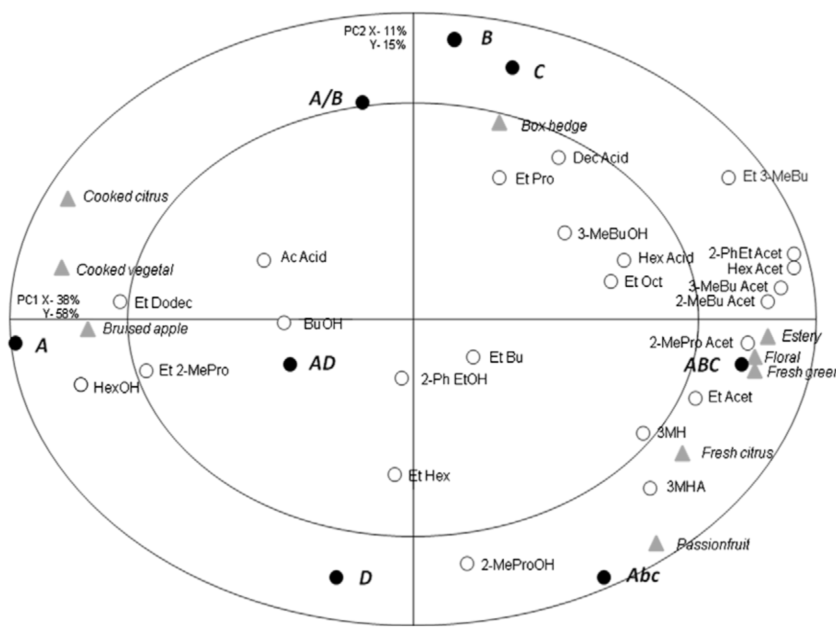


**Fig. 2.** Partial least squares regression (PLSR) of volatile compounds (○), significant sensory aroma attributes ($p$ < 0.07) (▲), and eight yeast inocula (●). Standardised mean values of three fermentation replicates (and two presentation replicates × 11 assessors for aroma attributes). *X*-variables are volatile compounds and *Y*-variables are aroma attributes. Percentage variance explained *X*, *Y*. Acet Acid, acetic acid; BuOH, butanol; Dec Acid; decanoic acid; Et Acet, ethyl acetate; Et Bu, ethyl butanoate; Et Dodec, ethyl dodecanoate; Et Hex, ethyl hexanoate; Et 3-MeBu, ethyl-3-methylbutanoate; Et 2-MePro, ethyl-2-methylpropanoate; Et Oct, ethyl octanoate; Et Pro, ethyl propanoate; Hex Acet, hexyl acetate; Hex Acid, hexanoic acid; HexOH, hexanol; 3MH, 3-mercaptohexan-1-ol; 3MHA, 3-mercaptohexyl acetate; 3-MeBuOH, 3-methylbutanol; 2-MeBu Acet, 2-methylbutyl acetate; 3-MeBu Acet, 3-methylbutyl acetate; 2-MePro Acet, 2-methylpropyl acetate; 2-MeProOH, 2-methylpropanol; 2-Ph EtOH, 2-phenylethanol; 2-PhEt Acet, 2-phenylethyl acetate. Treatments *A–D*. Single-strain fermentations of *Saccharomyces cerevisiae* or *S. cerevisiae/S. kudriavzevii*, strains A, B, C or D. Treatment *A/B*. Single-strain fermentation of hybridised yeast strains A and B. Treatment *AD*. Co-inoculation of two strains, A and D. Treatments *ABC* & *Abc*. Co-inoculations of three strains, A, B and C in differing proportions.

ability of the model. The yeast inocula are also shown in Fig. 2. The position of the sensory aroma attributes and volatile compounds in Fig. 2 indicates the degree of association of sensory rating with the concentration of chemical compounds. Those sensory attributes plotted closely to chemical compounds are likely to be related.

Samples on the right hand side of the biplot (Fig. 2), particularly Treatments *ABC* and *Abc* were rated highly in *estery*, *floral*, *fresh citrus, passionfruit* and *fresh green*, and also had high concentrations of many ester compounds, notably ethyl acetate, 2-methylpropyl acetate, 2- and 3-methylbutyl acetate, hexyl acetate and 2-phenylethyl acetate, and the volatile thiol compounds 3MH and 3MHA.

The sample on the left hand side of the biplot (Fig. 2), Treatment *A* was rated highest in *cooked citrus*, *cooked vegetal* and *bruised apple* and also had higher concentrations of ethyl dodecanoate, hexanol and acetic acid, and lower levels of acetate esters and volatile thiols. Treatments *B*, *C* and *A/B*, clustering in the upper quadrants of Fig. 2, were rated relatively highly in *box hedge*, and were intermediate in *passionfruit* and in concentrations of the volatile thiols. Treatment *A/B* was highest in acetic acid concentration. Treatments *D* and *AD* were intermediate in ratings of most sensory attributes and in concentrations of volatile compounds, although Treatment *D* was relatively high in *passionfruit*, *fresh citrus*, ethyl hexanoate and 2-methylpropanoic acid.

There were clear yeast strain effects on the concentrations of fermentation-derived compounds, acetate and ethyl esters, higher alcohols and volatile acids shown in the PLSR biplot. The data from the single-strain yeast inocula confirm that *Saccharomyces* yeasts differ in their production of fermentation-derived volatile compounds during fermentation (Fig. 2). These results are consistent with previous studies (King et al., 2008; Molina et al., 2009; Nikolaou et al., 2006; Swiegers et al., 2009). The volatile chemical composition of the hybrid yeast, Treatment *A*/*B* was relatively intermediate to its two parental strains, which has been reported for hybridised progeny from wine yeast backgrounds (Ranieri et al., 1998). It may be interesting to compare the wine aroma profile of the hybrid yeast strain to a blend of the parental single-strain wines after fermentation, which has been shown to result in an aroma profile intermediate to the single-strain components (Howell et al., 2006; King et al., 2008).

All co-inoculation treatments differed in their volatile chemical profiles from the single-strain wines (Fig. 2). Similar to other studies, the chemical profiles of the co-inoculated wines demonstrate that individual strains within the mixed cultures have contributed (Howell et al., 2006; King et al., 2008).

The two, three-yeast co-inoculations, Treatments *ABC* and *Abc* contain the same three single yeast strains in varying proportions. Treatment *ABC* had higher concentrations of 3MH (Fig. 1a) and esters (Fig. 2), and was rated higher in *estery*, and lower in *passionfruit* and *cooked vegetal* (Table 4) than Treatment *Abc*. These data indicate that proportions of the single-strain components in co-inoculations are important to final wine flavour.

The PLSR indicates that a number of sensory attributes were related to the chemical compounds measured. Assessment of the regression coefficients of the volatiles that were most important to particular sensory attributes, showed that the *estery* and *floral* attributes, which were well predicted by the chemical model, were each most strongly positively associated with the acetate esters measured, ethyl acetate, 2-methylpropyl acetate, 2- and 3-methylbutyl acetate, hexyl acetate and phenylethyl acetate, as well as 3MHA. Each of these compounds were significantly important to the model for *estery* as assessed by the jack-knife uncertainty test (Martens & Martens, 2000). Of the acetate esters measured, only 3-methylbutyl acetate, phenylethyl acetate and 3MHA (Fig. 1) were above their aroma detection thresholds.

The important components to the model that contributed negatively were ethyl dodecanoate, hexanol, 2-methyl propanoic acid and acetic acid, most likely masking the influence of the positive components. The *fresh green* attribute was strongly associated with those chemical compounds predictive of the attributes *estery* and *floral*. It is likely that methoxypyrazine compounds, considered primarily responsible for the green capsicum/bell pepper sensory properties in Sauvignon Blanc wines, would have correlated positively with the *fresh green* attribute rated in this study, however, these compounds were not measured.

The volatile thiol compounds 3MH and 3MHA were important to the *passionfruit* attribute according to the PLS model, together with ethyl hexanoate, ethyl acetate, 2-methylpropyl acetate and 2-methylpropanoic acid. The *passionfruit* model also included ethyl propanoate and acetic acid which contributed negatively. There was a negative association of the volatile thiol compounds with the attributes *cooked citrus*, *cooked vegetal* and *bruised apple*, which were positively associated with hexanol and acetic acid.

*Box hedge* was not modelled well, but was most strongly associated with the absence of 2-methyl propanoic acid. The volatile thiol 4MMP is known to have aromas reminiscent of box hedge, and is likely to have correlated strongly with the *box hedge* attribute and strengthened its loadings in the model, however, 4MMP was not measured in the study.

### *3.5. Consumer acceptance testing*

Yeast inocula were shown in this study to differ in their production of volatile thiols (Fig. 1) and fermentation-derived compounds (Fig. 2), and in sensory properties (Fig. 2), however, the question remained as to whether these sensory changes brought about by yeast were sufficient to affect wine quality, as perceived by consumers. Four of the eight yeast inocula, Treatments *A*, *D*, *AD* and *ABC* were selected for consumer acceptance testing, based on their differences in ratings for the sensory attributes in the descriptive sensory analysis.

The results of an ANOVA of the consumer testing data indicated that, for the 120 consumers tested, there was a marginal difference in overall liking among the four yeast inocula ($p = 0.06$). Overall, Treatment *AD* was the most liked wine, but only slightly greater than Treatments *A* and *D* which were relatively equally liked, while Treatment *ABC* was the least liked (Fig. 3).

Using hierarchical cluster analysis, four clusters of consumers were identified based upon their liking responses (Fig. 3). Clusters 1 and 4 significantly differed in their liking of the four yeast inocula ($p < 0.001$). Cluster 1 contained the majority of consumers (38%) with Treatment *AD* having a significantly higher liking score than the other yeast inocula and Treatment *ABC* liked the least. In contrast, consumers in Cluster 4 liked Treatment *ABC* more than Treatment *AD*, which was liked the least. In all clusters, Treatments *A* and *D* were relatively equally liked. In Clusters 2 and 3, the differences in yeast inocula were not enough to significantly alter consumer preference. Due to the small number of consumers in Cluster 3, differences in liking responses among the wines within this cluster cannot be interpreted.

There were some differences in wine usage behaviour across the four clusters. The majority of consumers in Cluster 1 drank wine more often than consumers in Cluster 4 (Table 5). Cluster 1 consumers also reported buying wine in the AUD$15–20 price range more frequently than Cluster 4 consumers (Table 5). There was no difference between Clusters 1 and 4 in purchase frequency for any other price categories. These data suggest that greater exposure to relatively higher priced wine may lead to consumers having different preferences compared to those who drink such wine less frequently, which was also suggested by a previous study (Lattey et al., 2010). There was no significant difference in liking or consumption of Sauvignon Blanc wines between the clusters. On average, consumers drank one to two glasses of Sauvignon Blanc wine per week, and 'moderately liked' Sauvignon Blanc wines.

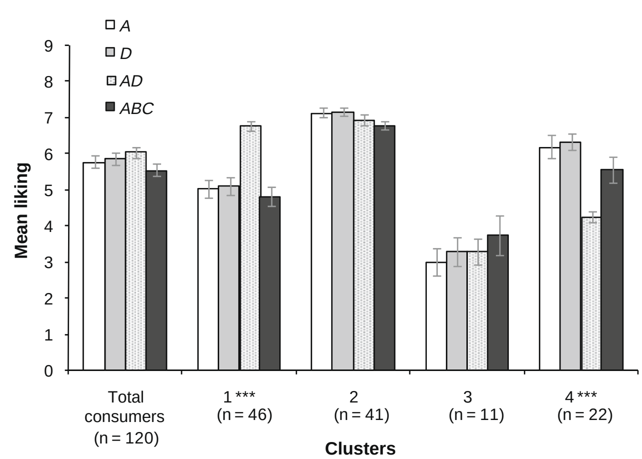


**Fig. 3.** Mean overall liking and mean liking scores of four identified clusters for consumer acceptance testing on a nine-point hedonic scale for four yeast inocula. Errors bars show the standard error of the mean. The number of consumers overall and per cluster shown in parentheses. Significant differences in wine preferences within a cluster are given as $^{***}p < 0.001$. Treatments *A* and *D*. Single-strain fermentations of *S. cerevisiae* or *S. cerevisiae*/*S. kudriavzevii*, strains A and D. Treatment *AD*. Co-inoculation of two strains, A and D. Treatment *ABC*. Co-inoculations of three strains, A, B and C in equal proportions.

**Table 5**
Percentages of consumers in each cluster for self-reported wine consumption and purchase price questions.

| Demographic data | Cluster percentages[a] | | | |
|---|---|---|---|---|
| | 1<br>$n = 46$ | 2<br>$n = 41$ | 3<br>$n = 11$ | 4<br>$n = 22$ |
| *Frequency of overall wine consumption*[*] | | | | |
| Less than once per week | 4.3 | 10.3 | 45.4 | 13.6 |
| 1–2 times per week | 28.3 | 12.8 | 18.2 | 31.8 |
| 3–4 times per week | 26.1 | 43.6 | 9.1 | 18.2 |
| 5 or more times per week | 41.3 | 33.3 | 27.3 | 36.4 |
| *Purchase frequency bottle price AUD$15–20*[**] | | | | |
| Rarely | 0.0 | 11.8 | 36.3 | 12.5 |
| Occasionally | 28.2 | 44.1 | 27.3 | 37.5 |
| Frequently | 71.8 | 44.1 | 36.4 | 50.0 |

[a] Percentage calculated based on column totals.
[*] Significance ($\chi^2$ test, $p < 0.05$).
[**] Significance ($\chi^2$ test, $p < 0.01$).

The results of the consumer acceptance testing indicated that more than half of regular wine-drinking consumers tested differed in their liking for the wines, resulting from the effect of the yeast inocula used to conduct alcoholic fermentation on the sensory properties of the wines. The main sensory differences among the wines were aroma/flavour attributes, rather than taste or mouthfeel, as indicated in Fig. 2, and it is most likely that aroma and flavour differences among the yeast inocula were driving the differences in consumer preference.

The Cluster 1 cluster group had relatively low liking scores for Treatment *A* compared to Treatment *AD*. Treatment A had the strongest *bruised apple* and 'cooked' type flavours, and the lowest 'fresh' fruit aroma (Fig. 2). Treatments *D* and *ABC* were also not highly liked by this cluster and these wines may have been too strong in *estery*, *floral*, *fresh citrus* and *passionfruit* for these consumers. In contrast, Treatment *AD*, most liked by this cluster, was intermediate in ratings in these attributes and had relatively high ratings for *box hedge*. The response of the consumers in Cluster 4 indicate that they prefer stronger flavours, liking both the strong *bruised apple* and 'cooked' flavour of Treatment *A*, and the *estery*, *floral* and *fresh citrus* characters of Treatments *D* and *ABC*. Cluster 4 consumers liked least the wine with medium intensity in most attributes.

It is noteworthy that the wine with highest ratings for *box hedge* was most liked by the majority of consumers. The wine rated highest in 'box hedge' attribute was also most preferred by winemakers in a preference ranking conducted by Swiegers et al. (2009). In this study *box hedge* had a relatively narrow range of sensory scores, indicating that the strain effect was somewhat limited for this attribute. However, it may be that consumers respond relatively strongly to wines with different levels of this attribute, which is often also characterised as cat urine-like. Further study is required to investigate this result.

Interestingly, the majority of consumers did not prefer the wine made using Treatment *ABC*, which had the highest *estery* and *floral* aroma, and highest concentrations of volatile thiols (Fig. 1). This study indicates that targeting yeast inocula that give a balanced sensory profile without an excessively high sensory level of particular flavours may be desirable.

## 4. Conclusion

A few short years ago there was debate as to whether yeast strains used to conduct wine fermentation had an effect on wine composition and aroma (Thorngate, 1999). Many studies have now shown this to be the case. The results of this study have demonstrated that the choice of yeast inoculum, using single or multiple yeasts, affects wine aroma composition and sensory properties, and that there are sufficiently large differences to affect consumer acceptance. The study highlights the importance of including consumer research to evaluate the impact of viticultural and oenological treatments upon wine quality. The results of the work will enable winemakers to modulate wine styles based on the optimal range of concentrations of volatile thiols in wines.

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## Addendum

This addendum contains additional results of a repeat consumer acceptance testing nine months after bottling, for the 2007 Sauvignon Blanc wines made using different yeast strains, reported in Chapter 3 (King et al. 2010).

The aim of the study was to determine whether the yeast strain(s) used to conduct fermentation continued to affect the consumer preference of Sauvignon Blanc wines.

## Materials and Methods

The 2007 Sauvignon Blanc wines made using different yeast strains were bottled in 375 mL clear glass bottles under inert gas, and sealed with roll-on tamper evident screw caps. Prior to bottling, a sulfur dioxide addition, in the form of potassium metabisulfite, was made to all wines to obtain a final free sulfur dioxide concentration of 25-30 mg/L. The wines were stored upright, away from light at approximately 15 °C (range 14-17 °C), until analysis. The consumer acceptance testing was repeated nine months after bottling for the four yeast inocula: single strains *A* and *D*; two-yeast co-inoculation *AD*, and three-yeast co-inoculation *ABC*. See Table 1 of King et al. (2010 – Chapter 3) for details of yeast inocula.

The procedures used for consumer acceptance testing are detailed in King et al. (2010 – Chapter 3). Briefly, 120 different consumers (47 female) were recruited and tested in the same three retail liquor stores across the Adelaide (Australia) metropolitan area as the three month time point. A portable table consisting of three booths was used to provide an isolated, standardised environment. The wines were served chilled at approximately 14 ºC, at a constant volume (30 mL), in ISO tasting glasses with randomly assigned three-digit codes. The wines were presented monadically, in a randomised, balanced order across all consumers. Consumers were asked to rate their liking for each wine on a categorical 9-point hedonic scale from ‘dislike extremely’ to ‘like extremely’. Paper score-sheets were used for data collection. Fruit and chocolate were provided as a token payment for completing the testing session. The Human Research Ethics Committee of The University of Adelaide (Adelaide, Australia) approved the methods of testing and data collection employed in this study.

Statistical software (JMP 5.1, SAS Institute, Cary, NC) was used for analysing all data. Consumer testing data were analysed using an analysis of variance (ANOVA) testing for the effects of yeast treatment and consumer. Hierarchical cluster analysis was performed using Ward’s method on the standardised data and a series of one-way ANOVAs were used for testing the effects of yeast treatment and consumer cluster.

## Results and Discussion

The results of the consumer testing nine months after bottling indicated that there was no significant difference in consumer preference for the wines made from different yeast strains ($p$=0.81) (Figure 1). There were also no differences in liking for the wines by any groups of consumers identified using cluster analysis (data not shown). This is in contrast to the results of the consumer testing at the three month time point for the same wines, where it was found that there was a marginal difference in overall liking among the four yeast inocula ($p$=0.06), and significant differences in liking of the wines for two of the four clusters identified using cluster analysis ($p$<0.001) (King et al. 2010 – Chapter 3). The mean liking scores of the four yeast inocula for the total populations at both time points are shown in Figure 1.

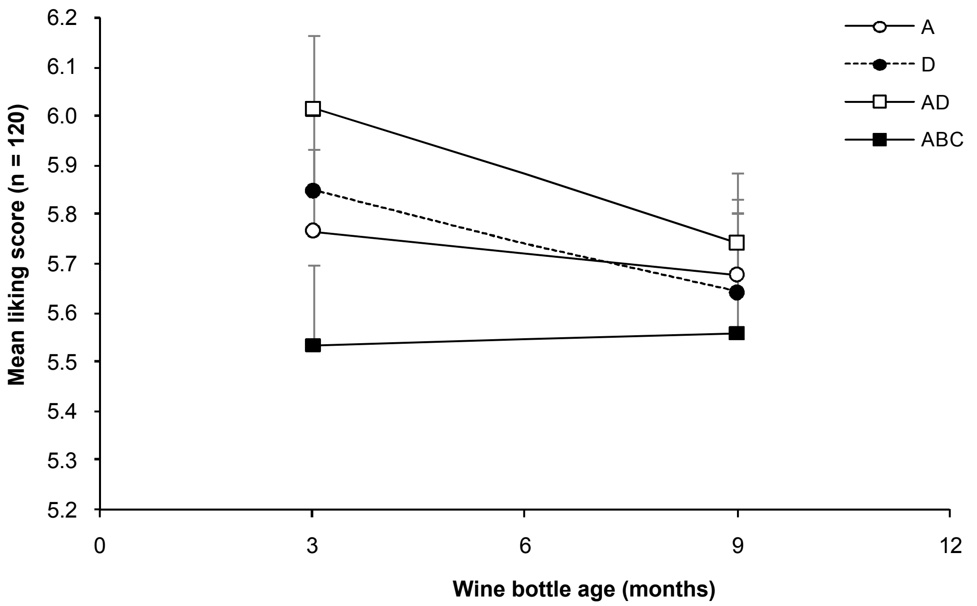


**Figure 1.** Comparison of mean consumer liking scores + standard error of the mean (n = 120) for four yeast inocula tested over two time points, three and nine months after bottling. The three month data is modified from King et al. (2010 – Chapter 3).

The mean liking scores of the yeast treatments decreased at the nine month time point, except Treatment *ABC*, which increased slightly compared to the liking at three months (Figure 1). For the nine month time point, all yeast treatments were rated on the 9-point hedonic scale between 5.5 and 5.8, the worded categories 'Neither like nor dislike' and 'Like slightly'.

After the consumer testing at nine months, seven experienced tasters, some of whom had participated in the previous tasting of the same wines, informally assessed the wines in a blind bench tasting. It was concluded that the wines were partially oxidised, with minimal fruit flavour or volatile thiol-related sensory descriptors, and detectable levels of 'bruised apple' aromas (data not shown).

Standard chemical analyses of the wines conducted nine month after bottling indicated that the wines had relatively low concentrations of free sulfur dioxide (Table 1), at critical levels according to Godden et al. (2001). Despite this, the wines had sufficient concentrations of total sulfur dioxide and low levels of browning colour (Table 1), according to the index of oxidation (420 nm absorbance), less than the commercially accepted maximum level of 0.18 au (Ribéreau-Gayon et al. 2006).

**Table 1.** Standard chemical analysis results of the nine month old 2007 Sauvignon Blanc wines made using different yeast inocula, averaged across triplicate fermentations with standard deviation in parentheses.

| Yeast inoculum | OD[a] 420 nm | Free $SO_2$[b] (mg/L) | Total $SO_2$[b] (mg/L) |
|---|---|---|---|
| *A* | 0.09 (0) | 9 (1) | 161 (5) |
| *D* | 0.07 (0) | 10 (0) | 143 (4) |
| *AD* | 0.08 (0) | 10 (1) | 145 (6) |
| *ABC* | 0.07 (0) | 11 (1) | 120 (2) |

[a] OD, optical density
[b] $SO_2$, sulfur dioxide

The wines in the present study are thought to have oxidised after the short time in bottle due to a combination of insufficient sulfur dioxide additions during the bottling process and the small bottle size, thus allowing faster rates of oxidation reactions. An oversight in the consumer acceptance testing methodology used in this study was the lack of assessment of the wine sensory profiles by the coordinators prior to conducting the consumer testing.

The consumer testing results suggest that consumers were reactive to the degree of oxidation of the nine month old wines made using different yeast inocula. This indicates that consumers are responsive to relatively small differences in wine sensory profiles, in particular wines with flatter sensory properties or enhanced levels of undesirable flavours have reduced consumer liking (Lattey et al. 2010, Prescott et al. 2004).

Of note, there were similar trends of liking between the two time points for the four yeast inocula, with Treatment *AD* remaining the most liked, and Treatment *ABC* the least liked by consumers at the nine month time point (Figure 1). This result suggests that had these wines not regrettably oxidised, there may have been continued differences in consumer liking. Further research is needed to understand whether yeast-derived flavour differences are retained after a longer period of bottle age, and whether those differences are sufficiently large to have a continued effect on consumer liking over time.

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## Chapter 4.

# Yeast strain-derived sensory differences are retained in Sauvignon Blanc wines after extended bottle storage

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## Preface

The results of our research, reported in Chapters 2 and 3, demonstrated that the yeast inocula used to conduct wine fermentation had an effect on the chemical composition and sensory profiles of young wines (King et al. 2008, King et al. 2010), and that sensory differences were sufficient to affect consumer preference (King et al. 2010). However, it is not known how long these differences may last beyond six months post-bottling.

A study was undertaken to investigate whether the yeast-derived flavour differences in young Sauvignon Blanc wines were retained after an extended period of bottle age. The 2006 Sauvignon Blanc wines made using different yeast strains and post-fermentation treatments, reported in Chapter 2 (King et al. 2008) were stored for three years, after which time chemical and sensory analyses were repeated. From a separate fermentation study conducted the previous year (2005), Sauvignon Blanc wines made using different strains with reported flavour differences after six months post-bottling (Swiegers et al. 2009) were also stored under the same conditions and the analyses repeated three years after bottling. These wines were included in the study to strengthen the overall conclusions, due to the limited number of replicates analysed for each treatment.

Brajkovich et al. (2005) had previously indicated that volatile thiols affect the sensory profiles of two year old Sauvignon Blanc wines. In an attempt to extend our understanding of the effect of volatile thiols and yeast strains, the wines in the present study were aged for a longer period of time.

Additional chemical data to the manuscript are presented as supplementary material at the end of the chapter.

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# Chapter 5.

## Assessing desirable levels of sensory properties in Sauvignon Blanc – consumer preferences and contribution of key aroma compounds

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## Preface

The results of consumer acceptance testing of Sauvignon Blanc wines made using different yeast strains in Chapter 3, demonstrated that the yeast strain used to conduct fermentation has an effect on consumer preference (King et al. 2010). However, it was unclear from the results of our research which sensory attributes were driving consumer preference for the wines. Also, the method of consumer testing used, with purposive and self-selected recruitment, and uncontrolled testing environments in Adelaide retail liquor stores, may have resulted in larger than normal variation. The generalisability of the results may thus, have been constrained.

A study was undertaken to further investigate the sensory attributes driving consumer preference in Sauvignon Blanc wines, and the volatile compounds and their levels responsible for these sensory attributes. To do this, volatile compounds were added to a neutral base wine to reflect the combinations and wide range of concentrations found in commercial Sauvignon Blanc wines. For a full list of the reported volatile data and references used to calculate the concentrations of the flavour additions, see Figure 3 in Chapter 1. A sensory descriptive analysis was conducted to characterise the differentiating sensory attributes, and a subset of the most diverse samples underwent acceptance testing by 150 consumers. Testing occurred in a central location sensory laboratory and recruitment was based on selection and availability from a database of regular wine-drinking consumers.

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**Assessing desirable levels of sensory properties in Sauvignon Blanc wines – consumer preferences and contribution of key aroma compounds**

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# Chapter 6.

# Characterisation of white wine consumers in South Australia – their wine usage and attitudes

## Preface

The consumer information collected from the 150 participants during acceptance testing, reported in Chapter 5 was analysed without including the preferences for the wine samples, similar to a marketing research study. The aim of this study was to better understand wine consumers in South Australian with different preferences for white wines. To do this, consumers were segmented using their self-reported liking of four white wine types, and levels of wine involvement and wine knowledge, which were then related to their demographics, and wine usage and attitudes, so as to gain insights into consumer behaviour.

## Introduction

Wine, for many consumers, is wrapped in an air of mystique. It is a multifaceted drink, not only providing enjoyment, but to some consumers also a story of its origin – its life from grapes to bottle.

In Australia, the domestic sales of white wine continue to surpass that of red wine (ABS 2010). However, domestic and international sales of Australian wine fell considerably in the 2007/08 period (AWBC and WFA 2007b). This is in part due to an increase in the volume and consumption of imported white wines in Australia. In particular, there has been phenomenal growth of New Zealand imports in the Australian white wine category, mainly due to Sauvignon Blanc wines (AWBC 2008). New Zealand Sauvignon Blanc wines now represent 70% of the total sales of bottled Sauvignon Blanc wine in Australia (Chappell 2009) and dominate at higher price points (AWBC and WFA 2007c).

Few studies to date have investigated the reasons behind consumer choices of white wines and the implications for the wine industry. Studies segmenting Australian wine consumers have thus far, focussed on psychographics (Bruwer and Li 2007, Johnson and Bruwer 2003), geographics (Lockshin et al. 2001), demographics and behaviours (Lockshin et al. 1997, 2001, 2006, Johnson and Bastian 2007). There is a need for Australian wine producers to better understand white wine drinkers and the reasons behind their purchasing decisions.

The aim of this study was to relate consumer demographics, and wine usage and attitudes with the factors that drive wine liking and purchase choices, so as to gain insights into consumer behaviour. To do this, consumers were segmented using their self-reported liking of four white wine types, and levels of wine involvement and wine knowledge.

## Methodology

### *Subjects and testing*

A cohort of 150 consumers from the Adelaide metropolitan district (South Australia, Australia) were recruited using online and paper newspaper advertisements, and screened using an online questionnaire and phone contact. Recruitment criteria was as follows: age over 18 years; regular white wine consumers (defined as drinking white wine at least once per week); regular white wine purchasers at AUD$10-20; equal numbers of males and females; equal distribution of age ranges, and no tertiary wine education.

Central location testing was conducted within two weeks of recruitment, in individual sensory booths. Participants were asked to evaluate blind their liking for seven white wines with and without flavour additions of important aroma compounds at realistic levels to mimic commercial Sauvignon Blanc wines. The methodology and results of the sensory tests are detailed in King et al. (*in press* – Chapter 5). A moderately-priced gift voucher was provided as a token payment for completing the testing session, as well as fruit and chocolate. The Human Research Ethics Committee approved the methods of testing and data collection employed in this study.

### *Demographic information, and wine usage and attitude questionnaire*

Questionnaires were used to collect data on participants' personal details, and usage and attitudes towards wine, including length of time drinking wine, average purchase price of wine, liking of four white wine types on a categorical 9-point hedonic scale anchored by 'dislike extremely' to 'like extremely', and consumption frequency of different white wine types using a 6-point category scale from 'never tried' to 'five or more glasses per week'.

Participants' levels of wine involvement and wine knowledge were measured using reliable and validated scales (Lockshin et al. 1997, 2001), on a categorical 7-point scale anchored by 'strongly disagree' to 'strongly agree', and included positively and negatively worded statements. Three segments of both wine involvement and wine knowledge were determined, where low was the lowest quartile, medium was the middle two quartiles and high was the highest quartile (Quester and Smart 1998). Involvement is the interest and enthusiasm that consumers have towards a product or a product category (Goldsmith and Emmert 1991). Subjective or self-perceived knowledge is an individual's perception of how much he/she knows about a product category (Raju et al. 1995).

The importance of 16 white wine purchase drivers was determined on an unstructured continuous line scale from 'not at all important' to 'very important' (0 to 10). Eighteen questions measured participants' attitudes towards wine were also asked, using positively and negatively worded statements and binary disagree-agree tick boxes (disagree=0, agree=1), based on Tragon Corporation's PROP™ methodology (Palo Alto, CA). FIZZ software (Version 2.1, Biosystemes, France) was used for the collection of data.

*Data analysis*

The data were analysed using a combination of descriptive statistics, one way ANOVA with Tukey post-hoc test, correspondence analysis, non-parametric analysis with Friedman's K-independent samples and cluster analysis using SPSS software (Version 13.0, SPSS Inc. IBM, IL, USA), JMP(Version 5.1, SAS Institute, Cary, NC, USA) and XLStat (Version 2006.06 Addinsoft, NY, USA).

**Results**

The wine usage and attitudes of 150 white wine consumers were investigated using consumers' wine involvement and wine knowledge, and liking for white wine types.

*Demographic and white wine behavioural information*

There was an even spread for gender and age of the 150 respondents tested (Table 1), reflecting the selection criteria. A larger percentage of respondents tested had achieved a high level of education and had high household incomes. Seventy-five percent of participants were born in Australia (Table 1), with the majority of other participants born in the United Kingdom (11%).

Over seventy percent of respondents reported drinking wine of any type for over ten years. As expected, length of time drinking wine was positively associated with age. Slightly more females than males reported drinking wine for between six and 20 years, and more males reported drinking wine for over 20 years.

**Table 1.** Demographic information of the 150 respondents participating in the study.

| Demographic information | Percentage |
|---|---|
| Gender | |
| Male | 51 |
| Female | 49 |
| Age | |
| 18-25 years | 13 |
| 26-35 years | 22 |
| 36-45 years | 18 |
| 46-55 years | 23 |
| 56 years plus | 24 |
| Highest level of education achieved | |
| High school certificate | 17 |
| Technical certificate/ apprenticeship/ diploma | 24 |
| Bachelors degree | 42 |
| Masters/ doctorate degree | 17 |
| Household income (pre-tax AUD) | |
| Less than $20,000 | 6 |
| $20,000-$39,999 | 8 |
| $40,000-$59,999 | 11 |
| $60,000-$79,999 | 17 |
| $80,000-$99,999 | 14 |
| $100-$149,999 | 25 |
| $150,000 plus | 18 |
| Country of birth | |
| Australia | 75 |
| Other | 25 |

Percentages based on column totals

Consumers were asked to rate their consumption and liking for four white wine types: Riesling, Australian Sauvignon Blanc, New Zealand Sauvignon Blanc and Chardonnay. Overall, New Zealand and Australian Sauvignon Blanc wines were the most liked white wines, with Riesling wines slightly lower and Chardonnay wine liked substantially less (Table 2).

There were significant strong positive correlations between consumption and liking for each of the white wine types ($r>0.60$, $p<0.001$). Sixty percent of respondents drank less than two glasses of Riesling wine per month, while 75% of respondents reported the same consumption frequency for Chardonnay wines. Over 50% of respondents reported drinking one or more glasses of Sauvignon Blanc wine from Australian and New Zealand per week.

Liking and consumption of Australian Sauvignon Blanc wine was moderately associated with liking and consumption of Sauvignon Blanc wine from New Zealand.

**Table 2.** Mean liking scores of four white wine types for 150 participants.

| White wine type | Mean liking score |
|---|---|
| New Zealand Sauvignon Blanc wine | $6.83^{a}$ |
| Australian Sauvignon Blanc wine | $6.77^{ab}$ |
| Riesling wine | $6.49^{b}$ |
| Chardonnay wine | $4.85^{c}$ |

Mean values with different superscripts within the column are significantly different based on Tukey HSD test ($p<0.05$).

Respondents typically reported spending, on average, AUD$10-25 on a bottle of wine of any type, with 10% of consumers purchasing wine over AUD$40. For an everyday bottle of white wine, consumers spent, on average, between AUD$9-16, whereas consumers spent more money on white wine purchased for a special occasion or a gift for someone (more than AUD$20) (Figure 1). Consumers indicated that they spent on average between AUD$17-24 for a bottle of white wine to be consumed at a weekend dinner or to expect a good bottle of white wine (Figure 1).

*Purchase drivers*

Consumers were asked to rate the importance of sixteen white wine purchase drivers on a line scale from ‘not at all important’ to ‘very important’, where the maximum value was 10. The mean scores for the purchase drivers are listed in Table 3. The grape variety, region/ country, brand name and recommendation by friends were considered the most important drivers for respondents when purchasing white wine, with technical comments by winemakers, alcohol level and traditional cork closure listed as the least important.

*Consumer attitudes*

The mean values of each wine attitude statement are shown in Table 4, where higher mean values indicate more overall agreement with the statement. There was high agreement among respondents with ‘I always pay great attention to the flavour of the wine I am drinking’, ‘I don’t mind purchasing wine that has a screw cap closure’, ‘wine makes a great gift’ and ‘you can buy good wines without spending a lot of money’. Respondents collectively disagreed that ‘wine is too expensive to enjoy very often’, ‘the best wines are the most expensive’, ‘I only drink when entertaining guests’ and ‘I find restaurant wine lists confusing and difficult to navigate’.

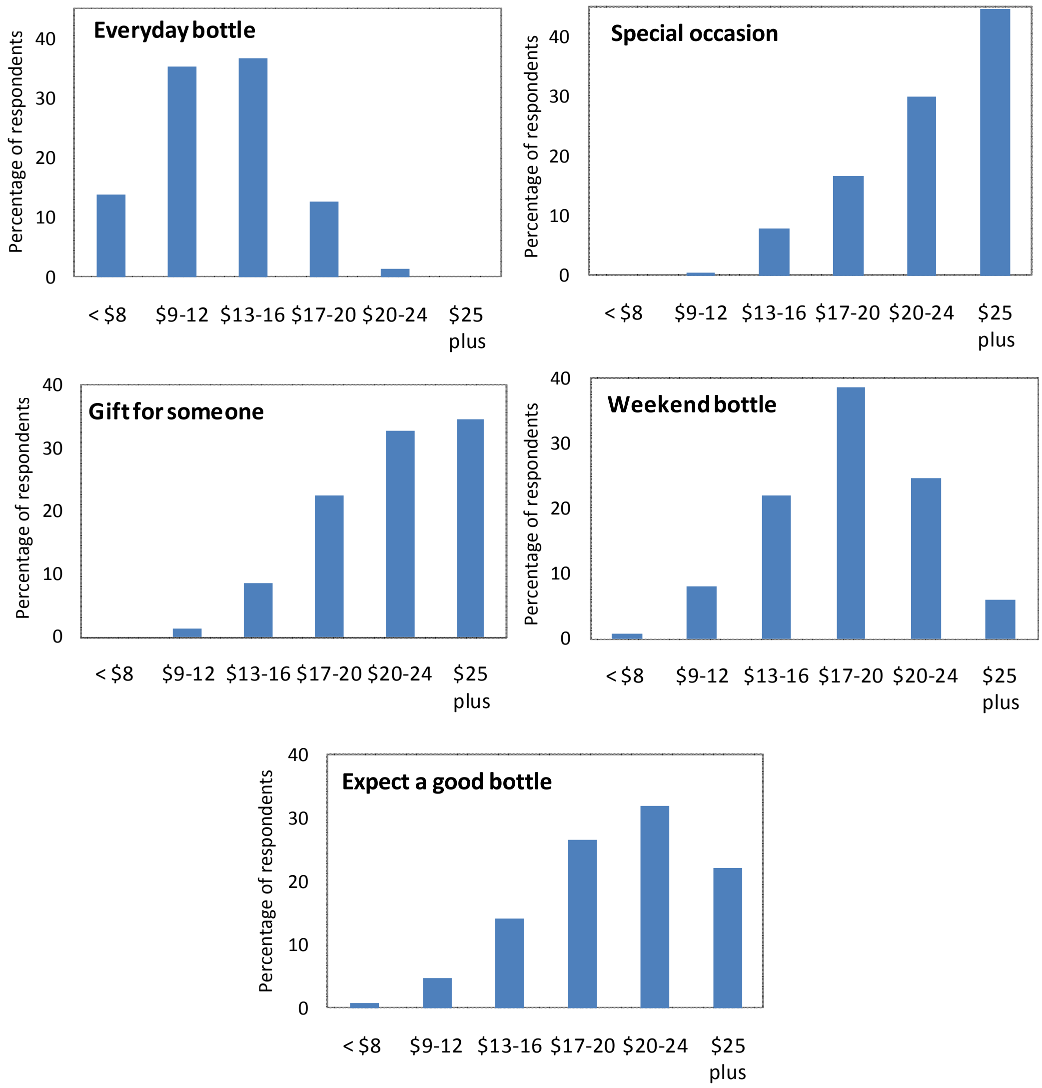


**Figure 1.** Typical purchase price (AUD$) of a bottle of white wine for different occasions by percentage of respondents.

**Table 3.** Mean scores for the purchase drivers of wine for respondents (n=150).

| Purchase driver | Mean score |
|---|---|
| Grape variety | 7.3[a] |
| Region/ country that the wine is from | 7.1[ab] |
| Brand name (producer, name of product) | 6.9[ab] |
| Recommendation by friends | 6.7[ab] |
| Trying something new and exciting | 6.4[b] |
| Good rating/ review by wine critic/ medals won | 6.3[b] |
| Vintage/ year grapes were harvested (age of wine) | 6.2[b] |
| Finding a wine on sale or special promotion discount | 6.2[b] |
| Recommendation by retailer or wine waiter | 5.1[c] |
| Knowing what to buy before shopping | 5.0[c] |
| Wine descriptions on the store shelf | 4.6[cd] |
| A tasting description on the back label | 4.4[cd] |
| Appearance of the label on the bottle | 4.3[cd] |
| Technical comments from the winemaker on the back label | 3.7[de] |
| Alcohol level | 3.3[e] |
| Purchasing a wine with a traditional cork closure | 1.8[f] |

Purchase driver mean scores with different superscripts indicate significant differences based on Tukey HSD test ($p<0.05$).

**Table 4.** Mean values of each attitude statement for respondents (n=150); higher mean values indicate more agreement with the statement.

| Attitude statements | Mean values |
|---|---|
| You can buy good wines without spending a lot of money | 0.97[a] |
| I don't mind purchasing wine that has a screw cap closure | 0.94[a] |
| Personally, I think that wine makes a great gift | 0.93[a] |
| I always pay great attention to the flavour of the wine I am drinking | 0.87[a] |
| I consider wine an everyday beverage | 0.69[b] |
| For me an evening meal is never complete without a glass of wine | 0.56[bc] |
| Sometimes I am overwhelmed by the number of brands there are when selecting a wine | 0.55[bc] |
| I like to drink wine to get a buzz. It makes me feel good | 0.47[cd] |
| I think of wine mostly for drinking with dinner | 0.37[de] |
| I prefer to drink wines recommended by friends | 0.30[ef] |
| I never know how to pair wines with food | 0.27[efg] |
| Drinking wine makes me feel sophisticated | 0.26[efg] |
| I don't like to open a bottle when I am by myself | 0.26[efg] |
| I don't know enough about wine to make it worth spending money for the better products | 0.20[fgh] |
| I find restaurant wine lists confusing and difficult to navigate | 0.12[ghi] |
| I typically only drink wine while entertaining guests | 0.09[hi] |
| Personally, I think the best wines are always the most expensive | 0.07[hi] |
| Wine is too expensive to enjoy very often | 0.02[i] |

Mean values of agreement statements sharing a letter within the column are not significantly different based on Tukey HSD test ($p<0.05$).

Wine involvement scores had a mean of 81% ±14 (±SD), with the lowest and highest quartile values of 71% and 93%, respectively, indicating that all consumers had a relatively high level of wine involvement. Subjective wine knowledge had a mean of 54% ±22, with 43% for the lowest quartile and 71% for the highest quartile.

The Cronbach Alpha co-efficients of each scale were 0.84 for wine involvement and 0.89 for wine knowledge, both of which were above the generally accepted score of 0.70 (Pallant 2001), indicating that the scales were reliable. Wine involvement and wine knowledge were strongly positively correlated ($r=0.67$, $p<0.05$), and thus, wine involvement will be discussed below, with reference made to wine knowledge.

**Table 5.** Wine involvement levels by percentage of respondents for gender, age, highest level of education achieved and length of time drinking wine ($p<0.05$), percentage of respondents in each segment shown in parentheses.

| | Low Involvement (20%) | Medium Involvement (61%) | High Involvement (19%) |
|---|---|---|---|
| Gender | | | |
| Male | 35 | 48 | 79 |
| Female | 65 | 52 | 21 |
| Age | | | |
| 18-26 years | 10 | 16 | 4 |
| 26-35 years | 17 | 25 | 17 |
| 36-45 years | 24 | 16 | 18 |
| 46-55 years | 28 | 23 | 18 |
| 56 years plus | 21 | 20 | 43 |
| Highest level of education achieved | | | |
| High school certificate | 28 | 15 | 11 |
| Technical certificate/ apprenticeship/ diploma | 21 | 23 | 29 |
| Bachelors degree | 41 | 40 | 47 |
| Masters/ doctorate degree | 10 | 22 | 13 |
| Length of time drinking wine | | | |
| < 2 years | 7 | 2 | 0 |
| 2-5 years | 3 | 8 | 4 |
| 6-10 years | 14 | 21 | 11 |
| 10-20 years | 35 | 23 | 14 |
| 20 years plus | 41 | 46 | 71 |

Percentages based on column totals

Demographic information for the wine involvement levels is shown in Table 5. The medium involvement segment contained equal numbers of both males and females, whereas the low involvement segment had more females than males, and the opposite was true for high involvement. Low and medium involvement segments contained similar proportions of respondents in each age group, whereas the majority of respondents in the high involvement segment were older than 46 years. The low involvement segment contained a higher proportion of respondents with high school certificates and apprenticeships/trade school qualifications, whereas, the medium and high involvement segments contained more respondents with University degrees. The majority of high involvement respondents had been drinking wine for a longer period (20 years plus) compared to other segments.

Information on wine usage for respondents with different levels of wine involvement is shown in Table 6. Respondents with high involvement liked Riesling and drank more Riesling wines than respondents with low and medium involvement. Similarly, high involvement respondents drank more Chardonnay wines and liked Chardonnay more than other involvement segments. Low involvement respondents liked and consumed more Sauvignon Banc wines than the other involvement segments, with no significant differences between the liking scores for Australia and New Zealand.

**Table 6.** Mean liking scores and percentage of wine consumption for three wine involvement segments, percentage of respondents in each segment shown in parentheses.

| | Low Involvement (20%) | Medium Involvement (61%) | High Involvement (19%) |
|---|---|---|---|
| Like Riesling wine | $5.8^{b}$ | $6.4^{b}$ | $7.7^{a}$ |
| Like Australian Sauvignon Blanc wine | $7.2^{a}$ | $6.6^{a}$ | $6.8^{a}$ |
| Like New Zealand Sauvignon Blanc wine | $7.3^{a}$ | $6.6^{a}$ | $6.9^{a}$ |
| Like Chardonnay wine | $4.6^{b}$ | $4.7^{b}$ | $5.6^{a}$ |
| Consumption of Riesling wine | | | |
| Don't drink | 24 | 13 | 12 |
| 1-2 glasses/ month | 57 | 45 | 28 |
| 1-2 glasses/ week | 14 | 28 | 40 |
| 3 or more glasses/ week | 5 | 14 | 20 |
| Consumption of New Zealand Sauvignon Blanc wine | | | |
| Don't drink | 11 | 14 | 4 |
| 1-2 glasses/ month | 20 | 46 | 44 |
| 1-2 glasses/ week | 40 | 24 | 40 |
| 3 or more glasses/ week | 29 | 16 | 12 |
| Consumption of Australian Sauvignon Blanc wine | | | |
| Don't drink | 10 | 5 | 7 |
| 1-2 glasses/ month | 14 | 45 | 29 |
| 1-2 glasses/ week | 59 | 32 | 54 |
| 3 or more glasses/ week | 17 | 18 | 10 |
| Consumption of Chardonnay wine | | | |
| Don't drink | 45 | 42 | 29 |
| 1-2 glasses/ month | 35 | 39 | 36 |
| 1-2 glasses/ week | 17 | 14 | 14 |
| 3 or more glasses/ week | 3 | 5 | 21 |

Mean values with different superscripts within a row are significantly different based on Tukey HSD test ($p$<0.05).
Percentages based on column totals

**Table 7.** Wine involvement levels by mean scores of white wine purchase drivers (0 to 10) and mean values of attitude statements (0-disagree, 1-agree) ($p$<0.05); percentage of respondents in each segment shown in parentheses; higher mean values indicate more agreement with the statement.

| | Low Involvement (20%) | Medium Involvement (61%) | High Involvement (19%) |
|---|---|---|---|
| White wine purchase drivers | | | |
| Recommendations by friends | $7.4^{a}$ | $6.5^{b}$ | $6.1^{b}$ |
| Vintage/ year grapes were harvested (age of wine) | $5.1^{b}$ | $6.2^{ab}$ | $7.1^{a}$ |
| Good rating/ review by wine critic/ medals won | $5.4^{b}$ | $6.6^{a}$ | $6.3^{ab}$ |
| Appearance of label on the bottle | $3.7^{ab}$ | $4.7^{a}$ | $3.4^{b}$ |
| Region/ country that the wine is from | $6.1^{b}$ | $7.2^{a}$ | $7.1^{ab}$ |
| Grape variety | $5.5^{b}$ | $7.5^{a}$ | $7.6^{a}$ |
| Attitude statements | | | |
| For me an evening meal is never complete without a glass of wine | $0.43^{b}$ | $0.52^{b}$ | $0.79^{a}$ |
| I consider wine an everyday beverage | $0.43^{b}$ | $0.73^{a}$ | $0.82^{a}$ |
| I prefer to drink wines recommended by friends | $0.36^{a}$ | $0.34^{a}$ | $0.11^{b}$ |
| Sometimes I am overwhelmed by the number of brands there are when selecting a wine | $0.79^{a}$ | $0.57^{b}$ | $0.29^{c}$ |
| I never know how to pair wines with food | $0.46^{a}$ | $0.26^{ab}$ | $0.11^{b}$ |
| I don't know enough about wine to make it worth spending money for the better products | $0.42^{a}$ | $0.17^{b}$ | $0.00^{b}$ |

Statements sharing the same superscript within a row are not significantly different based on Tukey HSD test ($p$<0.05).

Table 7 shows the purchase drivers and wine attitudes for respondents with different levels of wine involvement. Low involvement respondents put greater importance on the appearance of the label and recommendations by friends when purchasing white wine, whereas, highly involved respondents put more emphasis on the region/ country, grape variety, vintage, and ratings, reviews and medals. Low involvement respondents thought less of 'wine is an everyday beverage' and scored higher in 'not knowing enough about wine to make it worth spending money for a better product' compared to other segments. As involvement increased, there was a decrease in the feeling of 'being overwhelmed by the large number of brands available when selecting wine' and an increase in the 'ability to pair wine with food'. High involvement respondents agreed more that 'an evening meal is never complete without a glass of wine'.

*Segmentation of consumers on white wine liking*

As previously mentioned, consumers were asked to report their liking for four white wine types, Riesling, Sauvignon Blanc from Australia and New Zealand, and Chardonnay. The mean liking values of all 150 respondents were presented in Table 2. K-means cluster analysis was performed on consumer responses, and three clusters or segments of consumers were identified. The mean liking scores of the four white wine types for each segment are shown in Table 8. Liking scores of each wine type were significant different between the segments ($p<0.001$). The three segments were labelled based on their wine style preferences: *White wine likers* (Segment 1), *Sauvignon Blanc wine likers* (Segment 2) and *Riesling wine likers* (Segment 3). Consumers' demographic information, and wine usage and attitudes were significantly different ($p<0.05$) for the three segments of consumers.

**Table 8.** Mean liking scores of four white wine types for three segments of consumers clustered by K-means analysis based on their self-reported white wine liking, percentage of respondents in each segment shown in parentheses.

| White wine type | White wine likers (40%) | Sauvignon Blanc wine likers (35%) | Riesling wine likers (25%) |
|---|---|---|---|
| Riesling wine | $5.8^{b}$ | $6.3^{b}$ | $7.7^{a}$ |
| Australian Sauvignon Blanc wine | $7.2^{a}$ | $7.7^{a}$ | $4.9^{b}$ |
| New Zealand Sauvignon Blanc wine | $7.4^{a}$ | $7.8^{a}$ | $4.5^{b}$ |
| Chardonnay wine | $6.5^{a}$ | $2.5^{c}$ | $5.6^{b}$ |

Mean values with different superscripts within a row are significant different based on Tukey HSD test ($p<0.001$).

**Table 9.** Frequency of consumption of four white wine types by consumer segments, percentage of respondents in each segment shown in parentheses.

| Consumption of white wine types | White wine likers (40%) | Sauvignon Blanc wine likers (35%) | Riesling wine likers (25%) |
|---|---|---|---|
| Consumption of Riesling wines | | | |
| Don't drink | 22 | 17 | 3 |
| 1-2 glasses/ month | 58 | 48 | 21 |
| 1-2 glasses/ week | 17 | 27 | 39 |
| 3 or more glasses/ week | 3 | 8 | 37 |
| Consumption of Australian Sauvignon Blanc wines | | | |
| Don't drink | 5 | 0 | 18 |
| 1-2 glasses/ month | 32 | 31 | 47 |
| 1-2 glasses/ week | 45 | 48 | 26 |
| 3 or more glasses/ week | 18 | 21 | 8 |
| Consumption of New Zealand Sauvignon Blanc wines | | | |
| Don't drink | 12 | 4 | 34 |
| 1-2 glasses/ month | 38 | 23 | 55 |
| 1-2 glasses/ week | 28 | 46 | 11 |
| 3 or more glasses/ week | 22 | 27 | 0 |
| Consumption of Chardonnay wines | | | |
| Don't drink | 17 | 81 | 21 |
| 1-2 glasses/ month | 48 | 19 | 42 |
| 1-2 glasses/ week | 25 | 0 | 21 |
| 3 or more glasses/ week | 10 | 0 | 16 |

Percentages based on column totals

White wine likers (40%)

This segment contained the majority of respondents. All the white wine types were liked, with a slightly higher liking for both the Australian and New Zealand Sauvignon Blanc wines and the lowest liking for Riesling wines (Table 8). Similarly, these respondents reported the lowest consumption of Riesling wines and moderate consumption of the other wine types (Table 9). There was a relatively equal spread of gender and age categories in this segment, with a slightly higher number of middle-aged respondents, between 36 and 55 years (Table 10). The majority of respondents in this segment had low to moderate wine knowledge (Table 10). A higher percentage of consumers in this segment reported to be 'overwhelmed by the number of wine brands available' and 'not knowing enough about wine to make it worth spending money on the better products' (Table 11). These respondents also reported 'paying attention to the flavour of wine', similar to the other two segments (Table 11).

Sauvignon Blanc wine likers (35%)

Respondents in this segment had a similar liking for the white wine types to the previous segment, except for Chardonnay wines. Chardonnay wines were rated very low on the liking scale by respondents in this segment (Table 8), whereas the two Sauvignon Blanc wine types were reportedly liked the most. Following this, Sauvignon Blanc wine likers had the lowest consumption of Chardonnay wines and the highest consumption of Australian and New Zealand Sauvignon Blanc wines (Table 9). This segment contained more females than males and the highest number of younger respondents (less than 35 years of age), almost all of whom were born in Australia (Table 10). This segment contained a high percentage of respondents in the low knowledge level (Table 10).

Sauvignon Blanc wine likers put somewhat greater emphasis on recommendations by friends and less emphasis on ratings, reviews and medals when purchasing white wine (Table 11). These respondents significantly disagreed that 'wine is mostly for drinking with dinner' and that 'restaurant wine lists are difficult to navigate' (Table 11). Similar to the white wine likers, the majority of respondents agreed that they are 'overwhelmed by the number of wine brands available', and reported 'not knowing enough about wine to make it worth spending money on the better products', however, they 'paid attention to the flavour of the wine that they drink' (Table 11).

**Table 10.** Demographic characteristics of consumers and levels of wine knowledge in each segment based on white wine liking scores, percentage of respondents in each segment shown in parentheses.

| | White wine likers (40%) | Sauvignon Blanc wine likers (35%) | Riesling wine likers (25%) |
|---|---|---|---|
| Gender | | | |
| Male | 58 | 38 | 58 |
| Female | 42 | 62 | 42 |
| Age | | | |
| 18-26 years | 10 | 12 | 18 |
| 26-35 years | 15 | 35 | 18 |
| 36-45 years | 25 | 10 | 18 |
| 46-55 years | 23 | 29 | 13 |
| 56 years plus | 27 | 15 | 32 |
| Country of birth | | | |
| Australia | 65 | 92 | 68 |
| Other | 35 | 8 | 32 |
| Wine knowledge | | | |
| Low | 27 | 33 | 10 |
| Medium | 56 | 50 | 70 |
| High | 17 | 17 | 20 |

Percentages based on column totals

**Table 11.** Mean scores of wine purchase drivers (0 to 10) and mean values of wine attitude statements agreement (0-disagree, 1-agree) for consumer segments (*p*<0.05); percentage of respondents in each segment shown in parentheses; higher mean values indicate more agreement with the statement.

| | White wine likers (40%) | Sauvignon Blanc wine likers (35%) | Riesling wine likers (25%) |
|---|---|---|---|
| White wine purchase drivers | | | |
| Recommendations by friends | $6.5^{ab}$ | $7.2^{a}$ | $6.2^{b}$ |
| Vintage | $6.1^{ab}$ | $5.7^{b}$ | $6.9^{a}$ |
| Ratings/ reviews/ medals | $6.8^{a}$ | $5.3^{b}$ | $6.7^{a}$ |
| Alcohol level | $3.0^{b}$ | $2.6^{b}$ | $4.6^{a}$ |
| Attitude statements | | | |
| Sometimes I am overwhelmed by the number of brands there are when selecting a wine | $0.62^{a}$ | $0.61^{ab}$ | $0.37^{b}$ |
| I never know how to pair wines with food | $0.32^{ab}$ | $0.33^{a}$ | $0.11^{b}$ |
| Personally, I think the best wines are always the most expensive | $0.07^{ab}$ | $0.02^{b}$ | $0.16^{a}$ |
| I find restaurant wine lists confusing and difficult to navigate | $0.17^{a}$ | $0.04^{b}$ | $0.16^{ab}$ |
| I don't know enough about wine to make it worth spending money for the better products | $0.27^{a}$ | $0.24^{ab}$ | $0.05^{b}$ |
| I think of wine mostly for drinking with dinner | $0.43^{a}$ | $0.24^{b}$ | $0.45^{a}$ |
| I always pay attention to the flavour of the wine I am drinking | $0.90^{a}$ | $0.84^{a}$ | $0.87^{a}$ |

Statements sharing a superscript within a row are not significantly different based on Tukey HSD test (*p*<0.05).

Riesling wine likers (25%)

This segment contained the smallest number of respondents. Respondents in this segment had moderate liking scores for both of the Sauvignon Blanc wines and Chardonnay wines (Table 8). Riesling wines were liked and consumed the most by respondents in this segment (Tables 8 and 9), whereas, Sauvignon Blanc wines had lowest consumption (Table 9). There was a relatively even spread of males and females, and the highest percentage of older respondents, aged over 56 years (Table 10). The Riesling likers contained the highest percentage of respondents in the moderate and high knowledge levels (Table 10).

Respondents in this segment thought that the alcohol level and vintage were important when purchasing white wine (Table 11). They significantly disagreed that they 'don't know enough about wine to make it worth spending money for the better products' and significantly agreed that 'the best wines are always the most expensive' (Table 11). These respondents were also confident in 'pairing wine with food', 'paid attention to the flavour of wine' and were not 'overwhelmed by the number of wine brands' (Table 11).

**Discussion**

This study examined the preference and acceptability of four white wine types, and the levels of wine involvement and wine knowledge on the wine usage, attitudes and behaviours of South Australian white wine consumers.

There are limited studies segmenting consumers based on liking of grape varieties or wine types. Of those, all have conducted consumer sensory studies looking at consumer ratings of wine aromas and flavours. For white wines, this includes Riesling (Lattey et al. 2004), Chardonnay (Lattey et al. 2004, Yegge and Noble 2000) and Sauvignon Blanc (Lund et al. 2009). In the present study, self-reported liking of four white wine types, Riesling, Sauvignon Blanc from two countries and Chardonnay was used as a variable in a segmentation exercise that identified three segments. These varieties were chosen based on the top five white varieties sold in Australia in the 2007/08 period (AWBC 2008). Although grape variety is frequently listed as one of the most important purchase drivers (Goodman 2009, Jaeger et al. 2009), which was confirmed in this study (Table 3), to our knowledge, it has not previously been used to segment consumers in marketing research studies.

The first segment, 'white wine likers', had no particular preference for the four white wine types tested, i.e. they liked all types relatively equally, with a slight dislike for Riesling wines (Table 8). This is encouraging for the Australian domestic market, as these middle-aged consumers with medium levels of wine knowledge were generally comfortable drinking any wines.

*Sauvignon Blanc wine likers*

Young Australian females were the majority of Sauvignon Blanc wine likers, with low wine involvement (Table 10). This group of consumers liked Sauvignon Blanc wines from both Australia and New Zealand the most (Table 8) and had the highest consumption of Australian and New Zealand Sauvignon Blanc wines (Table 9) of all the segments.

It is reassuring that similar results were also found in the other segmentation exercises in this study. Low involvement and knowledge consumers were mainly females who drank one or more glasses of Australian and New Zealand Sauvignon Blanc wines per week (Table 6).

These consumers generally drink cheaper wine products from their safe brands (Spawton 1991) or from recommendations by friends, possibly because they reported being overwhelmed by the number of brands and not knowing enough about wine to make it worth spending money on the better products (Table 11). The lack of confidence of Sauvignon Blanc wine likers when purchasing wine might also be related, as consumers with low levels of knowledge also relied more on recommendations by friends when purchasing wine (Table 7), confirming the views of Johnson and Bastian (2007). Sauvignon Blanc wine drinkers also placed low importance on ratings, reviews and medals, which suggests that adorning the bottle with medal labels will not influence sales to this segment.

Low involvement Sauvignon Blanc wine likers were of the opinion that wine is not an everyday beverage and can be consumed without food (Tables 7 and 11), possibly because Sauvignon Blanc wines are marketed as refreshing, stand-alone wines, and also because in this context, these wines are more likely to be consumed in social settings with friends, such as in bars or clubs without food.

Sauvignon Blanc wines are usually drunk young, with fresh, vibrant fruit-forward or 'green' styles (Iland et al. 2009). These wines are considered by most to be simple and inexpensive. Sauvignon Blanc wines are popular, as they provide easy-to-appreciate and distinctive characteristics that "answer a need in modern wine consumers, who are perhaps more interested in immediate fruit than subtlety and ageing ability" (Robinson 2006).

When these same consumers rated their hedonic liking for white wine samples with varying levels of flavour additions, it was found that the majority of consumers who liked the 'green' characters in wines also reported liking and consuming New Zealand Sauvignon Blanc wines (King et al. *in press* – Chapter 5). 'Green' characters are typically an important part of the Sauvignon Blanc wine styles associated with New Zealand (Lund et al. 2009, Parr et al. 2007). These consumers also had lower wine knowledge levels, similar to the results of the segmentation exercises in the present study. These results indicate that consumers are able to recognise flavours in wine that they find desirable, regardless of their level of wine knowledge.

This was confirmed in the present study, as Sauvignon Blanc wine likers also reported paying attention to the flavour of the wine being drunk (Table 11). This is in contrast to the 'Generation Next' strategy in the 'Wine Australia: Directions to 2025' document, where wines are driven by innovation in marketing, products and packaging that appeal to social wine consumers who are less interested in wine flavour (AWBC and WFA 2007a). Our results indicate that the social wine consumers are in fact paying attention to the flavour of wine (Table 11) and thus, it is not only innovative extrinsic factors, such as packaging that appeal to this group of consumers. A potential marketing strategy then would be free tastings of wines, targeting this segment.

Of note, all consumers unanimously stated that they pay attention to the flavour of wines (Table 4). This should bring relief to wine producers who may feel that most consumers are not appreciating the sensory properties of their wines, and a note of caution to wine producers who might believe that consumers are not discerning in their palates and would not detect faults in wines.

The reported purchase price of white wines differed depending on the consumption occasion, with an everyday bottle of wine lower in price (AUD$9-16) than wine purchased for a special occasion, a gift for someone, a weekend dinner or to expect a good bottle of white wine, which was between AUD$17-24 (Figure 1). Growth of Australian Sauvignon Blanc wines in the domestic market was highest in the AUD$7-14.99 price point, compared with New Zealand Sauvignon Blanc wines, which were dominating at the AUD$15-19.99 price point as of 2008 (AWBC and WFA 2007c). This suggests that Australian Sauvignon

Blanc wines are purchased more as an everyday wine, while New Zealand Sauvignon Blanc wines are purchased more for social occasions. These results are in keeping with the findings of Panzera (2010), who identified a similar 'thirty-something' group of young consumers in Sydney (New South Wales, Australia), who regularly bought New Zealand Sauvignon Blanc wines in an attempt to impress in social occasions.

The Marlborough region in New Zealand is synonymous with Sauvignon Blanc (Parr et al. 2007), while Australia lacks association of any of its wine regions with this grape variety, which might warrant a higher price point. Some of Australia's principal wine regions producing quality Sauvignon Blanc wines, such as the Adelaide Hills, might benefit from the strong branding of the 'Regional Heroes' strategy in the 'Wine Australia: Directions to 2025', where wines from 'somewhere rather than from anywhere' will add and sustain interest for consumers by fostering a clear association between region and variety and/or style (AWBC and WFA 2007a).

Sauvignon Blanc wine likers reported drinking very little Chardonnay (Table 9). Current market figures suggest that the New Zealand white wine category is somewhat responsible for pushing Australian Chardonnay wines out of the Australian wine market (Chappell 2009). One wine industry report suggests that consumers developed an interest in aromatic, light white wine styles, such as Sauvignon Blanc, in a quest for sophistication (AWBC and WFA 2007b). Another study suggested that Sauvignon Blanc wine likers were not necessarily brand loyal, and were happy to try new and alternative varieties (Panzera 2010). This might provide an opportunity for Australian wine producers to target this segment with lighter, fresher, less oaked Chardonnay wine styles. The latest figures suggest that this may in fact be occurring, with consumption of Sauvignon Blanc wines in Australia decreasing and a resurgence of Australian Chardonnay wines (Greenblat 2010).

Our segment of Sauvignon Blanc wine likers were similar in demographics to the 'Millenials' or Generation *Y* consumers studied by Thach and Olsen (2006). They reported some of the traits of this young group of consumers: internet proficient, diversity conscious, positive and practical, have a belief in fun and responsibility, and are environmentally and socially aware (Thach and Olsen 2006). If our Sauvignon Blanc drinkers share these traits, then it opens the door for more marketing opportunities. For example, the use of social networks, or promoting a clean, green association with Australian wines.

*Riesling wine likers*

The last segment of consumers identified in this study were Riesling wine likers, who reported liking and consuming Riesling wines the most, followed by Chardonnay wines (Tables 8 and 9). These consumers were generally older, with higher levels of wine knowledge (Table 10). These consumers were more comfortable in matching food and wine, and thought that wine is mostly for drinking with dinner (Table 11), possibly because Riesling wines are marketed as a wine style to complement food. Most Riesling wines are known for their delicacy and complexity in style (Iland et al. 2009). Riesling wines also have a reputation for long-term aging (Iland et al. 2009).

Interestingly, high involvement Riesling wine drinkers considered the alcohol level, vintage and region/ country of wine important purchase drivers when choosing white wine, compared with the other white wine liking segments (Table 11), which somewhat supports the results of Hollebeek et al. (2007) and Johnson and Bastian (2007). Riesling consumers generally had higher wine knowledge and might therefore, be more interested in a holistic understanding of wines. It may also be because Riesling wines generally have lower levels of alcohol, are often drunk after a period of bottle age and Riesling styles often reflect the place in which it was grown (Iland et al. 2009). It is likely that Riesling wine likers are using these purchase drivers as indicators when seeking different styles of Riesling wines.

Liking and consumption of Riesling wines was found to increase as wine involvement and wine knowledge increased (Tables 6 and 10). This is in keeping with Robinson (2006), who stated that Riesling wines are considered an acquired taste that is not particularly palatable to young wine consumers who are looking for more strongly flavoured wine styles, such as Sauvignon Blanc wines. Chardonnay wine consumption also increased in popularity with increasing involvement and knowledge (Table 6). These results suggest that Riesling and Chardonnay wines are usually not consumed during the early or developmental stages of wine drinking. Such information could imply a gap in the marketplace for producers of these wines to develop more approachable styles and marketing suitable for emerging wine consumers.

Education is another strategy to enhance domestic sales of Australian white wines, by increasing the levels of wine involvement and wine knowledge of emerging wine consumers, also suggested by Johnson and Bastian (2007). This is in line with the strategic goals of the

Australian wine industry, contained within the 'Wine Australia: Directions to 2025' document (AWBC and WFA 2007a).

*Limitations of the study and future research*

The findings of this study can be extrapolated to the South Australian population, however, the sample size may restrict the generalisation of the results to the broader Australian populations. The recruitment method used in this study may have skewed the results of the participants to a higher level of wine involvement, due to the effort of registering their interest and travelling to the location of the testing. This is reflected in the high individual wine involvement scores.

Price was deliberately not included as a purchase driver of white wine in the present study, similar to Jaeger et al. (2009). Numerous other studies have conclusively demonstrated that price is one of the most important purchase drivers for wine choice (Quester and Smart 1998), particularly for low involvement and low knowledge consumers (Hollebeek et al. 2007, Johnson and Bastian 2007, Lockshin et al. 1997).

It would be interesting to compare the results of the present study with a larger number of consumers from other parts of Australia, such as the East coast, and expand the range of wine styles and varieties in the questionnaire, to obtain a clearer understanding of consumer preference.

**Conclusions**

To our knowledge, this is the first study to use white wine liking in a market segmentation exercise. The three white wine liking segments identified were used to investigate the possible effectiveness of some of the strategies in the 'Wine Australia: Directions to 2025' document (AWBC and WFA 2007a), for increasing sales of Australian wines in the domestic market.

The results of this study suggest that the 'Generation Next' strategy is insufficient for targeting emerging wine consumers, as young, low involved, 'social' consumers were not only influenced by wine's extrinsic packaging, but were also found to pay attention to the intrinsic sensory characteristics of wine, such that they could correctly identify wine flavours that they find desirable (King et al. *in press* – Chapter 5). This suggests that wine producers should not discount the influence that wine flavour has on purchase decisions for emerging wine consumers.

It may be possible for Australian wine producers to emulate the strong branding of New Zealand Sauvignon Blanc wine regions by incorporating the ‘Regional Heroes’ strategy for some of the primary Australian Sauvignon Blanc-producing wine regions, such as the Adelaide Hills. This might warrant a higher price point for Australian Sauvignon Blanc wines and a new level of prestige in social occasions.

The results of this study provide initial insights into the identification and characterisation of white wine consumer groups in South Australia who, through fostering, and tailored products and marketing communications, have the potential to become frequent consumers of a broad range of Australian wine styles in the domestic market.

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# Chapter 7.

## Concluding remarks and future perspectives

The overall aim of this thesis was to link control of primary fermentation to consumer preferences for Sauvignon Blanc wines.

Sauvignon Blanc wine was chosen as the basis for all studies in this thesis for two main reasons. Firstly, key aroma compounds derived in the grape berry, namely volatile thiols, are modulated during fermentation by yeast, which provides a clear link between varietal characters and oenological practices. Importantly, there is currently a strong market demand for Sauvignon Blanc wines in Australia and therefore, information regarding consumer preferences and behaviours towards this wine style are valuable to the Australian industry.

Not long ago, there was debate within the wine industry and research community regarding whether yeast strains used to conduct wine fermentation had an important effect on wine composition and aroma (Thorngate III 1999). The results of the work reported in this thesis have conclusively demonstrated that the choice of yeast inoculum, using single or multiple yeasts, affects wine aroma composition and sensory properties (King et al. 2008 – Chapter 2, King et al. 2010 – Chapter 3). These yeast-derived differences are also sufficient to affect consumer preference (King et al. 2010 – Chapter 3), and are retained in wine after an extended period of bottle age (King et al. *in press-a* – Chapter 4).

Yeasts have been shown to alter volatile thiol concentrations (Dubourdieu et al. 2006, Swiegers et al. 2009), to an even greater extent in some co-inoculated fermentations (King et al. 2008 – Chapter 2, King et al. 2010 – Chapter 3). Sauvignon Blanc grape juice from the same vineyard in the Adelaide Hills (Australia) was used for separate fermentation studies in 2005 (Swiegers et al. 2009), 2006 (King et al. 2008 – Chapter 2) and 2007 (King et al. 2010 – Chapter 3), which provides good longitudinal data on volatile thiol production over three vintages. 4MMP levels were highest in 2005, with lower levels reported in 2006, although wines from both vintages retained 4MMP levels after three years of bottle storage (King et al. *in press-a* – Chapter 4). The actual 3MH concentrations were higher in the 2006 and 2007 vintages, as was the range of 3MH concentrations observed between the treatments, probably owing to the use of co-inoculated fermentations as a means of enhancing volatile thiol levels. The highest concentrations and largest range of 3MHA was observed in the 2007 wines, again, probably due to metabolic interactions occurring in the co-inoculated fermentations.

Most of the co-inoculated fermentations in this study contained high levels of both 3MH and 3MHA. This was a surprising result, given that the yeast combinations were selected for their different volatile thiol modulation behaviours; with one strain more capable of 3MH release from its conjugate precursors and the other better at esterifying 3MH to produce 3MHA. It would be expected that once 3MH is metabolised to 3MHA, there would be less 3MH in the co-inoculated fermentations. Similar results were observed by Anfang et al. (2009) for co-inoculated fermentations of *Saccharomyces* and non-*Saccharomyces* yeasts. It may be that the yeasts in the co-inoculated fermentations are competing for nutrients, and thus, release more 3MH to obtain the nitrogen.

On the other hand, the results reported in this thesis indicate that co-inoculations do not increase the levels of the volatile thiol 4MMP in wine (King et al. 2008 – Chapter 2, King et al. 2010 – Chapter 3). Current knowledge suggests that 4MMP is not further metabolised by yeast once released from its conjugated precursors, and thus, yeast interactions in co-inoculated fermentations may not influence 4MMP. Follow up experiments may be required to investigate this further, as 4MMP concentrations were relatively low.

The hypothesis for the 3MH result implies that yeasts are sensing one another in co-inoculations. An investigation into metabolic interactions occurring between yeasts may reveal possible communication networks between different strains, such as quorum sensing (Chen and Fink 2006, Palková et al. 1997), and provide insights into the awareness and response of yeasts to their environments and competitors. Using volatile thiol release as an indicator for such interactions might provide a sensitive measure, due to relatively low concentrations produced during fermentation. Recent advances in the study of microbial population dynamics using techniques such as metagenomics (Konstantinidis et al. 2009) may also facilitate an improved understanding of contributions by different yeast in a wine fermentation.

Co-inoculated fermentations should be trialled in other grape varieties. Most of the work studying co-inoculations of multiple *Saccharomyces* yeasts has been conducted in white wine, particularly Chardonnay (Howell et al. 2006) and Sauvignon Blanc (King et al. 2008 – Chapter 2, King et al. 2010 – Chapter 3). The results of Chapter 2 showed that co-inoculated fermentations can sometimes result in a decrease of negative characteristics, such as volatile acidity (King et al. 2008). It seems important now to investigate whether co-inoculated fermentations have the same effect in other grape varieties, including red wines, particularly those that contain volatile thiol compounds, such as Cabernet Sauvignon. This would present its own challenges, such as higher fermentation temperatures, faster fermentation rates, higher sugar and alcohol environments, and colour and phenolic compositions. There is also the matter of oxidative handling, which adds another degree of complexity and difficulty.

The results of Chapters 2 and 3 highlighted the degree to which co-inoculated fermentations can differ in their production of volatile thiol concentrations and resulting wine styles (King et al. 2010, King et al. 2008). The co-inoculated wines with large differences in sensory profiles were initially used as the basis to measure consumer preference (King et al. 2010 – Chapter 3). Following this, the flavour addition experiment in Chapter 5 separated the influences of particular key aroma compounds on consumer preference (King et al. *in press-b*). The results from the flavour addition experiment demonstrated that a number of the sensory attributes driving consumer preference for Sauvignon Blanc wines are caused by volatile compounds modulated or produced by yeast (King et al. *in press-b* – Chapter 5). This confirms the results of the previous consumer testing in Chapter 3 (King et al. 2010), that consumer preference is influenced by yeast-derived sensory differences. There was also evidence of an optimal level of the less desirable volatile thiol descriptor, 'cat urine/sweaty'.

Another key aroma compound driving consumer preference was isobutyl methoxypyrazine and its associated 'green' characteristics, which are influenced by viticultural practices. The present study also demonstrated the strong suppressive effects of methoxypyrazines on volatile thiols. These results highlight the benefits of reconstitution and deconstruction tests, and the need to better understand interactive effects occurring between compounds.

Sauvignon Blanc consumers were identified as mainly young females with relatively low wine knowledge (Chapter 6). In hedonic tests, these consumers were able to recognise the sensory properties characteristic of wine styles they find desirable (King et al. *in press-b* – Chapter 5), namely New Zealand Sauvignon Blanc wines (Lund et al. 2009, Parr et al. 2007). Contrary to current market strategies (AWBC and WFA, 2007), these results suggest that consumers respond to specific flavours that they like, regardless of their knowledge level.

Fearne (2009) highlighted the need for integrated market intelligence and consumer insight. The work described in this thesis combined these aspects into studies of oenological practices, enabling development of wine styles by modulating wine aroma through control of primary fermentation.

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