Environmental Influences on Grape Aroma Potential

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Words of Wisdom...

Wine in life -

"Wine to me is passion. It's family and friends. It's warmth of heart and generosity of spirit. Wine is art. It's culture. It's the essence of civilization and the art of living."

Robert Mondavi (1913-2008)

"You have only so many bottles in your life, never drink a bad one."

Len Evans (1930-2006)

Wine in practice -

If you want continuity, you must start with a special vineyard. No matter how much you believe in the technology of wine-making, it takes a fine vineyard to produce fine wine."

André Tchelistcheff (1901-1994)

"In my opinion, the greatest grape is the noble Cabernet. Cabernet Sauvignon is the only variety that would be tolerated in heaven."

Jack Mann (1906-1989)

Declaration

I declare that this thesis is my own account of my research and contains as its main content work which has not previously been submitted for a degree at any tertiary education institution.

Anthony L. Robinson

26th May, 2011

Abstract

Understanding the source of wine volatile compounds and the mechanisms that influence their formation through grape growing, winemaking and storage is essential for wine businesses when developing strategies to produce wines with specific sensory attributes that appeal to target markets. The objective of this research was to develop a greater understanding of the environmental influences that drive flavour formation in grapes and translate this information into awareness of the limitations of site and region in producing wines to specification. A novel analytical method was developed utilising headspace solid-phase microextraction (HS-SPME) for the analysis of wine volatiles by comprehensive two-dimensional gas chromatography (GC × GC) time-of-flight mass spectrometry (TOFMS). The analytical technique was able to resolve and identify a substantially larger number of volatile compounds than current single dimensional GC-MS methodologies. While developing this method it became clear that there was a need to develop a greater understanding of wine matrix effects on SPME-based analyses of volatile compounds found in grape juices and wines of which ethanol and glucose had the greatest effect. Furthermore, the impact of shipping conditions in relation to wine composition and sensory characteristics was investigated to ensure sample integrity across the experiments. The HS-SPME GC × GC-TOFMS methodology was applied in conjunction with descriptive sensory analysis to field studies exploring the effects of site, viticultural management, and winemaking on wine composition and sensory characteristics. This study identified that site was a major influence on Cabernet Sauvignon wine composition and sensory characteristics leading to an extensive study exploring the composition and sensory attributes of a number of commercially produced Cabernet Sauvignon wines from ten wine growing regions of Australia. The results of the studies have enabled the integration of sensory and chemical data from

Australian Cabernet Sauvignon wines which has revealed potential chemical markers of sensory attributes and compositional characters that are associated with Australian wine regions.

Table of Contents

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Publications, Presentations, and Conferences

The following publications were derived from this work;

Peer Reviewed Publications

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Robinson, A., Boss, P., Heymann, H., Solomon, P., Trengove, R. (2011) 'Development of a sensitive non-targeted method for characterizing the wine volatile profile using headspace solid-phase microextraction comprehensive two-dimensional gas chromatography time-of-flight mass spectrometry', *Journal of Chromatography. A*, **1218**, 504-517.

Robinson, A., Mueller, M., Heymann, H., Ebeler, S., Boss, P., Solomon, P., Trengove, R. (2010) 'Effect of simulated shipping conditions on sensory attributes and volatile composition of commercial white and red wines'. *American Journal of Enology and Viticulture*, **61**, 337-347.

Robinson, A., Ebeler, S., Heymann, H., Boss, P., Solomon, P., Trengove, R. (2009) 'Interactions between wine volatile compounds and grape and wine matrix components influence aroma compound headspace partitioning', *Journal of Agricultural and Food Chemistry*, **57**, 10313-10322.

Conference Presentations

Eurosense – Vitoria-Gasteiz, Spain 2010

Machado, B., Heymann, H., Robinson, A., Torri, L. 'How many judges are required for Sensory Descriptive Analysis?'.

ASEV 61st Annual Meeting – Seattle, Washington, United States 2010

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INTERVITIS INTERFRUCTA IVIF-Congress – Stuttgart, Germany 2010 [Invited]
Robinson, A., Ebeler, S., Heymann, H., Boss, P., Solomon, P., Trengove, R. 'The role of ethanol and glucose on the headspace partitioning of volatile compounds'.
6th Symposium In Vino Analytica Scientia – Angers, France 2009
Robinson, A., Ebeler, S., Heymann, H., Trengove, R. 'Influence of wine and juice macromolecules on the headspace partitioning of volatile compounds'.
ASEV 60th Annual Meeting – Napa, California, United States 2009
Robinson, A., Graham, B., Boss, P., Solomon, P., Trengove, R. 'Volatile profiling of Cabernet Sauvignon wine using comprehensive two-dimensional gas chromatography'.
5th Symposium In Vino Analytica Scientia – Melbourne, Australia 2007
Robinson, A., Graham, B., Boss, P., Solomon, P., Trengove, R. 'Solid-phase microextraction of volatile compounds in wine – key matrix effects in headspace analysis'.

Conference Posters

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Robinson, A., Boss, P., Heymann, H., Solomon, P., Trengove, R. 'Understanding the role of geographic region in defining the sensory characteristics and chemical composition of Australian Cabernet Sauvignon wines'.
ASEV 60th Annual Meeting – Napa, California, United States 2009
Robinson, A., Ebeler, S., Heymann, H., Trengove, R. 'Effect of ethanol and glucose on aroma compound partitioning between the headspace and wine matrix'.
Wicks, M., Robinson, A., Heymann, H. 'Effect of simulated shipping conditions on sensory attributes and volatile compounds in six wines'.
13th Australian Wine Industry Technical Conference – Adelaide, Australia 2007
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Conferences

The following conferences were attended during the course of this PhD:
14th Australian Wine Industry Technical Conference (AWITC) – Adelaide, Australia 2010
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7th International Cool Climate Symposium (ICCS) – Seattle, Washington, United States 2010

INTERVITIS INTERFRUCTA IVIF-Congress – Stuttgart, Germany 2010

ASEV 60th Annual Meeting – Napa, California, United States 2009

CASSS GCXGC 2009 Symposium – Portland, Oregon, United States 2009

ASEV Unified Wine and Grape Symposium – Sacramento, California, United States 2009

13th Australian Wine Industry Technical Conference (AWITC) – Adelaide, Australia 2007

5th Symposium In Vino Analytica Scientia – Melbourne, Australia 2007

ASVO ‘Finishing the Job - Optimal ripening of Cabernet Sauvignon and Shiraz’ – Mildura, Australia 2006

1. Literature Review

1.1. Introduction

Chemists have been capable of isolating and analysing the composition of foods and beverages for centuries, attempting to identify and quantify those chemical compounds responsible for the human experience of smell and taste. An early pioneer was the Swedish chemist Carl Wilhelm Scheele (1742-1786) who isolated organic acids including citric, lactic, malic, and tartaric acids from lemons, milk, apples, and unripe grapes, respectively (Scheele and De Morveau, 2009). Wine has been an important product for such compositional studies partly due to its contribution to the world economy and culture, but also due to its complexity as a beverage which has inspired scientists. Although compositional information can provide us with information about the compounds contributing to the sensory perception of wines, it cannot replace people in their ability to translate the complex interactions of sight, smell, and taste which define the sensory experience of consuming wine; flavour is an interaction of consumer and product (Piggott, 1990). As the late Dr. Maynard Amerine noted, "Quality in wines is much easier to recognize than it is to define" (Amerine and Roessler, 1983).

Wine flavour perception is a complex notion, it is the culmination of multiple volatile and non-volatile compounds present in the product (Rapp and Mandery, 1986, Rapp, 1998, Ebeler, 2001, Polášková et al., 2008) and an equally complex receptor and perception system which is closely linked to neural systems in the brain used for learning, memory, emotion and language (Buck and Axel, 1991, Mori et al., 1999, Swiegers et al., 2005b, Shepherd, 2006, Auvray and Spence, 2008). This review presents a summary of literature relevant to the field of wine aroma research highlighting the current state of our knowledge concerning grape and wine composition and the analytical and sensory techniques used in this research field.

1.2. The origin of wine aroma

The origins of aroma and bouquet (to be referred to collectively as aroma) in wines have been of major interest over the last century with advances made through the development and utilisation of modern analytical techniques (Polášková et al., 2008, Ebeler and Thorngate, 2009) coupled to hybrid analytical / sensory methods (Guth, 1997b, López et al., 1999, Ferreira et al., 2000, Francis and Newton, 2005) or through multivariate statistical comparisons with descriptive sensory analysis (Noble and Ebeler, 2002, Lee and Noble, 2003, 2006, Escudero et al., 2007, Sáenz-Navajas et al., 2010). This has been particularly important in resolving interaction effects with the non-volatile matrix (Pineau et al., 2007, Sáenz-Navajas et al., 2010) and with other volatile compounds (Atanasova et al., 2005b, Escudero et al., 2007, Pineau et al., 2009) which may result in variations in the sensory character of the mixture due to enhancement and suppression effects. The sensation of flavour occurs when certain odour active molecules stimulate sensors in the mouth and nose which the brain collates to produce a flavour perception (Taylor, 1998). An alternative definition of flavour is the "combined sensory olfactory sensing by nose and mouth" (Coombe and McCarthy, 1997). The current understanding is that multiple sensory interactions occur in the perception of flavour (Auvray and Spence, 2008) where smell plays a particularly important role in the overall perception of the product (Shepherd, 2006, Shepherd, 2007). Smell is a biological and electrophysiological process which converts the molecular information of an odorant into a sensation (Hasin-Brumshtein et al., 2009). The human olfactory epithelium accommodates millions of olfactory sensory neurons which are attached to olfactory receptors (OR's) which are capable of detecting multiple compounds, due to common functional groups, while at the same time multiple receptors can recognise the same odour compound, due to multiple functional groups

(Firestein, 2001, Hasin-Brumshtein et al., 2009). As there are 347 potentially functional (OR) genes, (Zozulya et al., 2001, Gaillard et al., 2004), this allows humans to detect the thousands of odour compounds found in nature.

Aroma of wine is derived from:

- The direct contribution of grape-derived aroma compounds including monoterpenes, norisoprenoids, aliphatics, phenylpropanoids, methoxypyrazines, and volatile sulphur compounds (Coombe and McCarthy, 1997, Ebeler and Thorngate, 2009),
- Microbial derived secondary metabolites that are formed from catabolism of sugar, fatty acids, organic nitrogen compounds (pyrimidines, proteins and nucleic acids), and cinnamic acids found in grape (Chatonnet et al., 1992, Herraiz and Ough, 1993, Guitart et al., 1999, Hernández-Orte et al., 2002, Swiegers et al., 2005a),
- Contribution of oak derived aroma compounds during fermentation and storage of wine (Sefton et al., 1990, Gómez-Plaza et al., 2004) characteristic of the origin, seasoning, and heating of the wood (Francis et al., 1992, Cadahía et al., 2003, Fernández de Simón et al., 2010a), and
- Chemical changes associated with acid (Skouroumounis and Sefton, 2002, Versini et al., 2002) and enzyme catalysed (Günata et al., 1985, Sefton and Williams, 1991, Ugliano, 2009) modification of other non-aromatic grape constituents.
- Chemical modifications associated with oxidative processes in wine (Simpson, 1978, Escudero et al., 2002, Silva Ferreira et al., 2002) which are related to oxygen uptake due to winery operations, storage, and packaging materials (Karbowiak et al., 2009, Ghidossi et al., 2012).

While a number of aroma compounds have been identified, understanding of the role viticulture plays in their evolution remains limited. This may be attributed to time and cost limitations along with a large emphasis in the past on method development and compound discovery, and difficulty in identifying and quantifying grape components that contribute to wine aroma.

1.3. Volatile compound classes found in wine

The advent of gas chromatography and gas chromatography coupled to mass spectrometry has resulted in an expansion of the identification of aroma compounds in many foodstuffs, including wine (Ohloff, 1978). The major groups of aroma compounds found in wine are the monoterpenes, norisoprenoids, aliphatics, higher alcohols, esters, phenylpropanoids, methoxypyrazines, and volatile sulphur compounds (Francis and Newton, 2005, Ebeler and Thorngate, 2009). Some studies have investigated the composition of specific cultivars in an effort to better understand the origins of varietal aroma (Sefton et al., 1993, 1994, 1996, Schneider et al., 2002). In some instances these studies have been successful in distinguishing cultivars according to key compounds (Rosillo et al., 1999) or dominant groups of aroma compounds (Günata et al., 1985, Sefton et al., 1993). Although there have been significant developments in the identification of important odour active compounds, few have been able to identify a defining compound responsible for ‘varietal character’. It is apparent that ‘varietal character’ is dependent not on a particular compound but on the profile of odour active compounds present. What follows is a survey of the compounds found in wines grouped according to their functional groups.

1.3.1. Terpenes

Monoterpenes and sesquiterpenes are synthesized from isopentyl pyrophosphate (IPP) and dimethylallyl pyrophosphate (DMAPP) which are formed through the cytosolic

mevalonatic-acid (MVA) pathway from three molecules of acetyl-CoA (Newman and Chappell, 1999) and through the plastidial 2-C-methylerythritol-4-phosphate (MEP) pathway from pyruvate and glyceraldehyde-3-phosphate (Rohmer, 1999). Monoterpenes are formed from 2E-geranyl diphosphate (GPP) and sesquiterpenes are formed from 6E-farnesyl diphosphate (FPP) through the action of terpene synthases (*TPS*) (Lücker et al., 2004, Martin et al., 2010). A recent study documented that the *V. vinifera VvTPS* gene family contains the largest number of functionally characterized TPS for any species reported to date and functionally characterized 39 *VvTPS* gene products, demonstrating that the *VvTPS* gene family is capable of synthesising upwards of 21 different monoterpenes and 47 sesquiterpenes (Martin et al., 2010).

Monoterpenes are important contributors to the aroma of white wines made from muscat varieties and aromatic non-muscat varieties (Ribéreau-Gayon et al., 1975, Rapp, 1998, Mateo and Jimeńez, 2000) with correlations between floral sensory attributes and high levels of linalool and α-terpineol being well documented (De La Presa-Owens and Noble, 1997, Lee and Noble, 2003, Campo et al., 2005, Lee and Noble, 2006). Intensely flavoured 'muscat' cultivars, such as the Muscat de Frontignan, Muscat of Alexandria and Gewürztraminer are commonly grown in Australia and have been observed to contain high levels of free monoterpenes (Williams et al., 1981, Günata et al., 1985, Wilson et al., 1986). For example, research has identified that (Z)-rose oxide is an important impact aroma compound found in Gewürztraminer wines (Guth, 1997a) and is associated with the lychee aroma attribute common to this variety (Ong and Acree, 1999). Other 'aromatic' cultivars or non-muscat cultivars, of which the most common example in Australia is Riesling, contain lower levels of free monoterpenes (Dimitriadis and Williams, 1984, Günata et al., 1985, Razungles et al., 1993). A number of monoterpenes are subject to transformations under the pH and temperature conditions

found in juice and wine (Raguso and Pichersky, 1999) and thus grape biosynthesis may not explain all terpene metabolites found in wine. Analysis undertaken by Rocha and co-workers (Rocha et al., 2007) also indicates that not all terpenes have been identified in grapes and wines as the group tentatively identified 56 different monoterpenes in Fernão-Pires grapes from Portugal, of which 20 had not been previously identified in grapes.

Sesquiterpenes have gained little attention with respect to grape and wine analysis with only three major studies reporting multiple compounds namely Schreier and co-workers who identified 13 sesquiterpenes from Riesling, Traminer, Ruländer, Müller-Thurgau, Scheurebe, Optima, and Rieslaner grapes grown in Germany (Schreier et al., 1976); Coelho and co-workers who identified 18 sesquiterpenes from Baga grapes grown in Portugal (Coelho et al., 2006) and Parker and co-workers who reported 18 sesquiterpenes in Shiraz grapes (Parker et al., 2007). The last study identified α-ylangene as a candidate marker of pepper character in Australian Shiraz wines but was unable to confirm its aroma contribution to wine (Parker et al., 2007). Subsequent research identified the sesquiterpene rotundone as the potent aroma impact compound responsible for the pepper aroma in wines produced from *Vitis vinifera* cv. Shiraz (Siebert et al., 2008, Wood et al., 2008) as well as its presence in a number of other natural products including pepper (*Piper nigrum*), marjoram (*Origanum majorana*), oregano (*Origanum vulgare*), geranium (*Pelargonium alchemilloides*), nut grass (*Cyperus rotundus*), rosemary (*Rosmarinus officinalis*), saltbush (*Atriplex cinerea*), basil (*Ocimum basilicum*), and thyme (*Thymus vulgaris*) (Wood et al., 2008). It is obvious that terpenes play important roles in a number of different wine grape varieties and with important recent discoveries, such as rotundone, it is clear that this group of compounds will continue to be a focus of wine aroma research into the future.

1.3.2. Norisoprenoids

1.3.2.1. Introduction

Norisoprenoids (or apocarotenoids) are derived from carotenoids and are found commonly in nature (Baumes et al., 2002, Winterhalter and Rouseff, 2002). They consist of a megastigmane carbon skeleton and differ due to the position of the oxygen functional group being either; absent (Megastigmanes); attached to carbon 7 (Damascones); or attached to carbon 9 (Ionones) (Winterhalter and Rouseff, 2002). Although monoterpenes have been considered major contributors to grape and wine flavour, the norisoprenoids have attracted considerable attention as odorants in many food and fragrance products (Winterhalter and Rouseff, 2002). Norisoprenoids are abundant in aromatic cultivars (Strauss et al., 1987b, Winterhalter et al., 1990a, Marais et al., 1992, Schneider et al., 2001), are ubiquitous among grape cultivars, and they are thought to play an important role in wine aroma in many of the dominant wine varieties including Semillon, Sauvignon Blanc, Chardonnay, Merlot, Syrah, and Cabernet Sauvignon (Razungles et al., 1993, Sefton et al., 1993, 1994, 1996, Sefton, 1998).

1.3.2.2. Grape carotenoids

As norisoprenoids are derived from carotenoids, it follows that their abundance can be influenced by the carotenoid profiles of berries, Carotenoids fill an important photo-protective role in plant tissue by either scavenging singlet oxygen or by quenching the triplet state chlorophyll thereby preventing singlet oxygen formation (Demmig-Adams, 1990, Young, 1991, Demmig-Adams and Adams, 1996). Singlet oxygen is a powerful oxidising agent that can destroy cell components e.g. membranes and proteins. Additionally carotenoids are found to improve photosynthetic efficiency in higher plants as accessory light harvesters. This is achieved by absorption of light energy at 400-500 nm (not accessible to chlorophyll molecules) followed by singlet-singlet

energy transfer to chlorophyll molecules (Young, 1991). Carotenoids and xanthophylls are generated in chloroplasts but can also be generated in the chromoplasts (Baumes et al., 2002). In higher plants, chloroplasts transform into chromoplasts during maturation (Baumes et al., 2002) allowing further synthesis of new carotenoids. This is not the case in grapes where chloroplasts are not transformed into chromoplasts (Razungles et al., 1988, Razungles et al., 1996). Subsequently the levels of carotenoids, along with chlorophyll, decrease during maturation of grapes (Razungles et al., 1988, Razungles et al., 1993) where chloroplasts are lost (Hardie et al., 1996).

More than 600 carotenoids and xanthophylls, with a diverse range of structures, have been isolated from natural sources (Britton, 1995). Only a few of these, however, have been identified in grapes and wines. β-carotene and lutein constitute 85% of the total with neochrome, neoxanthin, violaxanthin, luteoxanthin, flavoxanthin, lutein-5,6-epoxide and zeaxanthin, and cis isomers of lutein and β-carotene being the next most abundant (Mendes-Pinto, 2009). Carotenoids accumulate in the leaves of *Vitis* (Skouroumounis and Winterhalter, 1994, Wirth et al., 2001) and prior to veraison in the grape exocarp (skin) (Razungles et al., 1988, De Pinho et al., 2001). Although carotenoids and xanthophylls are concentrated in the skins of grapes there is some dispute as to whether they carry over into juice during winemaking. It was previously thought that carotenoids were too lipophilic (Razungles et al., 1988) but subsequent research showed that they were present in the musts and wines of Port wine from the Douro Valley (De Pinho et al., 2001, Mendes-Pinto et al., 2005). This may be a function of the winemaking process where ethanol is added during the fermentation process (with the exocarp present) potentially increasing the solubility of these compounds (Mendes-Pinto, 2009).

1.3.2.3. Grape norisoprenoids and their formation from carotenoids

In 1970, the discovery of β-damascenone and β-damascone from Bulgarian rose oil (*Rosa damascene*) by Demole and co-workers (Demole et al., 1970) instigated further investigation into rose ketones. Rose ketones are a diverse group of aroma compounds possessing complex characters described as honey-like, flowery and ionone-like depending on the concentration (Skouroumounis and Sefton, 2002). Of particular importance are the remarkably potent norisoprenoids β-ionone (odour threshold in model wine; 0.09 µg/L, (Kotseridis et al., 1999b)) and β-damascenone (odour threshold in 10% ethanol; 0.05 µg/L, (Guth, 1997b)). Further, in wine research there has been additional interest in 1,1,6-trimethyl-l,2-dihydronaphthalene (TDN) associated with the kerosene bottle aged character of Riesling wines (Simpson, 1979, Winterhalter et al., 1990b) and more recently (E)-1-(2,3,6-trimethylphenyl)buta-1,3-diene (TPB) which may be associated with the floral, geranium, and tobacco characters of aged Semillon wines (Janusz et al., 2003, Cox et al., 2005).

Essentially, the formation of β-damascenone stems from the Xanthophyll cycle (or Violaxanthin cycle) which dissipates excess excitation energy in leaves during light exposure (Baumes et al., 2002). The epoxidation of zeaxanthin (derived from the major carotenoid β-carotene) to violaxanthin is catalysed by zeaxanthin epoxidase and occurs in conditions of low light (Baumes et al., 2002). This reaction is reversible in conditions of high light. The subsequent biogenesis of neoxanthin (the parent compound of β-damascenone (Skouroumounis and Sefton, 2002)) from violaxanthin occurs through the elimination of the 7´ proton resulting in the opening of the 5´, 6´ epoxide (Baumes et al., 2002).

Essentially, the formation of norisoprenoids occurs from the biodegradation (dioxygenase cleavage) of the parent carotenoid, enzymatic conversion to the aroma

precursor (polar intermediate), and finally the acid-catalysed conversion to the aroma-active compound (Winterhalter and Rouseff, 2002). Once formed, these compounds are then subject to further acid reaction during wine aging (Skouroumounis and Sefton, 2000). The specific enzyme systems involved in the degradation of carotenoids to form norisoprenoids were hypothesised in the 1990's and later (Razungles et al., 1993, Baumes et al., 2002, Winterhalter and Rouseff, 2002) but have only recently been described in *V. vinifera* (Mathieu et al., 2005). Oxidative cleavage of carotenoids leads to the production of norisoprenoids and is catalysed by a family of carotenoid cleavage dioxygenases (CCDs) (Mathieu et al., 2005, Walter et al., 2010). In addition, isophorone (C9), safranal (C10), and dihydroactinidiolide (C11) are also odour active compounds derived from carotenoid degradation although there is no evidence of this occurring in grapes. What is also of interest is that the CCD's involved in the cleavage of C_{40} carotenoids at 9-10 and 9'-10' double bonds are currently limited to the CCD1 and CCD4 enzyme classes which are located in the cytosol and plastid respectively (Walter et al., 2010). Subsequently it could be assumed that the CCD4 enzymes are more important than the CCD1's in the cleavage of carotenoids formed in the chloroplasts of grapes. Further research into this area is clearly required to better understand how the complement of CCD enzymes works *in vivo*. A recent study has also observed that grape cell cultures were able to metabolise the C_{13}-norisoprenoids β-ionone and dehydrovomifoliol to secondary norisoprenoid volatiles indicating that hydroxylases, oxidoreductases and glycosyltransferases, yet to be identified, may be involved in biotransformation of these carotenoid cleavage products (Mathieu et al., 2009).

β-damascenone has recently been noted as playing a particularly important indirect role in the aroma of wine. Recent research has suggested that the interactions of β-

damascenone together with IBMP (Pineau et al., 2007) and with other volatiles (Escudero et al., 2007) results in variations in the sensory character of the mixture due to enhancement and suppression effects. For example the combination of β-damascenone, β-ionone, dimethyl sulphide, and fruity esters enhance berry fruit character (Escudero et al., 2007). It is becoming clearer that volatile compounds interact to either enhance or mask particular aroma characteristics in wines (Atanasova et al., 2005b, Escudero et al., 2007, Pineau et al., 2007, Pineau et al., 2009).

It is apparent that norisoprenoids play an important role in aroma research particularly in the grape and wine field. A recent review documents research into the formation of norisoprenoids from carotenoids in grapes and provides a useful discussion on the contribution of norisoprenoids to wine aroma (Mendes-Pinto, 2009). Future research will need to move toward holistic studies to better understand interaction effects with particular interest in the role that norisoprenoids play in enhancing the fruit character of wines.

1.3.3. Phenylpropanoids

The biosynthesis of volatile phenylpropanoids have not been studied at any length in grapes *per se* while other plant systems have identified a number of organ and species specific dehydrogenases, reductases, methyltransferases, and acetyltransferases that are involved in the biosynthesis of volatile phenylpropanoids (Dudareva et al., 2004, Dudareva and Pichersky, 2006, Vogt, 2010). Volatile phenylpropanoids, such as phenylethanol, phenylacetaldehde, benzaldehyde, and benzylacetate, are derived from L-phenylalanine which is formed through the shikimic acid pathway in plastids. However, little is known about the complete biosynthetic pathways leading to their formation in plants (Dudareva and Pichersky, 2006). The phenylpropanoid pathway also leads to the formation of other important secondary metabolites in grapes including

hydroxycinnamates, stilbenes, lignin, lignan, aurones, flavones, isoflavonoids, as well as flavonoids, which include flavonols, tannins, and anthocyanins (Downey et al., 2006, Singh et al., 2010). The flavanoids are particularly important to the mouth feel properties of red wines (Gawel, 1998). The phenolic content of wine is dependent firstly on grape phenolic content which is influenced by a number of factors including variety (Harbertson et al., 2008), grape maturity (Kennedy et al., 2002), variations in water and nutrient availability, light and temperature environment, and changes in predation and disease pressures (Downey et al., 2006, Cohen and Kennedy, 2010). Secondly, it is based on the extractability of grape phenolics which is influenced by interactions with cell wall material (Bindon et al., 2010) and numerous red winemaking practices (Sacchi et al., 2005). It is likely that similar influences will vary the volatile phenylpropanoid content of wines.

Volatile phenylpropanoids deserve significant consideration because of their observed abundance in hydrolysates of Chardonnay juice (10-20% total hydrolysed volatile fraction, (Sefton et al., 1993)) and Tannat wine (51% total hydrolysed fraction, (Boido et al., 2003)) and their contribution to Cabernet Sauvignon and Merlot musts where they have been correlated with aroma attributes including dried fig, tobacco, and chocolate (Francis et al., 1998). One of the more interesting grape derived volatile phenylpropanoids is methyl anthranilate which is considered to be responsible for the distinctive ‘foxy’ aroma and flavour of the Washington Concord grape (*Vitis labrusca*) (Wang and Luca, 2005) and may also contribute to the aroma of Pinot noir (Moio and Etievant, 1995). First identified in grape juice in 1921 (Power and Chesnut, 1921) this compound has become a major compound used in the fragrance of perfumes and various cosmetics and it is the chief grape flavour compound in food, used extensively in the flavouring of soft drinks and of powder drinks (Wang and Luca, 2005).

It is also understood that volatile phenylpropanoids are contributed to wines through contact with other external sources. The most commonly recognised source is oak barrel maturation (*Quercus* sp.) (Spillman et al., 2004a, 2004b, Prida and Chatonnet, 2010) with over 50 volatile phenylpropanoids identified in the smoke from pyrolysed oak (Guillén and Manzanos, 2002). More recently it has been established that various phenylpropanoids can be passed onto grapes through exposure to smoke events (Kennison et al., 2007, Kennison et al., 2008, Hayasaka et al., 2010b) and these phenylpropanoids can also be released from non-volatile complexes in grape juices and wines (Kennison et al., 2008, Hayasaka et al., 2010b).

Saccharomyces cerevisiae metabolise aromatic amino acids, including phenylalanine and tyrosine, to produce substituted phenylpropanoids such as phenylethyl alcohol and 2-phenylethyl acetate (Rossouw et al., 2008, Rossouw et al., 2009) which are considered to play an important role in white wine aroma as they are typically found at concentrations above odour threshold (Guth, 1997a, López et al., 2003). Other phenyl-ethyl esters have been observed to change during maturation, for example previous research has identified that diethyl succinate increases with wine age in Airen white wines (Gonzalez-Viñas et al., 1996) and Spanish Cava (Francioli et al., 2003, Riu-Aumatell et al., 2006). However, other research has indicated that this increase in white wines does not occur at cooled storage temperatures of 0-5 °C over a 12 month period (Marais and Pool, 1980, Pérez-Coello et al., 2003). *Brettanomyces* sp. and its ascosporogenous form *Dekkera*, are well recognised for contributing to the volatile phenylpropanoid content of wines by breaking down hydroxycinammic acids (HCA) to vinyl and subsequently ethyl phenols (Chatonnet et al., 1992). *Brettanomyces* and *Dekkera* yeast use a phenolic acid decarboxylase (PAD) enzyme which converts HCAs to their vinyl derivatives, which are the substrates of a second enzyme, vinylphenol

reductase (VPR), whose activity results in the formation of ethylphenols (Harris et al., 2008). The enzymatic step that converts HCAs to their vinyl derivatives is present in a large number of bacteria, fungi, and yeast, but under oenological conditions it is predominantly *Brettanomyces* and *Dekkera* yeast that are capable of producing ethylphenols (Chatonnet et al., 1993, Suárez et al., 2007). Other studies have identified a number of lactic acid bacteria that are capable of decarboxylating PADs to vinyl phenols while very few are capable of forming ethyl phenols (Chatonnet et al., 1995, Couto et al., 2006), such as 4-ethylphenol and 4-ethylguiacol, which are considered to be detrimental to consumer acceptability of wine (Lattey et al., 2010).

Collectively, volatile phenylpropanoids and benzoids are a diverse group of volatile compounds contributed from a range of sources which make significant contributions to wine aroma.

1.3.4. Furanones

Furan derivatives, including furfural, 5-methylfurfural, are typically understood to be formed from pyrolysis of carbohydrates (Guillén and Manzanos, 2002) or from Maillard reactions (Cutzach et al., 1997, 1999) and have been noted to contribute toasty and caramel aromas to wine increasing the overall oak intensity irrespective of their low odour activity values (Prida and Chatonnet, 2010). The concentration of furans, in wine, from oak is dependent on the degree of toasting and oak surface area; however, oak species and seasoning has a varied and limited influence (Chatonnet et al., 1999, Cadahía et al., 2003, Fernández de Simón et al., 2010a, Fernández de Simón et al., 2010b). Other chemical conversions during wine maturation have also been observed such as the conversion of 2-ketobutyric acid, produced from the oxidative degradation of ascorbic acid, to Sotolon, a chiral furanone responsible for premature-aging flavour in dry white wines (Pons et al., 2010).

Furan derivatives have also been observed to play important roles in the aroma of a number of fruits including strawberry (*Fragaria* × *ananassa*) (Schieberle and Hofmann, 1997), blackberry (*Rubus* L. subgenus *Rubus* and *Rubus laciniatus*) (Klesk and Qian, 2003, Du et al., 2010), raspberry (*Rubus idaeus*) (Klesk et al., 2004), guava (*Psidium guajava*) (Steinhaus et al., 2009), and pineapple (*Ananas comosus*) (Tokitomo et al., 2005). One of the more important compounds in these studies, furaneol, was first identified in wines by Rapp and co-workers (Rapp et al., 1980) and has been identified in *Vitis* hybrid varieties including Baco and Villard noir, *V. lambrusca* varieties including Noah, Isabella, and *V. vinifera* including Carignan and Gewürztraminer (De Pinho and Bertrand, 1995, Ong and Acree, 1999). A recent study of Italian *V. vinifera* varieties (Genovese et al., 2005) has also suggested that furaneol is important to the aroma of Refosco and Primitivo (also known as Zinfandel in the US and Crljenak kaštelanski or Pribidrag in Croatia (Maletiæ et al., 2004)). Both furaneol and homofuraneol have low odour thresholds and have an additive and or synergistic role in conveying the fruity and caramel character of rosé wines (Ferreira et al., 2002, Masson and Schneider, 2009).

The furanones are clearly important contributors to wine aroma and have origins from both oak and fruit. However, further research is required to better understand the importance the various potential origins play in the concentrations and the diversity of these compounds in wine.

1.3.5. Fatty acid derivatives

In plants, a number of straight chain alcohols, aldehydes, ketones, acids, esters, and lactones that are derived from fatty acids are formed from α- or β-oxidation or through the lipoxygenase pathway (Schwab et al., 2008). The major aroma compounds derived from fatty acids in grapes tend to be the C_6 aldehydes and alcohols (Ferreira et al.,

1995, Dunlevy et al., 2009, Iyer et al., 2010) some of which are thought to be responsible for 'green' aromas in wines. The C_6 compounds are formed by the action of grape-derived lipoxygenase (LOX), hydroperoxide lyase (HPL), 3Z, 2E enal isomerase and alcohol dehydrogenase (ADH) enzymes which are synthesised, activated or released from compartments separate from their substrates when the grape is crushed (Schwab et al., 2008).

The other major grape-derived compounds with a fatty acid origin that are found in wines are the γ-(4) and δ-(5) lactones which are derived from their corresponding 4- or 5-hydroxy carboxylic acids. However, the enzymes involved in the synthesis of these compounds have not yet been determined in plants (Schwab et al., 2008) and little is known about their formation in grapes. δ-Lactones are generally discounted in importance compared to the λ-lactones which tend to have odour thresholds an order of magnitude lower for compounds of a similar molecular weight (Ferreira et al., 2000). Both γ- and δ- lactones have been identified in wine. However, their contribution to wine aroma has yet to be confirmed with one recent study suggesting that, although no single γ-lactone was found at concentrations above its odour threshold, they may contribute to the aroma of wine through synergistic effects (Cooke et al., 2009). In contrast, studies have correlated γ-nonalactone with aromas of prune in aged red wine (Pons et al., 2008) and numerous studies have correlated γ- and δ- lactones with the aroma of Botrytised wines from Sauternes (Bailly et al., 2009), Barsac, Loupiac (Sarrazin et al., 2007a), Campania (sweet Fiano wines) (Genovese et al., 2007), and Hungary (Tokaji Aszú) (Miklósy and Kerényi, 2004).

As less is known about the origins of fatty acid derivatives, compared to other grape derived volatile compounds, further research is warranted to better understand their formation and contribution to wine aroma.

1.3.6. Volatile acids, esters, and higher alcohols

It is well understood that yeast and bacteria derived volatile metabolites, which comprise volatile fatty acids, esters, higher alcohols, and carbonyls are derived from sugar and amino acid metabolism (Swiegers et al., 2005a). Many of these compounds can be produced by plants (Schwab et al., 2008), but wine research has focused on the contribution of microflora as the majority of volatile fatty acids, esters, and higher alcohols are absent in grape must and are produced during the fermentation process (Bell and Henschke, 2005, Swiegers et al., 2005a).

1.3.6.1. Volatile fatty acids

Yeasts produce short, medium, and long chain fatty acids with the short (fewer than 6 carbons) and medium (6-12 carbons) fatty acids comprising the volatile fatty acids. The majority of fatty acids produced by yeast are of the long chain type (longer than 12 carbons), specifically palmitic (C16) and stearic (C18) fatty acids (Tehlivets et al., 2007). However, these are too large to contribute to the aroma of wine. The short chain fatty acid acetic acid (C2) accounts for more than 90% of the volatile fatty acids in wine and is formed as a metabolic intermediate in the synthesis of acetyl-CoA from pyruvic acid (Bell and Henschke, 2005).

Short chain fatty acids potentially contributing to wine flavour include the branched chain fatty acids isobutyric and isovaleric acid and the straight-chained butyric, and propanoic acids (Francis and Newton, 2005), but the role these compounds play in wine sensory characteristics has not been studied extensively. However, isobutyric and isovaleric acids have been noted as markers of *Brettanomyces bruxellensis* spoilage and are thought to be capable of masking the ‘‘Brett character” attributed to 4-ethylphenol and 4-ethylguiacol which is somewhat counter-intuitive (Romano et al., 2009).

The medium chain fatty acids hexanoic (C6), octanoic (C8), decanoic (10) also contribute to wine aroma (Francis and Newton, 2005) and are dependent on anaerobic growth conditions, must composition, grape cultivar, yeast strain, fermentation temperature, and winemaking practices (Edwards et al., 1990, Bardi et al., 1999). Medium chain fatty acids are correlated with stuck and sluggish fermentations as they are inhibitory to *S. cerevisiae* as well as to some bacteria (Bisson, 1999). The inhibitory effect of medium chain fatty acids usually occurs under conditions of low pH, low temperature, and high ethanol concentrations (Viegas and Sá-Correia, 1995, 1997). However, another study has suggested that cell growth is arrested because fatty acid biosynthesis is prevented by the lack of oxygen and that elevated medium chain fatty acids are not the primary cause of stuck fermentation (Bardi et al., 1999).

1.3.6.2. Esters

It is widely understood that esters and acetates contribute to and enhance sweet fruity aromas in wines. For example, compounds including phenylacetaldehyde, ethyl cinnamate, ethyl dihydrocinnamate, 2-phenylethyl acetate in combination with linalool have been noted to enhance ripe fruit, honey, and sweet characters in neutral red wines (Escudero et al., 2007). Another recent study suggested that, in Bordeaux red wines, higher than average levels of ethyl propanoate, ethyl 2-methylpropanoate, and ethyl 2-methylbutanoate were involved in black-berry aromas while ethyl butanoate, ethyl hexanoate, ethyl octanoate, and ethyl 3-hydroxybutanoate conferred red-berry aromas (Pineau et al., 2009). It is of interest that most of these ethyl esters and acetates can also be found at similar or higher concentrations in white wines when compared to red wines (Guth, 1997b, Ferreira et al., 2000, Francis and Newton, 2005) suggesting that other intrinsic factors, such as the non-volatile wine matrix, play a role in releasing volatiles and defining the perception of these aromas (Pineau et al., 2007, Sáenz-Navajas et al.,

2010). This phenomenon has recently been investigated by Sáenz-Navajas and co-workers (2010) who assessed the perception of various reconstituted red and white wine samples suggesting that the non-volatile matrix exerts a powerful influence on the aroma perception of wine of a magnitude comparable to that of the volatile composition.

The most important esters and acetates in wine are considered to be the fatty acid ethyl esters and acetates including ethyl acetate, ethyl butyrate, ethyl hexanoate, ethyl octanoate, ethyl decanoate, hexyl acetate, isoamyl acetate, isobutyl acetate, and phenylethyl acetate (Guth, 1997a, Ferreira et al., 2000, Francis and Newton, 2005, Swiegers et al., 2005a). Esters are generally considered to be products of yeast metabolism through lipid and acetyl-CoA metabolism (Swiegers et al., 2005a). However, esters can also be produced through bacteria metabolism and chemical modifications. For example, ethyl lactate is known to be directly linked to the concentration of lactic acid produced through malolactic fermentation (MLF) (de Revel et al., 1999, Pozo-Bayón et al., 2005, Boido et al., 2009).

A number of studies have observed changes in ester concentrations in wines during maturation. For example, previous research has identified that diethyl succinate increases with wine age in Airen white wines (Gonzalez-Viñas et al., 1996) and Spanish Cava (Francioli et al., 2003, Riu-Aumatell et al., 2006). However, other research has indicated that this increase in white wines does not occur at cooled storage temperatures of 0-5 °C over a 12 month period (Marais and Pool, 1980, Pérez-Coello et al., 2003). Pérez-Coello and co-workers (Pérez-Coello et al., 2003) observed a decrease in ethyl esters and acetates during uncontrolled storage conditions and times (1, 2, 3, and 4 years and recently bottled wines) and as with Marias and Pool (Marais and Pool, 1980), found that wines that were stored chilled (0 and 10 °C) underwent fewer chemical

alterations thus retaining their youthful wine aromas. The loss of fruity and floral aromas in young white wine during storage is associated with the hydrolytic loss of acetates and esters (Marais and Pool, 1980, Ramey and Ough, 1980, Pérez-Coello et al., 2003) with similar results having been observed in red wines (Ough, 1985).

1.3.6.3. Alcohols

S. cerevisiae produces the majority of higher alcohols from sugar metabolism, producing α-keto acid precursors from pyruvate and acetyl CoA via the tricarboxylic acid (TCA) cycle (Crowell et al., 1961, Bell and Henschke, 2005, Swiegers et al., 2005a). Alternatively, higher alcohols are produced when the yeast catabolise amino acids via the Ehrlich pathway (Bell and Henschke, 2005, Swiegers et al., 2005a). Via this pathway the amino acids are completely consumed early during the yeast growth phase producing the corresponding higher alcohols later during the yeast stationary phase (Bell and Henschke, 2005, López-Rituerto et al., 2010).

The branched chain higher alcohols, including isoamyl alcohol and isobutyl alcohol, are synthesised from the branched chain amino acids, including leucine and valine, and have whiskey/malt/burnt and wine/solvent/bitter aromas respectively (Francis and Newton, 2005). The aromatic amino acids, including phenylalanine and tyrosine, produce aromatic alcohols, such as phenylethyl alcohol (Rossouw et al., 2008, Rossouw et al., 2009), which has a honey/spice/rose/lilac aroma (Francis and Newton, 2005) and is considered to play an important role in white wine aroma as it is typically found at concentrations above odour threshold (Guth, 1997a, López et al., 2003).

1.3.6.4. Factors influencing the production of fermentation-derived volatiles

Nitrogen plays an important role in the formation of volatile fatty acids, esters, and higher alcohols as research has identified that the concentration and speciation of assailable nitrogen is important in defining the volatile metabolites produced by yeast

(Hernández-Orte et al., 2002, Hernández-Orte et al., 2005). In turn numerous studies have identified that the addition of nitrogen to vineyards generally results in an increase in higher alcohols and esters in the resultant wines (Bell and Henschke, 2005). It has subsequently been suggested that, as these nitrogenous substrates are predominantly grape-derived, that the production of yeast-derived volatiles is essentially dependent, or at least modulated, by the initial grape composition (Keyzers and Boss, 2010).

Further to this, it is recognised that fermentation temperature plays an important role in the formation of yeast derived volatile compounds (Molina et al., 2007) and has an influence on the sensory characteristics of both white and red wines (Reynolds et al., 2001). There have also been a number of studies that have indicated that different yeast strains influence the volatile composition and subsequently the aroma of wine (Torrens et al., 2008, Callejon et al., 2010).

Clearly this is a complex area of research that explores elements of grape composition, in defining what is available for yeast metabolism, at the same time assessing how fermentation conditions, such as temperature, and yeast metabolism define the compliment of fermentation volatiles. Future research in this field will benefit from defining not only the differences observed but the magnitude of these variations in an effort to understand the critical operation points available to manipulate wine composition.

1.3.6.5. Pyrazines

The 3-alkyl-2-methoxypyrazines, including 2-isobutyl-3-methoxypyrazine (IBMP), 2-isopropyl-3-methoxypyrazine (IPMP), and sec-butyl-2-methoxypyrazine (SBMP) are often described as imparting sensory characteristics such as bell pepper, asparagus or pea (Sala et al., 2000), and are detectable at ng/L concentrations (Kotseridis et al., 1998). Initially identified in bell pepper (*Capsicum annum* var. *grossum*) (Buttery et al.,

1969), these green flavoured pyrazines also occur in chilli (*Capsicum annuum* var. *annuum*) (Mazida et al., 2005), pea (*Pisum sativum*) (Jakobsen et al., 1998), potato (*Solanum tuberosum*) (Oruna-Concha et al., 2001), and cheese (Neta et al., 2008). Although analytical detection of these compounds at such low concentrations has made their investigation difficult, it is now common knowledge that methoxypyrazines play an important role in the aroma of both the juice and wine of Sauvignon Blanc (Allen et al., 1991, Lacey et al., 1991) Cabernet Sauvignon (Allen et al., 1990, Allen et al., 1994), Cabernet Franc (Roujou de Boubée et al., 2000), Merlot (Sala et al., 2000), and Carmenere (Belancic and Agosin, 2007). Further to this, methoxypyrazines have been found at levels below their odour threshold in unripe Pinot noir, Chardonnay, and Riesling (Hashizume and Samuta, 1999).

It has been suggested that that the enzymatic methylation of hydroxypyrazine precursors to methoxypyrazines by *O*-methyltransferases (OMT) is an important factor in determining the level of methoxypyrazine accumulation in grape berries (Hashizume et al., 2001, Dunlevy et al., 2010). Recent research showed that the relative expression of *VvOMT1* in the skin and flesh tissue of Cabernet Sauvignon grapes was highest between 4 and 8 weeks post-flowering and declined to lower levels post-veraison (Dunlevy et al., 2010), and this coincided with the accumulation of methoxypyrazines in these berries. It is important to note that although methoxypyrazines occur in other grapevine tissues including the rachis (bunch stem), flowers, tendrils and roots, but not the leaves (Dunlevy et al., 2010), they are not translocated from these tissues to the fruit (Koch et al., 2010).

Most studies have addressed the management of alkyl methoxypyrazines through viticultural practices (Chapman et al., 2004b, Sala et al., 2004, Falcão et al., 2007) with particular emphasis on cluster light interception (Hashizume and Samuta, 1999) as

research has indicated that the content of alkyl methoxypyrazines in the wine depended primarily on the composition of the grapes (Roujou de Boubée et al., 2002). The exception to this proposition has been in the study of ladybug taint which is the contribution of 3-isopropyl-2-methoxypyrazine (IPMP), extracted from *Harmonia axyridis* (Pallas) (Galvan et al., 2008), which can increase the peanut, asparagus/bell pepper, and earthy/herbaceous aromas in red wines (Pickering et al., 2004).

Ryona and co-workers (Ryona et al., 2010) have recently proposed that IBMP is demethylated to 3-isobutyl-2-hydroxypyrazine (IBHP) during fruit maturation effectively reversing the final putative step of IBMP biosynthesis. This was based on the observation that IBMP is negatively correlated to stage of maturity and IBHP in bell peppers and in Cabernet Franc and Riesling grapes and that the loss of IBMP post-veraison was accompanied by an increase in IBHP (Ryona et al., 2010). However, without an explanation of the mechanism for the results of Ryona and co-workers (Ryona et al., 2010), these observations could also be attributed to the reduced expression of OMT in grapes post-veraison allowing IBHP to accumulate in the fruit while IBMP is reduced due to photodegradation as has been previously observed (Heymann et al., 1986, Hashizume and Samuta, 1999).

Pyrazines have been of particular interest to grape and wine research due to their low odour threshold and their correlation with herbaceous aromas in Cabernet Sauvignon and Sauvignon Blanc, both abundantly planted and important grape varieties in the international market. Further research into understanding the formation of these compounds in grapes will lead to a better ability to manipulate their concentration in wines for specific market segments.

1.3.7. Volatile sulphur compounds

Originally sulphur containing volatile compounds were associated with malodours mainly due to molecules such as hydrogen sulphide (H_2S), methylmercaptan (methanthiol), ethanethiol, and methionol. However, this is no longer the case with the discovery of a number of volatile thiols that impart pleasant herbaceous, fruity, mineral, smoky, and toasty aromas in wine (Dubourdieu and Tominaga, 2009). The major volatile sulphur compounds in wines are H_2S, methanthiol, dimethylmercaptans (dimethylsulphide, dimethyldisulphide, dimethyltrisulphide), methylthioesters (*S*-methyl thioacetate, *S*-methyl thiopropanoate, and *S*-methyl thiobutanoate), and liberated glutathione and cysteine polyfunctional thiols (4-mercapto-4-methylpentan-2-one (4MMP), 3-mercaptohexan-1-ol (3MH), and 3-mercaptohexyl acetate (3MHA)) (Swiegers and Pretorius, 2007, Dubourdieu and Tominaga, 2009, Roland et al., 2010). However, a myriad of other sulphur containing compounds have been identified in wines suggesting that there is still much to be discovered in this area (Mestres et al., 2000, 2002, Bailly et al., 2006, Sarrazin et al., 2007b, Dubourdieu and Tominaga, 2009).

Hydrogen sulphide can be generated by *S. cerevisiae* through the degradation of sulphur-containing amino acids (cysteine and glutathione), the reduction of elemental sulphur, or the reduction of sulphite or sulphate. H_2S production varies across yeast strains and due to the nitrogen status of the juice (Acree et al., 1972, Schutz and Kunkee, 1977, Giudici and Kunkee, 1994, Jiranek et al., 1995, Bell and Henschke, 2005, Linderholm et al., 2008, Kumar et al., 2010). It is generally understood that the addition of nitrogen, in the form of amino acids, with the exception of cysteine, or ammonium can reduce the production of H_2S by yeast. This is because these sources of nitrogen are precursors for *O*-acetylserine or *O*-acetylhomoserine synthesis which are

important in the synthesis of cysteine, methionine, and glutathione (Giudici and Kunkee, 1994, Jiranek et al., 1995, Linderholm et al., 2008). It has been demonstrated that the activity of *O*-acetylserine/*O*-acetylhomoserine sulfhydrylase (the enzyme responsible for incorporating reduced sulphur into organic compounds) is not the only factor important for reducing H_2S production, but rather the activity of a complement of enzymes involved in the synthesis of *O*-acetyl-L-homoserine and homocysteine that help to reduce H_2S production in *S. cerevisiae* (Spiropoulos and Bisson, 2000, Linderholm et al., 2008).

Methionine and cysteine are thought to be regulators of the sulphur reduction pathway. However, it is thought that, under the anaerobic conditions experienced during fermentation, cysteine concentrations may play a more important regulatory role in sulphate reduction (Linderholm et al., 2008). It has been shown that yeast respond to the addition of cysteine by increasing the production of H_2S in preference to methionol while the addition of methionine results in an increase in methionol in preference to H_2S (Moreira et al., 2002). This could be partly attributed to cysteine inhibiting serine *O*-acetyltransferase which lowers the cellular concentration of *O*-acetylserine required for induction of the sulphate reduction pathway (Ono et al., 1996, Ono et al., 1999) and / or cysteine repressing the genes which encode cystathionine β-synthase and cystathionine γ-lyase in addition to the genes involved in the sulphate reduction pathway (Hansen and Francke Johannesen, 2000). A number of other volatile sulphur compounds can be formed from reactions of H_2S with other organic compounds, for example ethanol or acetaldehyde and H_2S forms ethanethiol (Swiegers et al., 2005a). Dimethylsulfide (DMS) has been noted to increase black olive, truffle, and undergrowth sensory attributes in Syrah wines (Segurel et al., 2004) and has also been demonstrated to enhance the fruit aroma of red wines, which may be a function of complex

interactions with other volatile compounds including esters and norisoprenoids (Segurel et al., 2004, Escudero et al., 2007). However, it has not been thought to positively contribute to white wine aroma, enhancing asparagus, corn, and molasses characters although this could be considered 'complexing' (Goniak and Noble, 1987). DMS, along with methionol, diethyl sulphide, and diethyl disulphide increase in wine with age and with increased temperature and may contribute to the aroma of aged wines (Marais, 1979, Simpson, 1979, Fedrizzi et al., 2007).

Methionol, contributes to the raw potato or cauliflower character of wines, can be found in wines at concentrations up to 5 mg/L and is produced by either *S. cerevisiae* or *Oenococcus oeni* through the catabolism of methionine (Moreira et al., 2002, Ugliano and Moio, 2005, Vallet et al., 2008, Vallet et al., 2009). Methional, contributing to the cooked vegetable aroma of oxidised wines (Escudero et al., 2000), increases in white wines that are exposed to elevated temperatures and oxygen via a Strecker degradation of methionine to methional in the presence of a dicarbonyl compound or via direct peroxidation of methionol (Escudero et al., 2000, Silva Ferreira et al., 2002).

The more recently studied group of sulphur containing compounds are the polyfunctional thiols which impart pleasant fruity aromas to a range of varieties including Scheurebe, Sauvignon Blanc, Gewürztraminer, Riesling, Colombard, Petit manseng, Semillon, Cabernet Sauvignon, and Merlot (Darriet et al., 1995, Guth, 1997b, Tominaga et al., 1998, Tominaga et al., 2000a, Murat et al., 2001). These compounds have received significant attention in recent years as they are thought to be important to the varietal characteristics of wine aroma and are noted for low odour thresholds with 4MMP, 3MH, and 3MHA being detectable in wine at concentrations of parts per trillion (Tominaga et al., 1998, Tominaga et al., 2000a, Francis and Newton, 2005, Swiegers et al., 2005a, Swiegers and Pretorius, 2007, Dubourdieu and Tominaga, 2009).

For a long time the conjugated thiols were thought to be formed from the cysteine conjugates, but more recently it has been shown that glutathione precursors are an equally, if not more, important source of these pleasant smelling polyfunctional thiols (Subileau et al., 2008, Fedrizzi et al., 2009, Capone et al., 2010b, Grant-Preece et al., 2010, Roland et al., 2010). A recent study documented that the glutathione conjugated 3MH diastereomers were up to 35 times more abundant than their cysteine conjugated counterparts in juices of Sauvignon blanc, Riesling, Chardonnay, and Pinot Grigio with Sauvignon Blanc juices generally having the highest concentrations of the varieties studied (Capone et al., 2010b).

The conjugated thiols are produced in the grape, but there is little known about the mechanisms involved in their biosynthesis. One study has assessed the cysteine conjugated precursors of 4MMP, 4-mercapto-4-methylpentan-2-ol (4MMPOH), and 3MH in Sauvignon Blanc and identified that these precursors accumulate with increasing grape maturity (Des Gachons et al., 2000) and can vary due to site (Des Gachons et al., 2005). However, more viticultural research could be conducted to better understand the formation of these conjugated polyfunctional thiols in grapes.

The focus of grape and wine research has been on the release of polyfunctional thiols during fermentation, due primarily to the fact that yeast have had limited and varied capacity to liberate the polyfunctional thiols from their precursors (Subileau et al., 2008, Capone et al., 2010b). For example, estimates from the literature vary from 0.1-12% conversion of cysteine-3MH to 3MH (and 3MHA) (Subileau et al., 2008) representing only a small fraction of the polyfunctional thiols present in juice and leaving significant pools of both cysteine and glutathione precursors in finished wines (Capone et al., 2010b). It has been established that yeast strains having carbon-sulphur β-lyase activity release these polyfunctional thiols during fermentation (Howell et al.,

2005, Swiegers et al., 2007, Ugliano, 2009). However, the level of carbon-sulphur β-lyase activity varies due to yeast strain suggesting that yeast selection can be used, in part, to control the polyfunctional thiol content of wine (Dubourdieu et al., 2006, Swiegers and Pretorius, 2007).

It has been mentioned that 3MH decreases rapidly in red wines stored in barrel as it oxidises easily and is highly reactive with quinones (Dubourdieu and Tominaga, 2009). Further to this, it is well known that winemakers commonly use copper sulphate to remove H_2S from wines at the conclusion of fermentation or just prior to bottling, which has recently been noted to reduce 3MH in bottled Sauvignon Blanc (Ugliano et al., 2010). Future research into the longer term stability of these polyfunctional thiol compounds and potential reactivity with additives and fining agents is warranted.

Another group of pleasant smelling thiols are the sulphur containing furans including 2-methyl-3-furanthiol and 2-furanmethanethiol which may contribute to the toasty and roast coffee aroma characteristics of oak matured wines including Sauvignon Blanc, Chardonnay, Merlot, Cabernet franc, and Cabernet Sauvignon, and sweet Petit manseng wines (Tominaga et al., 2000b, Tominaga and Dubourdieu, 2006). The volatile sulphur compounds are a diverse group of highly odour-active compounds with multiple influences contributing to their presence in wine. They clearly make an important contribution to wine aroma and given the number of sulphur compounds identified in wine over the last decade, there is potentially still much to be discovered in this area (Dubourdieu and Tominaga, 2009).

1.3.8. Glycosylated aroma precursors

Glycosylated aroma precursors consist of a glycopyranosyl- (sugar moiety) and an aglycone (non-sugar moiety) linked by a β-glycosidic linkage (Williams, 1993). Glycosylated aroma precursor compounds have been identified in almost 170 plants

across nearly 50 families (Chassagne et al., 1998) and in a wide range of plant tissues including leaves, fruit, roots, petals, needles, woody tissues, and seeds (Winterhalter and Skouroumounis, 1997). Glycosylated aroma precursor content has also been assessed in numerous fruits including apricot (*Prunus armeniaca*) (Krammer et al., 1991, Salles, 1991), lulo (*Solanum quitoense*) (Duque et al., 2002), lychee (*Litchi chinensis*) (Chyau et al., 2003), mango (*Mangifera indica*) (Adedeji et al., 1992), mammea apple (*Mammea americana*) (Duque et al., 2002), peach (Krammer et al., 1991), quince (*Cydonia oblonga*) (Winterhalter and Schreier, 1988), and tomato (*Solanum lycopersicum*) (Buttery et al., 1990a, Marlatt et al., 1992). Plants produce a range of secondary metabolites including cannabinoids, flavonoids, diterpene sclareol, alkaloids, benzoxazinones, phenylpropanoids, cyanogenic glycosides, and glucosinolates as a self defence mechanism against pathogens and herbivores (Sirikantaramas et al., 2008). It has been suggested that glycosylation of lipophilic aroma compounds and subsequently compartmentalisation (Hardie et al., 1996) acts as a detoxification mechanism in plants (Sirikantaramas et al., 2008).

A number of reviews have dealt with the field of glycosylated aroma precursors in grapevine (Günata et al., 1993, Stahl-Biskup et al., 1993, Williams, 1993, Winterhalter and Skouroumounis, 1997). Commonly in grapes, a disaccharide forms with either α-L-arabinofuranosyl-, α-L-rhamnopyranosyl-, β-D-xylopyranosyl-, β-D-apiofuranosyl-, or β-D-glucopyranosyl- linked to position 6 of the glucose (Williams, 1993). This is of particular interest as all anthocyanin glycosides are found as mono-glycosides in *V. vinifera* (Ribéreau-Gayon, 1974) suggesting that aroma glycosylation occurs via specific pathways separate to that which glycosylates anthocyanins. The glycosylation of flavour compounds has been thought to increase the water solubility of the aglycone to improve storage in vacuoles (Hardie et al., 1996) and transport within the plant

(Winterhalter and Skouroumounis, 1997, Sirikantaramas et al., 2008). However, terpene alcohols and phenols do not always change in solubility due to the glycosylation of their hydroxyl groups (Stahl-Biskup et al., 1993). Günata and co-workers (Günata et al., 2002) found glycoconjugated norisoprenoids in the grape were synthesised independently of those produced in the leaves in Shiraz and Muscat of Alexandria. This indicates that glycosylated aroma precursors are synthesised *de novo* and not translocated to the fruit from other plant tissues.

One important topic of future research in this area will be the functional characterisation of glycosyltransferases (GT's) responsible for the biosynthesis of aroma precursors given the diversity of GT's already identified in other plant species (Lairson et al., 2008). For example, GTs in *Medicago truncatula* have been shown to be capable of glycosylating both flavanoids and triterpenes (Shao et al., 2005), GT's of *Arabidopsis thaliana* have been shown to have activity toward mono-, sesqui-, and diterpenes *in vitro* (Caputi et al., 2008), and, recently, GTs were identified in Valencia orange (*Citrus sinensis* L. Osbeck) capable of glucosylating terpenoids in leaf, flower, and fruit tissues (Fan et al., 2010). It is probable that grape GT's are likely to have similar properties. Various GT's have been identified that appear to be involved in the production of grape flavanoid glycosides (Offen et al., 2006, Ono et al., 2010). However, to date, no information exists regarding GT's involved in the synthesis of glycosylated aroma precursors in grape (Baumes et al., 2002, Mathieu et al., 2009).

It has been established that glycosidically bound aroma compounds are an important reserve of aroma in wine (Williams, 1993). This flavour reserve either evolves over time due to slow acid-hydrolysis (Skouroumounis and Sefton, 2002), or can be released by the application of exogenous fungal glycosidases (Günata et al., 1993). For example, the norisoprenoids 1,1,6-trimethyl-l,2-dihydronaphthalene (TDN) and vitispirane

isomers are typically found in Riesling wines that have been bottle aged (Simpson, 1979) and/or heated (Simpson, 1978) and have been shown to derive from glycosidic precursors (Winterhalter et al., 1990b, Winterhalter, 1991, Full and Winterhalter, 1994). Endogenous grape derived glycosidases (Aryan et al., 1987, Günata et al., 1990), exogenous yeast derived glycosidases (Gunata et al., 1986, Zoecklein et al., 1997, Ugliano et al., 2006), and bacterial glucosidases (Grimaldi et al., 2005b, 2005a) are also considered to play an important role in the release of these aroma precursors in wine but not in the fruit, presumably due to glucose inhibition (Günata et al., 1993) or compartmentation.

Grape derived glycosidases are located in the pulp and juice fraction of the grape (Aryan et al., 1987) and have characteristics similar to those of *S. cerevisiae*. *Saccharomyces* derived β-glucosidase, α-rhamnosidase and α-arabinosidase activity have been observed to increase during the exponential yeast growth phase (first 24 hours of fermentation) and rapidly decrease within the following 3 days (Delcroix et al., 1994). This initial increase is likely to be correlated to the synthesis and excretion of glycosidases by yeast (Delcroix et al., 1994). Glycosidase activity is subject to the influence of pH, temperature, and the presence of ethanol, glucose, phenols, polyphenols, and cations (Günata et al., 1993). Consequently, the impact of glycosidases on the release of aroma molecules from precursors is dependent on the stability and activity of these enzymes in the juice or wine medium. The yeast intracellular pH (5-6) is highly favourable to yeast glycosidase stability (Delcroix et al., 1994). In contrast the activity of *S. cerevisiae* β-glycosidase is reduced by 90% at a pH of 3.0 (similar to juice or wine pH) after 90 minutes (Günata et al., 1993). This reduced activity has also been observed in grape derived β-glycosidase (Aryan et al., 1987). In contrast, β-glucosidase found in *Debaryomyces hansenii* has been observed to have a

similar activity to *Saccharomyces* glycosidase except they are stable at wine pH (Yanai and Sato, 1999). Further, β-glycosidases derived from *Aspergillus niger*, commonly associated with *Botrytis cinerea* bunch rot (Nair, 1985, Zahavi et al., 2000, Hocking et al., 2007), lose only 20% activity under similar conditions suggesting they too are relatively stable at juice and wine pH (Günata et al., 1993). Additional investigation into the stability and activity of other microbially derived glycosidases deserves attention.

Temperature is known to play an important role in the activity of enzymes due to reaction kinetics and enzyme stability. The maximum activity of *S. cerevisiae* derived β-glycosidases occurs at temperatures of 40-50° C (Delcroix et al., 1994) which is similar to the results reported for the *A. niger* enzymes (Günata et al., 1993). Importantly, the activity of these enzymes is relatively low at 30° C (~20% of maximum) and rapidly decreases approaching 60° C (Delcroix et al., 1994). Consequently, hydrolysis of glycosides by β-glycosides is likely to be slow during fermentation and wine storage due to the low temperatures (10-20° C). Ethanol has been observed to significantly reduce the activity of β-glycosidase derived from grape leaves, grapes, and almond emulsin (Aryan et al., 1987). However, yeast derived β-glycosidases from *S. cerevisiae* (Delcroix et al., 1994) and *A. niger* (Aryan et al., 1987) have shown losses of only 10% and 20% activity respectively at concentrations of 15% ethanol.

The activity of β-glycosidase is usually competitively inhibited by the presence of glucose. It is interesting to note that the activity of *S. cerevisiae* derived β-glucosidase is only slightly reduced at glucose concentrations found in juice (Günata et al., 1993, Delcroix et al., 1994). Günata and co-workers (Günata et al., 1993) observed a loss of 30% activity while Delcroix and co-workers (Delcroix et al., 1994) reported a reduction

of only 20% activity. The variation between reported values is likely to be due to variations in experimental conditions such as glucose concentration (90 g/L and 100 g/L respectively). In contrast, *A. niger* derived β-glycosidase is inhibited significantly by glucose. Günata and co-workers (Günata et al., 1993) observed a 38% reduction in activity at concentrations of glucose considered 'dry' in winemaking.

In summary, endogenous glycosidases have poor stability in juice and wine due to the low pH, and their ability to liberate conjugated aroma compounds is significantly inhibited by ethanol concentrations found in wine. In contrast, exogenous glycosidase are more stable at juice pH, barely inhibited by ethanol but are strongly inhibited by glucose. Consequently, a large proportion of glycosides initially present in the grape remain after winemaking. Thus application of commercial enzyme preparations to dry wines or slow acid hydrolysis during wine maturation (Williams, 1993) can have important consequences on the final aroma profile of the wine. Further information about the activity of glucosidases can be found in a recent review (Maicas and Mateo, 2005).

1.4. The role of the grape and grape ripening in wine composition

The idiom that you 'cannot make a silk purse out of a sow's ear' is commonly used to explain that the qualities of wine are indicative of the qualities of the materials, particularly the grapes, used in its production. The simplest illustration of this is that a red wine cannot be made from white grapes and it can be argued that the same premise exists with respect to the aroma and taste characteristics of wine varieties, vintages, regions, and producers. This has been noted by many wine authorities but is well captured by the late Len Evans, Australian wine commentator and wine judge, who borrowed from Gertrude Stein by saying "Perhaps I've been tasting too long but to me

wine smells of itself. A Rose is a rose is a rose. Cabernet from Coonawarra smells like Cabernet from Coonawarra" (Evans, 2007).

Current knowledge about the processes that occur during grape ripening suggests that an optimum level of maturity occurs when flavour accumulation is greatest (Hardie and Obrien, 1988, Coombe and McCarthy, 2000). However, this may be a gross simplification given that the disappearance of undesirable flavour compounds will also be involved. The ripening of grapes involves many processes including translocation, accumulation and metabolism of principal components within the berry:

- The uptake of sucrose from leaves via the phloem followed by its cleavage and storage as D(+)-glucose and D(-)-fructose (Coombe, 1992, Davies and Robinson, 1996),
- Phloem dilution of L-(+)-tartaric acid, synthesised from ascorbic acid pre-veraison (DeBolt et al., 2006), and metabolism of L-(-)-malic acid (Ruffner, 1982b, 1982a, Sweetman et al., 2009),
- Accumulation of amino acids, particularly arganine and proline, coupled to a decline in ammonium (Kliewer, 1968, Stines et al., 2000, Bell and Henschke, 2005),
- Decreased synthesis of phenols, and accumulation of condensed tannins in the skin and seeds (Downey et al., 2003a, Downey et al., 2006),
- Accumulation of flavanols, anthocyanins (in red cultivars), and leuco-anthocyanins (in white cultivars) in the skins (Boss et al., 1996, Dokoozlian and Kliewer, 1996, Downey et al., 2003b), and
- Changes in concentration and diversity of aroma precursors and volatile compounds (Reynolds and Wardle, 1989, Lacey et al., 1991, Razungles et al., 1993, Dunlevy et al., 2009).

Collectively these components characterise the abundant organic compounds found in grapes that are used in wine production. In many cases these components are subject to further biological and chemical modifications through the course of vinification and maturation. However, they essentially establish the basis of wine composition.

1.4.1. Environmental Influences on Grape Aroma Formation

1.4.1.1. Climate

Climate encompasses environmental conditions of sunlight, temperature, humidity and rainfall, all of which play important roles in the growth and development of the vine. The levels of aroma and aroma precursor compounds are found to vary between and within climates (Marais et al., 1991, Schneider et al., 2002). Levels of lutein, β-carotene (Marais et al., 1991) and 1, 1, 6-trimethyl-1, 2-dihydronaphthalene (TDN) (Marais et al., 1992) have been found at higher concentrations in wines from warmer climates (South Africa) compared to cooler climates (Germany). On the other hand, the reverse relationship is observed with higher levels of methoxypyrazines in Sauvignon Blanc from cool climates (New Zealand) compared to warm climates (Australia) (Lacey et al., 1991). These observations may be explained by the variation in hours of sunlight and possibly temperature (Gerdes et al., 2002).

1.4.1.2. Season

The unique conditions of climate vary from year to year and it is commonly accepted worldwide that vintage has a major influence on the composition of fruit. Studies of aroma composition of Chardonnay (Sefton et al., 1993), Sauvignon Blanc (Sefton et al., 1994), Semillon (Sefton et al., 1996), Merlot noir (Kotseridis et al., 1998) and Melon B (Schneider et al., 2001) have confirmed this observation on an analytical level. It follows that the current environmental issue of global warming and climate change is of

major importance to viticulture. This will not be discussed here but has been addressed well in a review by Schultz (Schultz, 2000).

1.4.1.3. Sunlight

It has been suggested that quality and not intensity of light regulates the accumulation of norisoprenoid compounds (Bureau et al., 1998, Schultz, 2000). These studies indicate that exposure to blue-green light (and potentially far red wavelengths) results in synthesis of carotenoids (Bureau et al., 1998) as a photo-protective defence in vines (Young, 1991, Baumes et al., 2002). In addition, degradation of these carotenoids is enhanced by exposure to these same wavelengths of light (Bureau et al., 1998). The subsequent loss of carotenoids in grapes is observed once chloroplasts are lost and carotenoid synthesis ceases (Baumes et al., 2002).

Exposure of fruit to sunlight favours accumulation of glycosylated norisoprenoids, monoterpenes, and other non-terpene aglycones (Reynolds and Wardle, 1989, Gerdes et al., 2002, Schneider et al., 2002). This increased accumulation of glycosides in grapes may be a factor of temperature and light exposure on enzyme activity within the fruit (Gerdes et al., 2002). On the other hand, light exposure has been observed to reduce the concentration of free methoxypyrazines (Hashizume and Samuta, 1999).

1.4.2. Grape Maturity

Carotenoids that accumulate prior to veraison degrade sharply post veraison (Razungles et al., 1988, Marais et al., 1991, Razungles et al., 1993). This rapid degradation occurs for β-carotene, lutein and violaxanthin while neoxanthin levels decrease steadily (Razungles et al., 1996). The subsequent accumulation of norisoprenoids is inversely proportional to the degradation of these carotenoids and positively correlated with sugar accumulation (Strauss et al., 1987b, Razungles et al., 1993). This relationship also exists for monoterpenes (Wilson et al., 1984). Studies of Sauvignon Blanc, Cabernet

Sauvignon, Cabernet Franc, Carmenere, and Merlot have observed a decrease in free methoxypyrazines with maturity (Allen et al., 1990, Lacey et al., 1991, Sala et al., 2000, Belancic and Agosin, 2007). It has been suggested that although changes in concentration of aroma compounds and sugar accumulation occur simultaneously, they may not be mutually dependent (Reynolds and Wardle, 1989).

1.4.3. Water and Canopy Management

Vineyard practices including canopy management and imposed water stress are recommended ways of manipulating fruit light interception, to attain the desired varietal aroma composition. Reduced vine water status is thought to alter carotenoid composition (Oliveira et al., 2003). Water status can influence canopy density (Hardie and Martin, 2000) and consequently fruit light exposure as discussed previously. Crop thinning (a common practice in vineyards) has been observed to increase levels of glycosylated terpenes and aliphatics (Bureau et al., 2000), but no effect has been observed on the concentration of glycosylated norisoprenoids (Bureau et al., 2000). This may be explained by the independent biosynthesis of norisoprenoids in berries rather than their translocation from leaves (Günata et al., 2002).

1.4.4. Pathogenesis

The common fungus, *B. cinerea*, which causes 'Grey bunch rot' of grapes, has been observed to transform monoterpenes (Bock et al., 1988) and norisoprenoids (Schoch et al., 1991) in grape juice. Additionally, glycosidases derived from *A. niger*, although used commonly in analysis of grape glycosides (Winterhalter and Skouroumounis, 1997), are known to generate oxidative artefacts of aromas when present at high concentrations (Sefton and Williams, 1991). Although *A. niger* glycosidase is inactive at high concentrations of glucose (Günata et al., 1993) it does suggest that the

associated infection of *A. niger* with *B. cinerea* (Nair, 1985) could have compounding effects on the varietal aroma composition of infected grapes.

1.4.5. Non-vineyard influences - wine maturation conditions

A loss of fruity and floral aromas in young white wine during storage is associated with the hydrolytic loss of acetates and other esters (Marais and Pool, 1980, Ramey and Ough, 1980, Pérez-Coello et al., 2003). This could also be compounded by the loss of monoterpenes, such as linalool, due to increased storage temperature which has previously been observed in citrus juices (Perez-Cacho and Rouseff, 2008) and has been attributed to the coinciding increase in α-terpineol (Pérez-López et al., 2006). The loss of linalool and increase in α-terpineol has also been observed in heated black currant juice (Varming et al., 2004, 2006). It is suggested that the transformation of linalool to α-terpineol occurs through the protonation of linalool's hydroxyl group (Haleva-Toledo et al., 1999). Under acid conditions, as is the case in wine, it is generally understood that linalool is produced as an intermediate in the formation of α-terpeniol and other products from the thermal degradation of geraniol (Baxter et al., 1978, Skouroumounis and Sefton, 2000). Silva Ferreira and co-workers have previously observed that the degradation of linalool, and formation of linalool oxides, was significantly greater at 45 °C when compared to 15 °C in white wine (Silva Ferreira et al., 2002). The enhancement of aged characters in wine have been correlated with the oxidative formation of methional and phenylacetaldehyde (Silva Ferreira and Guedes De Pinho, 2004) and increases in TDN and vitispirane (Simpson, 1979) due to acid hydrolysis of aroma precursors (Francis et al., 1994, Versini et al., 2002). Silva Ferreira and co-workers have shown that temperature and pH are particularly important to the formation of both TDN and vitispiranes (Silva Ferreira and Guedes De Pinho, 2004). It has also been observed that p-cymene can be produced through heated acid hydrolysis

of aroma precursor fractions from grapes (Williams et al., 1982b, Schneider et al., 2001). Other research has also identified that the low level formation of ethyl carbamate (urethane), primarily from ethanol and urea in wine (Monteiro et al., 1989, Stevens and Ough, 1993, Kodama et al., 1994), follows first order kinetics and is accelerated by storage of wine at high temperatures (Hasnip et al., 2004). These studies emphasize the importance of storage conditions on the maintenance of fresh aromas in wines.

1.4.6. Analytical Chemistry of Aroma & Flavour Precursors

Essential to the understanding of various influences on wine flavour is the ability to purify, identify and measure the compounds responsible for the sensory attributes experienced by a wine consumer. Multiple techniques have been utilised for characterising wine composition including flame atomic absorption spectrophotometry (AAS) and flame atomic emission spectrophotometry (AES) (Frías et al., 2003), inductively coupled plasma mass spectrometry (ICP-MS) (Baxter et al., 1997), liquid chromatography (LC) (Bellomarino et al., 2009), gas chromatography (GC) (Marengo et al., 2002), UV, visible (Vis), near-infrared (NIR) and mid-infrared (MIR) spectroscopy (Liu et al., 2006, Cozzolino et al., 2010), nuclear magnetic resonance (NMR) spectroscopy (Brescia et al., 2002), and electronic nose (EN) (Cynkar et al., 2010). However, the majority of studies assessing volatile aroma compounds have predominantly used GC methods coupled to either a flame ionisation detector (FID), nitrogen phosphorous detector (NPD), or a mass spectrometer (MS) type detector which includes quadropole (QMS), ion trap (ITMS), triple quadrapole (QQQ-MS), or time-of-flight (TOF-MS) style detectors.

1.4.7. Sample preservation

At any stage of sample preparation it is important to preserve the compounds of interest. Essentially control of temperature, oxygen, and the activity of enzymes are the

keys to reducing formation of oxidative artefacts. In addition, preservation of conjugated compounds is important in determining aroma potential. Acid hydrolysis of glycoconjugates is not likely to happen rapidly at juice pH and ambient temperature (Skouroumounis and Sefton, 2000) but cold storage of grapes is preferable. Further, enzyme hydrolysis by most native enzymes is inhibited in juice environments (Günata et al., 1993) but enzyme inhibitors can be added to the extract as a precaution (Razungles et al., 1993). Verhoeven and co-workers have previously documented the formation of Maillard products following the immediate thermal desorption of a liquid SPME sample from strawberry and apple fruit (Verhoeven et al., 1997). This study identified the importance of washing the SPME fibre when conducting liquid SPME analysis of samples high in carbohydrates and or amines. More recently Čajka and co-workers (2007) noted that significantly different chromatograms were formed from honey samples conditioned at temperatures above 60 °C while optimising a HS-SPME method (Čajka et al., 2007).

1.4.8. Liquid extraction methods

In most early studies, volatile and glycosylated aroma precursors were isolated from plant extracts, fruit juice, de-alcoholised wine, and other liquid media either by selective retention on Amberlite XAD resins (typically XAD-2 (Günata et al., 1985)), on C-18 reversed phase silica adsorbent (Williams et al., 1982a), or by simple liquid/liquid extraction. These techniques allow the isolation of aroma and aroma precursor compounds free of sugars and organic acids (Günata et al., 1985). The compounds of interest were selectively eluted with organic solvents of varied polarity (Mateo et al., 1997, Guyot-Declerck et al., 2000). Once these eluates are collected they can be dried and concentrated for analysis. These were simple and effective methods.

However, there is little scope for automation limiting sample sizes and the methods involve contact with potentially hazardous organic solvents.

1.4.9. Static headspace (SHS) and dynamic headspace (DHS) methods

The greatest advantage of SHS and DHS methodologies is that they directly sample the volatile composition of the sample headspace which can then be directly related to the aroma of the sample. SHS involves sampling the headspace, at equilibrium, typically using a syringe while DHS involved flushing the headspace of the sample vial with inert gas. In either case the liberated volatiles are usually captured in a cold trap or adsorbent such as Tenax prior to injection onto a GC (Rosillo et al., 1999). SHS has been used effectively to determine partition coefficients of analytes in aqueous ethanol solutions (Conner et al., 1994, Conner et al., 1998, Athès et al., 2004). These methods are simple but have poor reproducibility, they have bias towards high and medium volatile compounds, have limitations in detecting trace analytes, and are often unrepresentative of the sample composition (Ortega-Heras et al., 2002). SHS and DHS are less sensitive and less selective methods for headspace analysis when compared to solid-phase microextraction (SPME) (Kataoka et al., 2000).

1.4.10. Headspace Solid-phase Micro Extraction (HS-SPME)

HS-SPME has been increasingly utilised in volatile flavour analysis since it was introduced as a technique by Janusz Pawliszyn in the 1990's (Arthur and Pawliszyn, 1990, Arthur et al., 1992, Zhang and Pawliszyn, 1993, Pan et al., 1995, Steffen and Pawliszyn, 1996). The primary advantage of this technique is that it combines analyte extraction and pre-concentration in a single step without significant sample preparation. A number of grape and wine profiling studies have used HS-SPME to better understand the role of various compounds in differentiating varieties, regions, and wine vintage (Marengo et al., 2002, Câmara et al., 2007, Setkova et al., 2007c) and the technique has

been repeatedly documented as a sensitive, reproducible, automated method for pre-concentration of wine volatiles prior to analysis (Howard et al., 2005, Câmara et al., 2006, Setkova et al., 2007b). Various parameters are routinely optimized in the development of HS-SPME techniques for the analysis of ethyl esters, acetates, acids and alcohols (Siebert et al., 2005), monoterpenes, and norisoprenoids (Câmara et al., 2006), methoxypyrazines (Hartmann et al., 2002), thiols, sulphides, and disulphides (Mestres et al., 1999a, Mestres et al., 1999b), furfural derivatives, phenolic aldehydes, volatile phenols, and oak lactones (Carrillo et al., 2006) in wine. Most methods described within the literature explore parameters such as fibre type, sample temperature, salt concentration, agitation speed, and extraction time as part of method development and optimisation (Sala et al., 2000, Rocha et al., 2001, Silva Ferreira and Guedes De Pinho, 2003, Howard et al., 2005, Câmara et al., 2006, Carrillo et al., 2006, Setkova et al., 2007b). This agrees with a protocol for SPME method development that has recently been published (Risticevic et al., 2010). The following sections address the relevant parameters that need to be considered for wine sample preparation for HS-SPME volatile analyses.

1.4.10.1. SPME Fibre type

SPME fibres are coated with various single or mixed polymers that vary in polarity, thickness, and length. Firstly, the mechanisms of extraction differ between single or liquid phases, which absorb analytes into the entire fibre coating, and mixed or solid phases, which adsorb analytes to the surface of the fibre coating. This has implications with regards to sensitivity and time to reach equilibrium (Risticevic et al., 2010). Secondly, the polarity of the fibre coating allows the user to target specific compounds based on their affinity for the fibre understanding the principle that ‘like dissolves like’ (Risticevic et al., 2010). Common phases, or mixtures of, that are used for the analysis

of volatile compounds in wine include polydimethylsiloxane (PDMS), polyacrylate (PA), divinylbenzene (DVB), and carboxen (CAR) (Rocha et al., 2001, Howard et al., 2005, Setkova et al., 2007b, Risticevic et al., 2010).

1.4.10.2. Sample temperature

Increasing the sample temperature can increase analyte partitioning into the headspace and thus increase the sensitivity of the HS-SPME method (Risticevic et al., 2010). However, it has also been observed that elevated temperature can modify monoterpenes (Varming et al., 2004, 2006, Perez-Cacho and Rouseff, 2008), esters and acetates (Marais and Pool, 1980, Ramey and Ough, 1980, Pérez-Coello et al., 2003), and release volatiles from glycosylated aroma precursors (Silva Ferreira and Guedes De Pinho, 2004). While no studies to date have addressed the role of temperature in modifying volatiles in a wine sample, it has been recently noted that different chromatograms were observed from honey samples that had been conditioned at temperatures above 60 °C, compared to those kept at room temperature, while optimising a HS-SPME method (Čajka et al., 2007). It is possible that similar results could occur if wine or grape juice samples were also heated. This aspect of HS-SPME method development needs to be addressed. However, when trying to relate HS-SPME results to data obtained by sensory panels, it would seem sensible to use temperatures between 30-40 °C to align with conditions experienced by the tasters.

1.4.10.3. Salting out

The salting-out or Setschenow effect describes how a non-electrolyte, in this case an organic volatile compound, decreases in solubility following the addition of an electrolyte to the solution (Mazo, 2006), in this case sodium chloride. It is interesting to note the reported concentration of salt considered optimal varies between 100 and 350 g/L for wine samples (De La Calle García et al., 1998, Rocha et al., 2001, Azenha and

Vasconcelos, 2002, Rodríguez-Bencomo et al., 2002, Castro Mejías et al., 2003, Demyttenaere et al., 2003, Castro et al., 2004, Siebert et al., 2005, Câmara et al., 2006, Setkova et al., 2007b). Given that the Setschenow effect is related to the preferential association of electrolytes with the solvent with respect to the non-electrolyte solute, it can be assumed that once the electrolyte reaches saturation the further addition of electrolyte will not cause a greater effect. By extrapolating from the raw data presented by Farelo and co-workers (Farelo et al., 2004) it is observed that in a 13% ethanol solution at 30 °C sodium chloride reaches saturation at ~274 g/L. Dry white or red table wines are characterised by an alcohol content ranging between 10 and 15% ethanol by volume where sodium chloride is saturated at 292 and 262 g/L, respectively. Thus, concentrations of sodium chloride between 250 and 300 g/L will generally accommodate the alcohol content of wine products at or around ambient temperatures.

1.4.10.4. Sample agitation

Mechanical agitation plays an important role in accelerating mass transfer of molecules from the liquid into the headspace. First, it works by increasing mixing of molecules within the liquid, creating a relatively homogenous mixture at any point in time (Zhang and Pawliszyn, 1993). Second, agitation increases the rotational velocity of the liquid forcing the liquid towards the sides of the container and thus increasing the liquid-gas interface surface area. Zhang and Pawliszyn (Zhang and Pawliszyn, 1993) discussed the rate of diffusion from the liquid to the headspace with respect to Fick's first law, refer to Equation 1.1.

Equation 1.1. Fick's Law of diffusion

$$J = -D\frac{\Delta\phi}{\Delta x}$$

where J is the diffusive flux, D is the diffusion coefficient, ϕ is the concentration, and x is the position.

The rate of agitation produces turbulent diffusion in the liquid and increases the exchangeable surface area with the gas phase. Zhang and Pawliszyn (1993) indicated that the diffusive flux of a compound was dependent on the concentration gradient but they did not address the diffusion coefficient in great detail. The rate of mass transfer between liquid and gas is directly proportional to the area of the gas-liquid interface. As agitation speeds increase, the surface area is maximised such that it is approximately equal to the internal circumference of the vessel by the height, refer to Equation 1.2.

Equation 1.2. Difference in surface area from stationary to highly agitated

$$\Delta A \approx \frac{H}{0.5r}$$

where ΔA is the change in surface area, H is the height of the vessel, and r is the internal radius of the vial.

As a consequence, the greater the agitation speed the greater the effective surface area for the transfer of volatiles across the gas-liquid interface. In addition to this, a higher level of agitation will result in a constant concentration of volatiles at the gas-liquid interface due to continuous effective mixing.

1.4.11. Extraction time

The extraction time is the major limiting step in HS-SPME method development, where the objective is to establish an equilibrium point between the SPME fibre and the headspace.

There are three scenarios that are generally considered (Risticevic et al., 2010);

- High throughput methods require that the extraction time is proportional to the separation and detection time requirements i.e. if the user intends to complete a chromatographic run in 5 minutes (Setkova et al., 2007b) then the extraction time is only likely to be as long or slightly longer than 5 minutes (taking into account cool down time). Short extraction times are usually pre-equilibrium

conditions and are subject to time control related errors thus, automation control is essential (Risticevic et al., 2010).

- High sensitivity methods may require longer extraction times to establish equilibrium between the SPME fibre and the headspace for analytes with higher molecular mass due to slower transfer rates. In some instances HS-SPME extraction times of 120 minutes have been used for wine volatile analysis (Câmara et al., 2006).
- Good reproducibility is paramount in quantitative and semi-quantitative analysis and thus equilibrium conditions should be used as these reduce timing related errors. The exception to this is where precise automation is available and the user can demonstrate that the errors incurred due to timing are minimal (Risticevic et al., 2010).

If we consider Graham's Law of diffusion, Equation 1.3;

Equation 1.3 Graham's Law of diffusion

$$K_m = \frac{1}{2}.m_m.v_m^2$$

where K_m is the kinetic energy of the molecule, m_m is the mass of the molecule, and v_m is the velocity of the molecule. Assuming that the kinetic energy of any molecule is constant at any given temperature and pressure then Equation 1.3 can be simplified to Equation 1.4;

Equation 1.4 Graham's Law of diffusion relationship between molecule velocity and mass

$$v_m \propto \sqrt{\frac{1}{m_m}}$$

It is then understood that the diffusion of the molecule in the gaseous phase towards the SPME fibre is dependent on the molecular mass of the molecule. Consequently it is expected that the analysis of higher molecular weight compounds would require a

longer extraction time compared to low molecular weight compounds which experience faster diffusion rates in the headspace comparatively.

Thus, the optimum extraction time depends on the nature of the analysis. High-throughput necessitates shorter extraction times but may forego the benefits of increased sensitivity for particular compounds while longer extraction times have a greater likelihood of the samples reaching equilibrium.

1.4.12. Stir-bar sorptive extraction (SBSE)

Stir-bar sorptive extraction was developed in 1999 (Baltussen et al., 1999) and works on the same principal of SPME where the analyte of interest partitions between the sample matrix and the extraction phase. The major advantage of SBSE over SPME is that it is coated with 25-125 μL of PDMS compared with 0.5 μL of PDMS on a SPME fibre which allows for a substantial increase in sensitivity (Lancas et al., 2009). However, the extraction method is currently limited in respect to stationary phase types, and specialised inlets and sampling stations are required on the GC-MS instruments.

1.5. Gas chromatographic methods

1.5.1. GC-MS

Gas-liquid chromatography (GC) was developed by James and Martin in 1952 (James and Martin, 1952) and, with the introduction of fused-silica capillary columns by Dandeneau and Zerenner in 1979 (Dandeneau and Zerenner, 1979), GC has transformed chemical separations and chemical analysis. GC separates the volatile aroma compounds by boiling points and polarity and is the method of choice for analysing volatile compounds found in grape and wine samples.

Mass spectrometry (MS) was discovered by J. Thomson at the turn of the last century but was developed by Aston in 1919 who demonstrated the existence of isotopes in non-radioactive elements (Aston, 1919). A mass spectrometer determines the mass of a

molecule by measuring the mass-to-charge ratio (m/z) of its ion. Modern commercial GC-MS instrumentation combines high resolution separation, compound ionisation resulting in unique mass spectral fragmentation patterns, and selective and sensitive mass detection.

GC-MS has significant advantages in compound identification over other analytical techniques due to extensive mass spectral and retention index databases (Stein, 1999, Babushok et al., 2007). There are numerous reviews that discuss various aspects of wine composition that in-turn refer to GC-MS as making a significant contribution to current knowledge in the field (Ebeler, 2001, Hayasaka et al., 2005, Polášková et al., 2008, Ebeler and Thorngate, 2009). It is apparent that future understanding of grape and wine composition will continue to employ GC-MS methodologies that incorporate further advances in separation capacity and improvements in sensitivity.

1.5.2. GC×GC-TOFMS

The development of comprehensive two-dimensional gas chromatography (GC×GC) (Liu and Phillips, 1991) has been followed by numerous reviews discussing the principals and experimental design of GC×GC (Ong and Marriott, 2002, Dallüge et al., 2003, Górecki et al., 2004). These reviews have shown that GC×GC offers enhanced separation efficiency, reliability in qualitative and quantitative analysis, capability to detect low quantities, and information on the whole sample and its components. In more recent years, there has been a shift towards the use of this technique in the analysis of real-life samples including food and beverages, and samples from environmental, biological, and petrochemical studies (Adahchour et al., 2008).

GC×GC and SPME techniques have been successfully coupled for volatile profile analysis of a range of food and beverages including honey (Čajka et al., 2007), coffee (Ryan et al., 2004), cachaça (Cardeal et al., 2008), pepper (Cardeal et al., 2006), and

ginger (Shao et al., 2003) to name a few. The combination of HS-SPME and GC×GC-TOFMS techniques has provided a major advantage in analysing complex samples where the number of analytes may be large or the analytes of interest are present at trace levels – as is the case with wine. A number of publications have emerged in the grape and wine field that have utilized HS-SPME and GC×GC as a technique (Ryan et al., 2005, Rocha et al., 2007, Ryona et al., 2008, Ryona et al., 2009, Perestrelo et al., 2010, Ryona et al., 2010, Schmarr et al., 2010). However, the majority of these studies have used the method for targeted analysis (Ryan et al., 2005, Ryona et al., 2008, Ryona et al., 2009, Perestrelo et al., 2010, Ryona et al., 2010) with only two publications to date utilizing the technique for volatile profiling (Rocha et al., 2007, Schmarr et al., 2010).

Rocha and co-workers (Rocha et al., 2007) used GC×GC to analyse monoterpenes in grapes and identified 56 monoterpenes in the Fernão-Pires variety, of which 20 were reported for the first time in grapes. This highlighted the advantage that structured chromatographic separation can provide compound classification and compound identity confirmation. There continues to be new aroma compound discoveries in the grape and wine research field with recent discoveries including (E)-1-(2,3,6-trimethylphenyl)buta-1,3-diene (TPB) (Cox et al., 2005) and 1(2H)-azulenone, 3,4,5,6,7,8-hexahydro-3,8-dimethyl-5-(1-methylethenyl)- ((-)-Rotundone) (Wood et al., 2008). It is anticipated that GC×GC will provide significant advantages in the identification of new and novel compounds which were previously unresolved using traditional one-dimensional chromatography.

1.6. Analysis of Glycoconjugates

Generally speaking, there has been little research investigating the speciation of glycoconjugates compared to the analysis of the volatile aglycones released from the

glycosylated aroma precursors. The following provides some information of experimental approaches taken and instrumentation employed in the analysis of glycoconjugates.

1.6.1. Indirect Analysis of Glycoconjugates

Hydrolysis under controlled conditions by acid (Williams, 1993) or enzyme (Günata et al., 1993) is used to liberate the aglycone and sugar moiety. Essentially the two methods are indirect and involve the measurement of hydrolytically liberated components. Determination of liberated sugar moieties can provide an indication of the conjugate speciation, mono- or di-glucoside, arabinofuranoside, rhamnopyranoside etc. (Williams, 1993). Determination of the aglycone component provides quantitative and qualitative data about the speciation of bound aroma compounds.

Williams and co-workers (Williams et al., 1995) proposed that a rapid form of glycoconjugate analysis of wine was possible through determination of the glycosyl glucose (G-G assay) by enzymatic assay of glucose and fructose. The assay is rapid, accurate, and precise (Williams et al., 1995), and has been used in further research for determination of aroma potential in grapes (Zoecklein et al., 1998, Escalona et al., 1999). The determination of glycosyl glucose provides a measure of abundance of conjugated compounds but provides no qualitative information about the speciation of aroma compounds.

Most research has centred on the measurement of the liberated aglycone compounds through GC-MS or GC olfactometry (GC-O) techniques. GC-MS has been previously discussed in section 1.5. . GC-O was originally proposed by Fuller and co-workers in 1964 and makes use of the human nose as a detector for the compounds eluting from the chromatographic column, typically a fused silica capillary column (Fuller et al., 1964, Acree et al., 1984). The method has been promoted as a useful tool in

determining the sensory character of some flavour compounds (Deibler et al., 1999, Kotseridis and Baumes, 2000, Aznar et al., 2001, Friedrich and Acree, 2002). Although useful in characterising aroma compounds and for initial investigations, GC-O and AEDA may not allow for the extrapolation of the sensory contribution of an aromatic compound to the wine sample (Barbe et al., 2008). This can be attributed to interaction effects with the non-volatile matrix (Pineau et al., 2007, Sáenz-Navajas et al., 2010) and with other volatile compounds (Atanasova et al., 2005b, Escudero et al., 2007, Pineau et al., 2009) which may result in variations in the sensory character of the mixture due to enhancement and suppression effects. GC-O also tends to focus on potent aroma compounds, which chromatograph well at low concentrations, while abundant compounds tend to overload the chromatographic column resulting in broad peaks that elute in the effluent over a long period of time presenting only a fraction of the compound to the operator to smell.

1.6.2. Direct Analysis of Glycoconjugates

While the more common method of glycoconjugate analysis has been to measure the aglycone and/or the sugar moiety after controlled hydrolysis, some studies have directly measured the glycoconjugates. Glycoconjugates have been isolated and fractionated using liquid chromatography (LC), high performance liquid chromatography (HPLC), counter current chromatography (CCC), and supercritical fluid extraction (SFE) (Strauss et al., 1987a, Winterhalter et al., 1990a, Bonnländer et al., 1998, Palma et al., 2000). Glycoconjugates have been analysed directly by HPLC-MS and or tandem MS (MS/MS) methodology (Hayasaka et al., 2010a) and matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) MS (Nasi et al., 2008). There are opportunities to utilise other analytical techniques including high resolution (HR) TOF-MS and fourier transform ion cyclotron resonance (FT-ICR) MS. However, these

techniques have not yet been employed for the analysis of glycoconjugated aroma compounds.

1.7. Sensory Evaluation of Wine

Sensory evaluation stems from the experimental psychology field of psychophysics which explores how human responses are elicited by chemical and physical stimuli. Early work in this field by researchers including Ernst Weber and Gustav Fechner investigated the mathematical relationship between the physical and perceptual magnitude of stimuli which is still the subject of cognitive research (Dehaene, 2003). Where psychophysics might focus experimental research on understanding how humans respond to stimuli, sensory evaluation focuses on utilising human subjects to explore the sensory properties of stimuli. Sensory evaluation has been defined as a scientific method used to evoke, measure, analyse, and interpret those responses to products as perceived through the senses of sight, smell, touch, taste, and hearing (Lawless and Heymann, 2010). Historically sensory evaluation in the food, beverage, and fragrance industries was conducted by product experts such as the cheese maker, winemaker, or perfumer who had training and extensive knowledge of how raw materials and processing affected the finished product quality (Sidel and Stone, 1993). Although these product experts are still commonly used in industry today, the use of specialised sensory panels for conducting discriminative, descriptive, and affective evaluations of products has many more advantages. This is because it is generally recognised that the judgments of a panel are generally more reliable than the judgments of an individual, there is less potential risk that the single expert might be ill / retire / die / or be otherwise unavailable to make decisions, and most importantly the opinions of the expert may or may not reflect what consumers want in a product (Sidel and Stone, 1993). Sensory panels are used for;

- Discrimination tests: to determine whether two products are perceptibly different from one another due, for example, to a modification in the production process or the identification of a defect. These tests are commonly quick to conduct and require little training but provide little additional detail about the differences that exist or the relative impact of the difference (Peryam and Swartz, 1950, Lawless and Heymann, 2010),
- Descriptive tests: to obtain a more detailed description of the sensory attributes of a product. These tests assist in identifying which attributes vary due to a modification to the product or comparisons between products but they typically require additional time and panel training (Murray et al., 2001, Lawless and Heymann, 2010),
- Consumer tests: where it is determined if a consumer likes a product, prefers it to another product, or finds the product acceptable based on its sensory characteristics. These tests are different to market research where the extrinsic factors such as brand, region, price, and awards can effect wine choice (Lockshin et al., 2006, Lawless and Heymann, 2010).

Wine is a highly diversified food product being made from numerous grape varieties, grown in a diverse range of environments worldwide, and utilising a multitude of permutations in viticultural management and winemaking techniques that have been developed over the course of centuries. As such, the sensory characteristics of wines are highly varied and standardised terminology is used to communicate the sensory attributes of wine products between winemakers, marketers, consumers, and researchers (Noble et al., 1984, Noble et al., 1987). As an example, a Cabernet Sauvignon wine can smell of blackberry, raspberry, mint, eucalyptus, bell pepper, asparagus, oak, tobacco, and/or a combination of other terms that may or may not be indicative of the viticultural

environment, management, winemaking practices, packaging material (i.e. in the case of cork taint), and storage conditions that the wine has been derived from. At the same time, a Cabernet Sauvignon wine can elicit sweet, sour, and bitter taste sensations along with varied textural descriptors (Gawel et al., 2000) that characterise the tactile sensation of astringency associated with red wines generating a drying, puckering, or rough mouth-feel sensation (Gawel, 1998). However, standardised terminology is not always used by wine writers and consumers who frequently use everyday language to infer relationships between the wine product and the sensory properties of other common food products and smells.

1.7.1. Descriptive analysis of wine

Noble and Ebeler stated "Without sensory evaluation, even precise information about the volatile composition in the nasal passages cannot predict the flavour of the system as perceived by humans" (Noble and Ebeler, 2002). Descriptive sensory analysis is one of the most powerful tools for conducting product comparisons and for determining relationships between sensory properties of foods and beverages to their composition or consumer liking (Murray et al., 2001, Lawless and Heymann, 2010). There are a number of different methods for conducting descriptive analysis including the Flavour Profile Method, Texture Profile Method, Quantitative Descriptive Analysis™, the Spectrum™ method, Quantitative Flavour Profiling, and Free Choice Profiling where their comparable advantages and disadvantages have been previously discussed in a review of the field (Murray et al., 2001). Descriptive sensory analysis has been utilised extensively in the wine industry over the last thirty years following pioneering work in the area conducted by Anne Noble and co-workers at the University of California, Davis in the late 1970's and early 1980's (Arnold and Noble, 1979, Schmidt and Noble, 1983, Aiken and Noble, 1984, Heymann and Noble, 1987, Noble and Shannon, 1987).

Numerous studies have utilised descriptive sensory analysis to explore differences in the sensory characteristics of single variety wines including Cabernet Sauvignon (Heymann and Noble, 1987), Chardonnay (Arrhenius et al., 1996), Pinot noir (Guinard and Cliff, 1987), and Zinfandel (Noble and Shannon, 1987) from California, Seyval Blanc from Missouri (Andrews et al., 1990), Cabernet Franc from Niagara (Hakimi Rezaei and Reynolds, 2010), Riesling from Germany (Fischer et al., 1999), Malbec from Argentina (Goldner and Zamora, 2007), Albariño from Spain (Vilanova and Vilariño, 2006), Touriga Nacional and Tinta Roriz from Portugal (Falqué et al., 2004), and Sauvignon blanc from New Zealand and other countries (Lund et al., 2009). However, studies have predominantly utilised descriptive sensory analysis to explore the sensory impacts of various viticultural and oenological treatments (Lesschaeve, 2007) with examples including the influence of oak (Francis et al., 1992, Cano-López et al., 2008), fermentation with different yeast strains (Eglinton et al., 2000, Soden et al., 2000), wine storage temperature conditions (Francis et al., 1994, De La Presa-Owens and Noble, 1997), closure types (Godden et al., 2001, Skouroumounis et al., 2005a, Skouroumounis et al., 2005b), grapevine diseases including *Botrytis cinerea* and *Uncinula necator* (powdery mildew) (Stummer et al., 2003, Sivertsen et al., 2005, Stummer et al., 2005), and viticultural elements including grapevine water status, crop yields, and canopy management (Reynolds et al., 1996, Chapman et al., 2004a, Chapman et al., 2005) to name a few. Although many of these studies primarily explored the sensory differences between imposed treatments, current studies tend to explore the relationships between these sensory differences and wine composition and / or the sensory aspects of wine associated with consumer preferences (Francis and Newton, 2005, Lesschaeve, 2007). Recent examples include identification of sensory attributes that drive consumer and expert acceptance of Shiraz and Cabernet Sauvignon

wines (Lattey et al., 2010), assessment of the relationship between sensory and chemical data for oak derived compounds found in French and Spanish wines (Prida and Chatonnet, 2010), modelling of wine mouth feel attributes using metabolomic data (Skogerson et al., 2009), comparisons between vine vigour status with tannin and sensory data (Cortell et al., 2008), assessment of the relationships between red wine textural characteristics and the chemical composition of Shiraz wines (Gawel et al., 2007), comparisons of volatile components of sweet Fiano wines and sensory data (Genovese et al., 2007), and comparisons between volatile compositional data and sensory data of Chardonnay wines (Lee and Noble, 2006). Future work may also consider aspects of wine and food interactions given that an understanding of how people enjoy pairing wine with food combinations is commonly discussed in the popular literature (Madrigal-Galan and Heymann, 2006). In all cases, descriptive sensory analysis produces multivariate data in relation to a single sample set. Various multivariate statistical techniques have been utilised in the field to explore relationships between descriptive sensory data to compositional and / or consumer sensory data including multivariate analysis of variance (MANOVA), principal component analysis (PCA), canonical variate analysis (CVA), generalised procrustes analysis (GPA) and partial least squares (PLS) regression. The use of multivariate statistics requires further understanding of the limitations of the techniques used and is well discussed in a review by Noble and Ebeler and in the text *Sensory Evaluation of Food* by Lawless and Heymann (Noble and Ebeler, 2002, Lawless and Heymann, 2010).

1.7.2. Interaction effects

Previously, wine sensory research has focussed on correlating descriptive sensory and quantitative analytical data in order to successfully identify odour compounds that contribute to the overall aroma perception of wine (Guth, 1997a, 1998, Kotseridis and

Baumes, 2000, Ferreira et al., 2001, Ferreira et al., 2002, Escudero et al., 2004). The use of sensory evaluation to elucidate the impact of complex aroma compound interactions including masking and enhancing effects is likely to improve our understanding of the perceived aroma of wine (Atanasova et al., 2005a). For example, recent sensory research has shown that ethanol exerts a suppression effect on 'fruity' notes in model wine solutions (Grosch, 2001, Escudero et al., 2007, Le Berre et al., 2007). This has been considered to be due to the increased solubility of the volatiles in the solution by ethanol (Le Berre et al., 2007) and due, in part, to the inhibition of the volatile compound odour activity by ethanol (Grosch, 2001). For example, ß-damascenone is recognised universally as a potent wine aroma compound (Skouroumounis and Sefton, 2002, Pineau et al., 2007) due to its low aroma threshold of 2 ng/L (Buttery et al., 1990b) in water or 50 ng/L (Guth, 1997a) in 10% aqueous ethanol. A range of threshold values for model wines have been reported over the years and are well documented in a recent publication by Pineau and co-workers (Pineau et al., 2007). In this study the research group identified that the odour threshold (OT) for β-damascenone in red wine was 7000 ng/L or 1000 fold higher compared to an OT of 50 ng/L in aqueous ethanol (Pineau et al., 2007) indicating that wine components, other than ethanol, are important to aroma perception. Another recent study suggests that the OT for this compound in water is 13 ng/L, compared to 2 ng/L which is the most frequently referenced OT (Buttery et al., 1990b), with the recognition threshold of 56 ng/L (Czerny et al., 2008). This observation highlights the difficulty associated with accurately determining the OT for specific compounds and that distinct differences in OT values can be attributed to interactions with the major wine components. Understanding the factors that influence the release of volatiles from the wine matrix is of major importance to understanding wine aroma perception (Plug and Haring, 1994). It has been suggested that at relatively

low concentrations, ß-damascenone has the ability to mask the 'herbaceous' aroma associated with 2-isobutyl-3-methoxypyrazine (Pineau et al., 2007) and the ability to enhance the 'berry fruit' aromas in red wines (Escudero et al., 2007, Pineau et al., 2007). It has previously been suggested that changes in threshold values may arise from changes in the headspace partition coefficient of a compound either as a result of a change in solubility or an interaction with other solute components (Conner et al., 1998). This is consistent with the different odour thresholds reported in water, aqueous ethanol model solutions, and model white and red wines (Pineau et al., 2007). Clearly interaction effects support the use of holistic approaches, such as descriptive analysis, in the sensory assessment of wine products where enhancement and suppression effects complicate the assessment of volatile components in isolation.

1.8. Concluding Comments

It is apparent that the aroma of wine is dependent not on a particular compound but on the profile and interaction of odour active compounds present. The potential aroma of wine is also dependent on the release of aroma compounds from their odourless precursors during wine maturation (Günata et al., 1993, Williams, 1993) and the modification of volatiles due to chemical changes. To the author's knowledge, there is a significant deficiency of analytical information related to the influence of viticulture on wine aroma in the regions of Western Australia, or indeed anywhere.

A recent critical review (Polášková et al., 2008) suggested that future developments in understanding differences in the sensory attributes of wines will be due to: (1) development of improved and high throughput analytical methods that will allow monitoring of a large number of volatiles including those present at low concentrations; (2) improved understanding of the relationships between chemical composition and sensory perception, including an emphasis on the mechanisms of how odorants and

matrix components interact chemically to impact odorant volatility and overall flavour perception of wines; and (3) multidisciplinary studies using genomic and proteomic techniques to understand flavour and aroma formation in the grape and during fermentation. The current study addresses the first recommendation from this publication and outlines a comprehensive analytical technique for the analysis of the wine volatile profile. This work subsequently addresses the second recommendation as while developing this method it became clear that there was a need to develop a greater understanding of wine matrix effects on SPME-based analyses of volatile compounds found in grape juices and wines. The successful application of this technique to a small number of commercial wines clearly demonstrates that the optimized method can resolve and identify a large number of compounds and could be used in the future to differentiate wines based on their volatile profile. This study provides a foundation for future metabolomic studies investigating flavour and aroma formation in the grape and during fermentation. Understanding the source of wine volatile compounds and the mechanisms that influence their formation through grape growing, winemaking and storage is essential to wine businesses when developing strategies to produce wines with specific sensory attributes that appeal to target markets.

2. Interactions between wine volatile compounds and grape and wine matrix components influence aroma compound headspace partitioning.

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2.1. Introduction

Understanding the factors that influence the release of volatiles from the wine matrix is of major importance to understanding wine aroma perception (Plug and Haring, 1994). The sample matrix can be defined as the components of a sample other than the component of interest (McNaught and Wilkinson, 1997). In the assessment of volatiles in grape juice and wine, the matrix predominantly consists of ethanol (in wine), and non-volatile components including sugars, organic acids, amino acids, phenolic compounds, proteins, and inorganic ions in water.

Headspace Solid-phase Microextraction (HS-SPME) has been increasingly utilised in volatile flavour analysis since it was introduced as a technique by Janusz Pawliszyn in the 1990's (Arthur and Pawliszyn, 1990, Arthur et al., 1992, Zhang and Pawliszyn, 1993, Pan et al., 1995, Steffen and Pawliszyn, 1996). The primary advantage of this technique is that it combines analyte extraction and pre-concentration in a single step. The combined effect of the sample matrix components on the measurement of volatile compounds must be understood to accurately characterise the composition of grape and wine volatiles.

In recent years a number of studies have optimised the HS-SPME sampling conditions required to sample grape and wine matrices for target analytes. These analytes include

ethyl esters, acetates, acids and alcohols (Siebert et al., 2005), monoterpenes, and norisoprenoids (Câmara et al., 2006), methoxypyrazines (Hartmann et al., 2002), thiols, sulphides, and disulphides (Mestres et al., 1999a, Mestres et al., 1999b), furfural derivatives, phenolic aldehydes, volatile phenols, and oak lactones (Carrillo et al., 2006). However, the application of this technique for quantitative analysis has necessitated greater understanding of the matrix influences on volatile compound partitioning into the headspace and subsequent sorption by the SPME fibre.

Most methods described within the literature explore the parameters of fibre type, incubation time, temperature, salting concentration, and degree of agitation as part of their development (Sala et al., 2000, Rocha et al., 2001, Silva Ferreira and Guedes De Pinho, 2003, Howard et al., 2005, Câmara et al., 2006, Carrillo et al., 2006, Setkova et al., 2007b). Commonly, an internal standard is utilised allowing the researcher to compensate for the matrix effects of the solution, presuming that volatile compounds partition into the headspace in equivalent ratios.

Research by Câmara and co-workers (Câmara et al., 2006) and Hartmann and co-workers (Hartmann et al., 2002) using model aqueous solutions showed that increased ethanol concentrations reduce the amount of analyte absorbed onto SPME fibres. Conner and co-workers (Conner et al., 1998) reported that below 17% (v/v), concentrations typical of table wines, ethanol in water forms a mono-dispersed aqueous solution which has limited capacity to retain hydrophobic volatile compounds in solution. This observation is supported by Athès and co-workers (Athès et al., 2004) and Conner and co-workers (Conner et al., 1994) who demonstrated that increasing ethanol concentration in model aqueous solutions reduced the headspace partition coefficient of some volatile alcohols, aldehydes, and esters.

This matrix influence on headspace partitioning of volatiles is expected to have a major impact on the sensory perception of the wine. Recent sensory research has shown that ethanol exerts a suppression effect on 'fruity' notes in model wine solutions (Grosch, 2001, Escudero et al., 2007, Le Berre et al., 2007). This has been considered to be due to the increased solubility of the volatiles in the solution by ethanol (Le Berre et al., 2007) and due, in part, to the inhibition of the volatile compound odour activity by ethanol (Grosch, 2001). Understanding this effect is particularly important when trying to discern which volatile compounds are considered to be contributing to the perception of wine aroma.

Previously, wine sensory research has focussed on correlating descriptive sensory and quantitative analytical data in order to successfully identify odour compounds that may contribute to the overall aroma perception of wine. The role of these odour compounds is commonly confirmed through reconstitution and omission experiments (Guth, 1997a, 1998, Kotseridis and Baumes, 2000, Ferreira et al., 2001, Ferreira et al., 2002, Escudero et al., 2004). The use of sensory evaluation to elucidate the impact of complex aroma compound interactions including masking and enhancing effects is likely to improve our understanding of the perceived aroma of wine (Atanasova et al., 2005a). For example, ß-damascenone is recognised universally as a potent wine aroma compound (Skouroumounis and Sefton, 2002, Pineau et al., 2007) due to its low aroma threshold of 2 ng/L (Buttery et al., 1990b) in water or 50 ng/L (Guth, 1997a) in 10% aqueous ethanol. A range of threshold values for model wines have been reported and are well documented in a recent publication by Pineau and co-workers (Pineau et al., 2007). It has been suggested that at relatively low concentrations, ß-damascenone has the ability to mask the 'herbaceous' aroma associated with 2-isobutyl-3-methoxypyrazine (Pineau

et al., 2007) and the ability to enhance the 'berry fruit' aromas in red wines (Escudero et al., 2007, Pineau et al., 2007).

It has previously been suggested that changes in threshold values may arise from changes in the headspace partition coefficient of a compound either as a result of a change in solubility or an interaction with other solvent components (Conner et al., 1998). This is consistent with the different odour thresholds reported in water, aqueous ethanol model solutions, and model white and red wines (Pineau et al., 2007).

The objective of this study was to observe the influence that major grape and wine matrix components have on the partitioning of volatile compounds into the headspace of model solutions and to study the effect of varied ethanol concentrations in commercially available wines. An additional benefit of this study was that we would be able to observe the impact that the matrix has on the headspace partitioning of impact odour compounds such as β-damascenone and 2-isobutyl-3-methoxypyrazine.

2.2. Materials and Methods

2.2.1. Analytical reagents and supplies

Polydimethylsiloxane (PDMS) SPME fibers, 100 µm 23 ga, were purchased from Supelco (Bellefonte, PA, USA). Prior to initial use, all new fibres were conditioned for 30 minutes at 250 °C as per the manufacturer's recommendations. Amber glass, screw threaded, 20 mL headspace vials with magnetic screw caps and white PTFE / blue silicone (thickness 1.3 mm) septa were purchased from Alltech (Alltech Corp, Deerfield, IL, USA). The following chemicals were purchased; pure Ethanol (200 proof) (Gold Shield, Hayward, CA, USA), D-Glucose anhydrous (Fisher Scientific, Fair Lawn, NJ, USA), (+)-Catechin, 98 %, L-Proline, Potassium hydrogen tartrate, 99 % (Sigma, St. Louis, MO, USA), and Glycerol (EMD Chemicals Inc., Gibbstown, NJ, USA). Milli-Q water (Millipore, Bedford, MA, USA) was purified to a level of 18 MΩ.

Characterisation of matrix interactions was performed using artificial matrices spiked with a stock mixture of volatile chemical standards prepared in pure ethanol. These chemical standards and their respective concentrations after dilution in the artificial matrix solutions are listed in Table 2.1, and will be commonly referred to as the volatile standard mix. The artificial matrices are described in section 2.2.7. . A C8–C20 alkane standard mixture, used for determination of Kovats retention indices (RI) was obtained from Fluka (Sigma–Aldrich, St. Louis, MO, USA). Studies with commercially available wines were conducted using a 2006 vintage Australian Chardonnay (14.0 % ethanol vol/vol) and a 2005 vintage Australian Cabernet Sauvignon (14.0 % ethanol vol/vol).

2.2.2. Instrumentation

All experimentation was conducted using a Gerstel MPS2 autosampler with agitator (Baltimore, MD, USA) coupled to an Agilent 6890N gas chromatograph with an Agilent 5975 inert mass selective detector (Little Falls, DE, USA). The GC oven was equipped with a 30 m DB-WAX capillary column with an ID of 0.25 mm and a film thickness of 0.25 µm (J&W Scientific, Folsom, CA, USA) with a 0.75 mm ID SPME inlet liner (Supelco, Bellefonte, PA, USA).

2.2.3. Chromatographic conditions

The injector was held at 250 °C in the splitless mode with a purge-off time of 1 minute, a 50 mL/min split vent flow at 1 minute and a 20 mL/min gas saver flow at 5 minutes. Ultra high purity (UHP) Helium (Praxair, Danbury, CT, USA) was used as the carrier gas at a constant flow rate of 1.2 mL/min. The temperature program was 40 °C for 1 minute, 5 °C/min to 185 °C, then 40 °C/min to 240 °C, held for 3.62 minutes with a total run time of 35 minutes. The transfer line and ion source were maintained at 240 and 230 °C respectively. The detector collected masses between 40 and 240 amu with a scan rate of 6.61 scans/sec.

Table 2.1 Volatile chemical standards used for the characterisation of wine matrix effects.

Compound	CAS	Concentration (µg/L)	Manufacturer[ϕ]	Purity	MW	LogD[§]	Unique Ion[¥]	Cluster[ϑ]	RT (min)	RI[₦] (calc)	RI[€] (lit)	Ref RI (lit)
Ethyl 2-methylbutyrate	7452-79-1	2068	Aldrich	99%	130.18	2.12	102	1	4.417	1048	1056	A
Ethyl 3-methylbutyrate	108-64-5	2184	Aldrich	98%	130.18	2.12	88	1	4.689	1064	1068	B
Isoamyl acetate	123-92-2	2108	Aldrich	98%	130.18	2.12	43	1	5.763	1120	1125	B
Limonene	5989-27-5	21	Sigma-Aldrich	97%	136.23	4.45	93	3	7.386	1193	1194	B
Ethyl hexanoate	123-66-0	209	Sigma-Aldrich	99%	144.21	2.83	88	1	8.359	1233	1238	B
Hexyl acetate	142-92-7	213	Aldrich	99%	144.21	2.83	43	1	9.323	1272	1269	B
Anisole	100-66-3	2216	Aldrich	99.7%	108.14	2.13	108	1	10.944	1337	1355	B
1-Hexanol	111-27-3	20036	Sigma-Aldrich	99.9%	102.17	1.94	56	1	11.468	1357	1354	B
Ethyl octanoate	106-32-1	22	Aldrich	99%	172.26	3.90	88	4	13.416	1435	1438	B
2-Isobutyl-3-methoxypyrazine	24683-00-9	209	Pyrazine Specialties	99%	166.22	2.61	124	2	15.610	1525	1527	B
Linalool	78-70-6	2064	Merck	98%	154.25	3.28	71	4	16.230	1551	1554	B
Ethyl decanoate	110-38-3	21	Aldrich	99%	200.32	4.96	88	2	18.309	1640	1647	B
Ethyl benzoate	93-89-0	251	Aldrich	99%	150.17	2.73	105	4	18.775	1660	1654	C
Nerol	106-25-2	2014	Sigma-Aldrich	97+%	154.25	3.28	93	2	21.910	1802	1793	D
2-Phenylethyl acetate	103-45-7	2212	Aldrich	99%	164.20	2.30	104	2	22.079	1810	1809	E
β-Damascenone	23726-93-4	226	SAFC Supply Solution	1.1-1.3% in ethanol	190.28	4.04	69	2	22.212	1816	1820	D
α-Ionone	127-41-3	217	Aldrich	90%	192.30	3.86	121	2	22.827	1845	1840	A
Phenylethyl alcohol	60-12-8	20206	Sigma	99%	122.16	1.36	91	4	24.080	1906	1903	D
β-Ionone	79-77-6	210	Sigma-Aldrich	95+%	192.30	3.85	177	2	24.632	1933	1932	D
Eugenol	97-53-0	2224	Aldrich	99%	164.20	2.20	164	2	28.952	2149	2167	D

[ϕ] Manufacturer: Aldrich, Milwaukee, WI, USA; Sigma-Aldrich, St. Louis, MO, USA; Sigma, St. Louis, MO, USA; SAFC Supply Solution, St. Louis, MO, USA; Merck, Darmstadt, Germany; Pyrazine Specialties, Atlanta, GA, USA. [§] LogD: Distribution coefficient at pH 3.0 and 25 °C calculated using Advanced Chemistry Development (ACD/Labs) Software V8.14 for Solaris (© 1994-2009 ACD/Labs). [ϑ] Cluster: compounds that respond similarly to optimisation parameters determined by hierarchal cluster analysis as described in 2.2.4. ; [¥] Unique ion (*m/z*): used for peak area determination; [₦] RI: retention indices calculated from C8-C20 n-alkanes. [€] RI: retention indices reported in the literature for polyethylene glycol (PEG) capillary GC columns. Ref RI (lit) are as follows: A (Bianchi et al., 2007), B (Riu-Aumatell et al., 2006), C (Goodner, 2008), D (Beck et al., 2008), E (Stein, 1999).

2.2.4. Optimisation of SPME extraction time

Samples were incubated at 30 °C with agitation at 500 rpm for
5 minutes and allowed to rest for an additional 5 minutes prior to extraction. The headspace was sampled for 1, 2, 5, 10, 15, 20, 25, 30, 45, and 60 minute periods with the vial at ambient temperature (25 °C ± 2 °C). The fibre was desorbed in the inlet at 250 °C for 1 minute. The fibre was then re-conditioned in the inlet for a further 4 minutes to prevent analyte carry over between samples. The relative responses of compounds were assessed in relation to the specific optimisation parameter through hierarchical cluster analysis using a minimal variance algorithm (Ward, 1963). Compound cluster membership (compounds that responded similarly to the optimisation parameters) was then analysed using a one-way analysis of variance (ANOVA) to determine whether compound clusters responded differently to the specified optimisation parameter (Table 2.1). Cluster means were then plotted against the extraction time.

2.2.5. GC-MS Data analysis software

GC-MS interrogation and spectral deconvolution was conducted using AMDIS Ver. 2.65 (Build 116.66) (National Institute of Standards and Technology, Gaithersburg, MD, USA) (Stein, 1999) using a component width of 32 scans, two adjacent peak subtraction, and high sensitivity, resolution, and shape requirements. Compound mass spectral data were compared against the NIST 2005 Mass Spectral Library and calculated retention indices were compared to published retention indices (Lee and Noble, 2003, Selli et al., 2004, Riu-Aumatell et al., 2006, Bianchi et al., 2007, Beck et al., 2008, Goodner, 2008, Babushok and Zenkevich, 2009) for identity confirmation. Peak area integration of unique masses was conducted using MSD Chemstation (G1701-90057, Agilent).

2.2.6. Statistical analysis software

Statistical analysis was conducted using JMP version 7.0.1 (SAS Institute Inc., Cary, NC, USA). Figures and tables were generated using Microsoft Office Excel 2007 (Microsoft Corporation, Redmond, WA, USA).

2.2.7. Experimental Design

Potassium hydrogen tartrate was added to all model solutions at a rate of 6 g/L creating a super saturated solution at 25 °C (Berg and Keefer, 1958). The addition of potassium hydrogen tartrate provided buffering capacity to the solution and for all practical purposes provides a pH of 3.57 ± 0.02 (Lingane, 1947). Each solution was spiked with the volatile standard mix at 10 µL/mL to give a final concentration, listed in Table 2.1, of each compound. All samples were analysed in triplicate with the exception of experiment 2.2.8. where samples were analysed in duplicate. Sample sequence order was randomised within replicate blocks using a random number generator (http://www.random.org) in all experiments.

2.2.8. Interaction effects of major grape and wine matrix components

A full-factorial design was used to assess the influence of ethanol (14% vol/vol), glucose (240 g/L), glycerol (10 g/L), proline (2 g/L), catechin (50 mg/L) and their interactions on volatile partitioning. The concentrations used were intended to reflect the higher concentration ranges reported in *Vitis vinifera* grapes and table wines (Kliewer, 1967, Rankine and Bridson, 1971, Collins et al., 1997, Goldberg et al., 1998, Stines et al., 1999). The results were analysed using a five-way analysis of variance (ANOVA) testing the effects of ethanol, glucose, glycerol, proline, catechin and all two-way interactions. Least Squares (LS) means of peak area relative to the mean peak area observed in the water matrix, ± the Standard Error (SE), were plotted for significant two-way interactions.

2.2.9. Influence of ethanol concentration

Ethanol is a major component of the wine matrix. An artificial matrix with ethanol concentrations of 10, 11, 12, 13, 14, 15, 16, 17, and 18% vol/vol was spiked with the volatile standard mix to observe if there was a clear difference in partitioning of volatile compounds at varied concentrations of ethanol. Peak area was normalised to the average of that observed in Milli-Q water and the results were analysed using ANOVA. Where values were significantly different, the bivariate data was fitted to a linear-fit curve. A Student t-Test was used to test the significance of the curve slope for each volatile compound.

2.2.10. Influence of glucose concentration

Glucose is a major component of the grape juice matrix. An artificial matrix with glucose at 160, 180, 200, 220, 240, 260, 280, 300, 320 g/L was spiked with the volatile standard mix designed to determine if glucose at typical juice concentrations influenced the partitioning of volatile compounds. Results were treated and analysed in the same way as the ethanol concentration study.

2.2.11. Influence of ethanol and glucose on SPME linearity

Quantitative SPME methodology commonly generates a standard calibration curve, using the optimised SPME extraction methodology, to determine the compound concentration from the sample peak area. To achieve this, compounds of interest are typically spiked, at known concentrations, into a model solution that reflects the sample matrix. Standard curves for the compounds in the volatile standard mix were generated in 240 g/L glucose and 14% vol/vol ethanol and compared to Milli-Q water to determine the slope of the calibration curves. Dilutions of the volatile standard mix were made to cover a 200-fold range in concentration. Results were treated and analysed in the same way as the ethanol concentration study.

2.2.12. Influence of ethanol concentration on wine volatile partitioning

The ethanol concentration of one red and one white wine were manipulated by dilution with ethanol and Milli-Q water to reflect the ethanol range of the synthetic wines. Although many compounds were identified in the wine samples only a selection of 20 target compounds were analysed as they were common between both wines. Table 2.4 lists these target compounds. A number of compounds used in the standard volatile mix were not detectable in the wine samples. Peak area was multiplied by the dilution factor and normalised to the average of that observed in the undiluted wine sample. Wines were also diluted with a 14% vol/vol ethanol solution to compare the dilution effect while maintaining the ethanol concentration. Results were analysed in the same way as the ethanol concentration study.

2.3. Results and Discussion

2.3.1. Optimisation of SPME extraction time

Figure 2.1 shows that peak area increased with increasing extraction time for all compounds with the exception of limonene. Limonene was the only compound belonging to Cluster 3 and its peak area was not significantly different between extraction times of 1 and 60 minutes. Compounds belonging to Cluster 1 (please refer to Table 2.1 for cluster membership) increased significantly to a maximum peak area at 5 minutes while compounds belonging to Cluster 4 showed no significant increase in peak area after 15-20 minutes. Compounds belonging to Cluster 1 typically had lower molecular weights and eluted earlier in the chromatogram compared to compounds in Clusters 2 and 4. Compounds belonging to Cluster 2 increased steadily with increasing extraction time but did not appear to reach a maximum in the extraction time range assessed. It is likely that compounds belonging to Cluster 2 are being refreshed from the solution as they are depleted from the headspace by the SPME fibre. These results are

consistent with previous studies (Roberts et al., 2000, Jung and Ebeler, 2003, Setkova et al., 2007b). An extraction time of 15 minutes was considered adequate to establish equilibrium between the fibre and the sample headspace for most compounds, minimising additional repartitioning from the solution to the headspace.

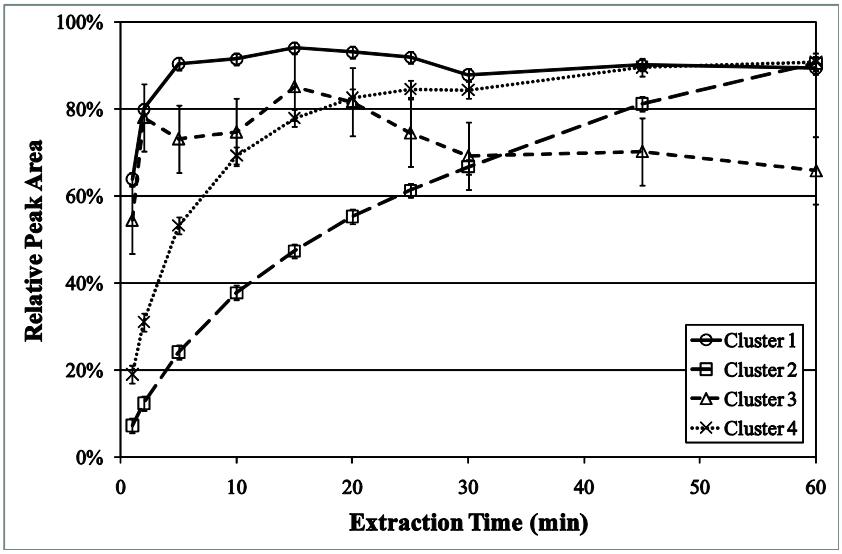

Figure 2.1 SPME extraction time optimisation. Data points represent the LS means (± SE) for compounds belonging to Clusters 1-4, please refer to Table 2.1 for compound cluster membership. Peak areas are relative to the maximum peak area observed in the water matrix.

2.3.2. Interaction effects of major grape and wine matrix components

All compounds were influenced by one or more of the matrix components assessed (Table 2.2). Limonene was unique as it was only significantly affected by the presence of ethanol (Table 2.2). Proline was found to significantly influence three compounds. The magnitude of these influences, however, were approximately 1-2% (data not presented) indicating that it had no real effect. All compounds, with the exception of limonene, were affected by glucose, ethanol and the two-way interaction between glucose and ethanol. Figure 2.2 shows that ethanol caused a reduction in relative peak area while the presence of glucose resulted in an increase in relative peak area for all compounds.

Table 2.2 Significance Values for Standard Least Squares Analysis of Variance for main effects of catechin (CAT), ethanol (ETH), glucose (GLU), glycerol (GLY), proline (PRO) and all two-way interactions. Values marked in bold italics are significant at $p \leq 0.05$.

Compound	CAT	ETH	GLU	GLY	PRO	CAT* GLY	CAT* PRO	ETH* CAT	ETH* GLY	ETH* PRO	GLU* CAT	GLU* ETH	GLU* GLY	GLU* PRO	PRO* GLY
Ethyl 2-methylbutyrate	0.325	***<0.001***	***<0.001***	0.806	0.433	***<0.001***	0.534	0.071	0.680	0.117	0.097	***<0.001***	0.485	0.742	0.161
Ethyl 3-methylbutyrate	0.930	***<0.001***	***<0.001***	***0.021***	0.711	0.540	0.817	0.589	0.936	0.813	0.220	***<0.001***	***0.023***	0.921	0.104
Isoamyl acetate	0.896	***<0.001***	***<0.001***	0.234	0.314	***<0.001***	0.629	0.143	0.265	0.217	0.091	***<0.001***	0.361	0.931	0.113
Limonene	0.827	***<0.001***	0.505	0.793	0.352	0.530	0.172	0.819	0.687	0.262	0.675	0.072	0.562	0.858	0.334
Ethyl hexanoate	0.469	***<0.001***	***<0.001***	0.663	0.219	***<0.001***	0.895	0.096	0.790	0.283	0.226	***<0.001***	0.581	0.740	0.473
Hexyl acetate	0.505	***<0.001***	***<0.001***	0.892	0.554	***<0.001***	0.561	0.146	0.473	0.121	0.238	***<0.001***	0.760	0.929	0.542
Anisole	0.366	***<0.001***	***<0.001***	0.668	0.134	***0.002***	0.584	0.106	0.524	0.193	0.176	***<0.001***	0.699	0.922	0.169
1-Hexanol	0.066	***<0.001***	***<0.001***	***0.028***	0.730	0.271	0.301	0.167	0.082	0.313	0.061	***<0.001***	0.266	0.713	0.307
Ethyl octanoate	0.457	***<0.001***	***<0.001***	0.531	0.578	***0.002***	0.438	0.618	0.561	0.650	0.183	***<0.001***	0.296	0.795	0.566
2-Isobutyl-3-methoxypyrazine	0.064	***<0.001***	***<0.001***	***0.017***	0.140	***0.035***	0.196	0.543	***0.038***	0.795	0.369	***<0.001***	0.061	0.994	0.820
Linalool	0.095	***<0.001***	***<0.001***	***0.002***	0.080	***0.038***	0.113	0.635	***0.008***	0.310	0.308	***<0.001***	0.104	0.439	0.844
Ethyl decanoate	***0.020***	***<0.001***	***<0.001***	***0.027***	***0.033***	0.737	***0.031***	0.233	0.395	0.624	***0.009***	***0.008***	0.193	0.057	0.473
Ethyl benzoate	0.199	***<0.001***	***<0.001***	***0.024***	0.060	***0.024***	0.245	0.922	0.053	0.656	0.363	***<0.001***	***0.043***	0.968	0.650
Nerol	0.058	***<0.001***	***<0.001***	***<0.001***	0.218	0.090	0.093	0.797	***<0.001***	0.790	0.383	***<0.001***	0.343	0.341	0.437
2-Phenylethyl acetate	***0.048***	***<0.001***	***<0.001***	***<0.001***	***0.041***	0.088	0.127	0.535	***0.002***	0.642	0.422	***<0.001***	***0.013***	0.766	0.831
β-Damascenone	***0.046***	***<0.001***	***<0.001***	***0.003***	0.124	***0.032***	0.164	0.488	***0.006***	0.636	0.611	***<0.001***	0.122	0.954	0.821
α-Ionone	***0.041***	***<0.001***	***<0.001***	***0.012***	0.324	***0.035***	0.365	0.674	***0.010***	0.428	0.643	***<0.001***	0.752	0.778	0.621
Phenylethyl alcohol	***0.010***	***<0.001***	***<0.001***	0.093	***0.007***	0.839	0.166	0.636	0.116	0.768	0.490	***<0.001***	***0.005***	0.792	***0.047***
β-Ionone	***0.039***	***<0.001***	***<0.001***	***0.042***	0.484	***0.045***	0.629	0.518	***0.016***	0.485	0.583	***<0.001***	0.726	0.755	0.465
Eugenol	***0.014***	***<0.001***	***<0.001***	***<0.001***	0.177	0.087	0.202	0.805	***<0.001***	0.637	0.440	***<0.001***	0.267	0.990	0.696

The combination of ethanol and glucose resulted in a slightly increased relative peak area when compared to ethanol in isolation. However, it is unlikely that both of these matrix components would be found together in table wines at the concentrations used. The magnitude of the ethanol effect was typically larger for the higher molecular weight compounds, in particular the potent aroma compounds such as 2-isobutyl-3-methoxypyrazine, β-damascenone, α-ionone, and β-ionone while the magnitude of the glucose effect was unrelated to molecular weight.

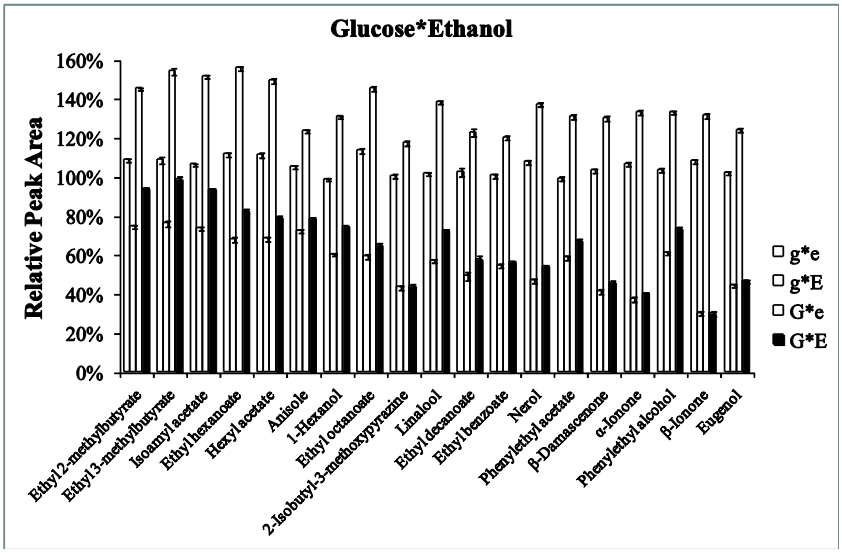

Figure 2.2 Compounds significantly influenced by an interaction between glucose and ethanol. Data points represent the LS means of peak area relative to the mean peak area observed in the water matrix (± SE). Capital letters denote presence of the matrix component while lower case letters denote absence; G corresponds to Glucose and E corresponds to Ethanol.

Significant two-way interactions were observed between ethanol and glycerol (Table 2.2) for 2-isobutyl-3-methoxypyrazine, linalool, nerol, 2-phenylethyl acetate, β-damascenone, α-ionone, β-ionone and eugenol. Figure 2.3 reiterates the observation that ethanol plays an important role in reducing relative peak area but also shows that glycerol can significantly increase the relative peak area in the absence of ethanol. However, the magnitude of this increase is small in comparison to the impact of ethanol. Glycerol has no significant effect in the presence of ethanol and it is unlikely

that both of these matrix components would be found in isolation at the concentrations used since they are both products of yeast primary metabolism. Two previous studies have concluded that glycerol, between the range of 5-50 g/L in aqueous ethanol, had no impact on volatile partitioning which is consistent with the results of this experiment (Fischer et al., 1996, Lubbers et al., 2001). Further, increasing the glycerol content of Chardonnay wine was found not to change the overall flavour perception (Lubbers et al., 2001). As such, glycerol is not likely to have a significant role in the volatile partitioning of aroma compounds in wine.

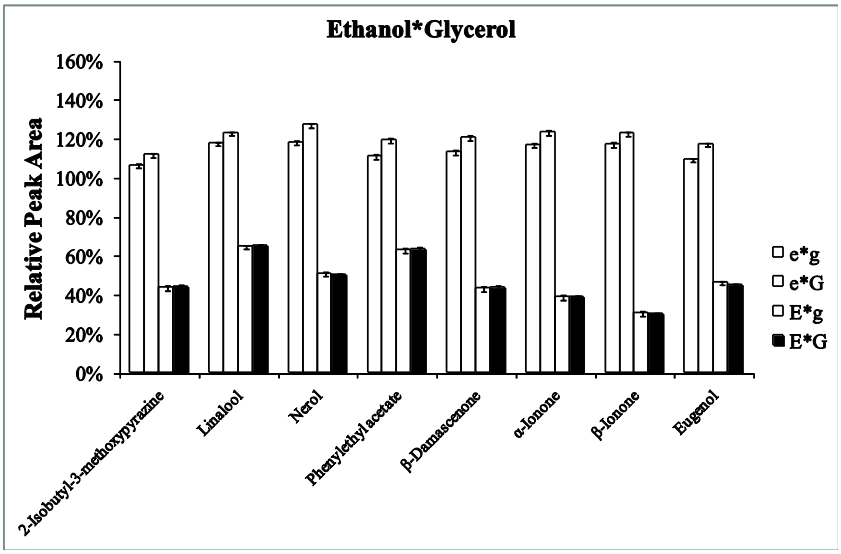

Figure 2.3 Compounds significantly influenced by an interaction between ethanol and glycerol. Data points represent the LS means of peak area relative to the mean peak area observed in the water matrix (± SE). Capital letters denote presence of the matrix component while lower case letters denote absence; E corresponds to Ethanol and G corresponds to Glycerol.

Significant two-way interactions were observed between catechin and glycerol for a number of compounds (Table 2.2); however, there were mixed effects. Ethyl-2-methylbutyrate, isoamyl acetate, ethyl hexanoate, hexyl acetate, anisole and ethyl octanoate had significantly higher relative peak areas with either glycerol or catechin compared to neither glycerol nor catechin or both glycerol and catechin (Figure 2.4). Solutions with glycerol had significantly higher relative peak areas for 2-isobutyl-3-

methoxypyrazine, linalool, ethyl benzoate, β-damascenone, α-ionone, and β-ionone compared to solutions without glycerol or if there were glycerol with catechin.

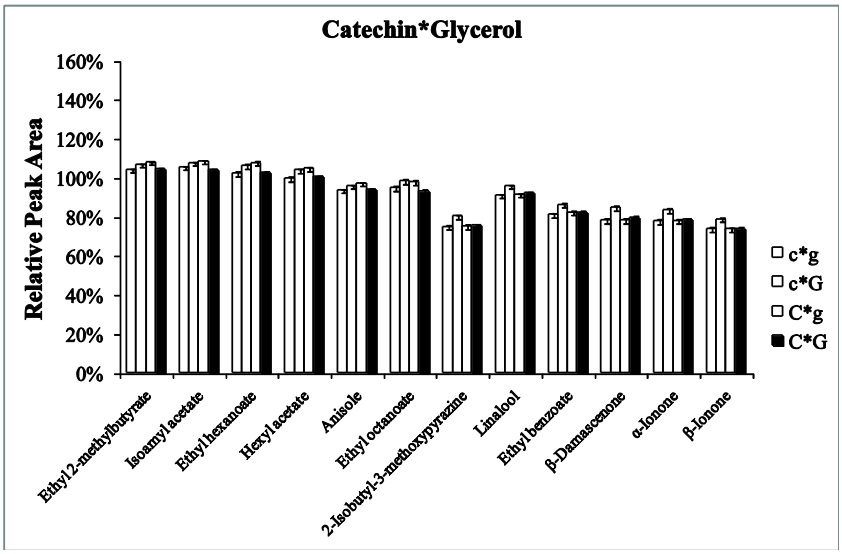

Figure 2.4 Compounds significantly influenced by an interaction between catechin and glycerol. Data points represent the LS means of peak area relative to the mean peak area observed in the water matrix (± SE). Capital letters denote presence of the matrix component while lower case letters denote absence; C corresponds to Catechin and G corresponds to Glycerol.

Previous research has indicated that catechin, at concentrations between 0-5 g/L, reduced the relative activity coefficient of benzaldehyde, isoamyl acetate, and ethyl hexanoate by ~5-10% (Dufour and Bayonove, 1999). In this study, nuclear magnetic resonance (NMR) spectroscopy was used to determine that the relative activity coefficient reduction in the presence of catechin was caused by hydrophobic aroma-phenolic interactions. Gallic acid has also been shown to reduce the partitioning and perceived aroma intensity of 2-methylpyrazine (Aronson and Ebeler, 2004) which has been attributed to increased π-π stacking between the galloyl ring and the aromatic ring of the aroma compounds (Jung et al., 2000). The results presented in Figure 2.4 neither reaffirm nor disprove these previous observations. The magnitude of the effect for the catechin and glycerol interaction was 4-7%, diminishing the importance of this matrix interaction as compared to the effect of ethanol and glucose presented in Figure 2.2.

The reduced impact of catechin compared to previous research could be attributed to the significantly lower concentration used in this study and the addition of other matrix components which may change the intermolecular interactions of catechin. However, it is difficult to explain the causative nature of the matrix interactions between glycerol and catechin and further research is warranted to better understand the role of wine phenolic compounds in aroma-phenolic interactions.

2.3.3. Influence of ethanol concentration

Increasing concentrations of ethanol decreased the relative peak area for all compounds (Table 2.3). Previous HS-SPME optimisation studies assessing aqueous ethanol model solutions and alcoholic beverages have indicated that ethanol reduces the efficiency of HS-SPME (Mestres et al., 1998, Whiton and Zoecklein, 2000, Câmara et al., 2006). It has been suggested that this reduced efficiency is due to ethanol directly competing with analytes for SPME binding sites (De La Calle García et al., 1998, Ebeler et al., 2000, Wardencki et al., 2003). However, SHS methods have been used effectively to determine partition coefficients of analytes in aqueous ethanol solutions (Conner et al., 1994, Conner et al., 1998, Athès et al., 2004). One study compared phase ratio variation (PRV), vapour phase calibration (VPC), and liquid calibration static headspace (LC-SH) SHS methods and showed that regardless which SHS method employed, increasing the ethanol concentration in solution leads to lower partition coefficients for ethyl hexanoate and isoamyl alcohol (Athès et al., 2004). This study did not utilise SHS as it is a less sensitive and less selective method for headspace analysis compared to SPME (Kataoka et al., 2000) and with the increasing use of SPME as a routine automated technique, the authors felt that HS-SPME would be a useful technique for studying the interactions between volatile compounds and the non-volatile matrix components.

The effect of increasing ethanol was particularly pronounced for 2-isobutyl-3-methoxypyrazine, β-damascenone, α-ionone, and β-ionone which had relative peak areas of 46, 49, 45 and 37% respectively at 14% ethanol vol/vol compared to water. Whiton and Zoecklein found that ethyl acetate, ethyl hexanoate, hexyl acetate, ethyl decanoate, 2-phenylethyl alcohol, 4-ethyl guiacol and 4-ethyl phenol showed a decrease of 20-30% with β-ionone decreasing by nearly 50% between 11 and 14% ethanol (Whiton and Zoecklein, 2000). In a more recent study, Câmara and co-workers observed that 12% ethanol vol/vol decreased the peak area (relative to the octan-3-ol internal standard) of β-ionone, β-damascenone, and α-ionone by ~40, 60, and 30% respectively (Câmara et al., 2006). The actual change in relative peak area would be significantly larger than this as it is expected that the octan-3-ol internal standard would also be affected by the change in ethanol concentration. It is also difficult to ascertain if the observed effect of ethanol in these two studies also reflects the addition of sodium chloride to the matrix which is known to significantly weaken the water-ethanol hydrogen bonding structure (Nose et al., 2004). The recent use of an in-fibre standard, which is loaded directly into the SPME fibre coating prior to the sample extraction step, has been successfully used to correct for matrix effects (Wang et al., 2005, Niri and Pawliszyn, 2007, Setkova et al., 2007a, Setkova et al., 2007b) and may be a useful solution in qualitative or semi-quantitative analysis for comparing samples with varied ethanol content.

For each analyte, increasing ethanol in the matrix was negatively correlated with analyte peak area and was linear over the range 10-18% vol/vol. Table 2.3 lists the slope values for relative peak area with slope values ranging from -2.01% for ethyl-2-methylbutyrate to -3.38 for α-ionone.

Table 2.3 Linear Fit Slope values reflecting the percentage change (± SE) in peak area (relative to the average peak area measured in the water matrix) per 1.0% vol/vol change in ethanol (over the range 10 – 18% ethanol), 10 g/L change in Glucose (over the range 160 – 320 g/L), and 200 fold change in analyte concentration in 14% ethanol, 240 g/L glucose, and Milli-Q water respectively. Linear Fit Slope values marked in bold italics are significant at $p \leq 0.05$.

	Linear Fit Slope (Analyte)		Linear Fit Slope (Δ Analyte)		
Compound	Δ Ethanol	Δ Glucose	Ethanol	Glucose	H2O
Ethyl 2-methylbutyrate	***-2.01 ± 0.15***	***0.66 ± 0.19***	***58.83 ± 0.67***	***129.69 ± 3.71***	***98.61 ± 1.76***
Ethyl 3-methylbutyrate	***-2.35 ± 0.15***	***1.54 ± 0.37***	***62.86 ± 1.13***	***132.37 ± 4.85***	***97.95 ± 3.05***
Isoamyl acetate	***-2.46 ± 0.16***	***0.69 ± 0.29***	***61.48 ± 0.69***	***129.04 ± 3.82***	***98.31 ± 2.38***
Limonene	***-2.21 ± 1.03***	-0.59 ± 0.91	***75.09 ± 1.21***	***89.90 ± 3.51***	***99.07 ± 2.74***
Ethyl hexanoate	***-2.85 ± 0.24***	0.14 ± 0.35	***52.09 ± 0.47***	***139.76 ± 1.96***	***99.18 ± 1.77***
Hexyl acetate	***-2.78 ± 0.24***	0.13 ± 0.33	***53.88 ± 0.41***	***136.95 ± 2.30***	***99.05 ± 1.86***
Anisole	***-2.53 ± 0.20***	0.25 ± 0.19	***62.09 ± 0.49***	***115.43 ± 2.05***	***99.41 ± 1.35***
1-Hexanol	***-2.06 ± 0.13***	***1.34 ± 0.12***	***52.81 ± 0.46***	***127.35 ± 3.53***	***98.63 ± 2.04***
Ethyl octanoate	***-3.07 ± 0.25***	-0.15 ± 0.53	***42.57 ± 0.34***	***122.61 ± 0.54***	***99.59 ± 0.73***
2-Isobutyl-3-methoxypyrazine	***-3.07 ± 0.24***	-0.16 ± 0.31	***38.81 ± 0.54***	***110.96 ± 1.98***	***99.73 ± 1.85***
Linalool	***-2.63 ± 0.22***	***0.8 ± 0.25***	***51.42 ± 0.52***	***131.98 ± 3.14***	***99.2 ± 1.84***
Ethyl decanoate	***-2.69 ± 0.27***	-0.27 ± 0.52	***40.71 ± 0.35***	***97.82 ± 3.66***	***99.28 ± 2.50***
Ethyl benzoate	***-3.19 ± 0.28***	-0.11 ± 0.30	***48.72 ± 0.61***	***113.21 ± 2.10***	***99.58 ± 1.67***
Nerol	***-2.29 ± 0.40***	0.03 ± 0.21	***39.23 ± 0.36***	***128.46 ± 1.13***	***100.15 ± 0.49***
2-Phenylethyl acetate	***-2.87 ± 0.33***	0.54 ± 0.36	***55.05 ± 0.70***	***126.15 ± 2.40***	***99.67 ± 1.77***
β-Damascenone	***-3.35 ± 0.31***	-0.27 ± 0.38	***37.19 ± 0.63***	***121.45 ± 1.97***	***100.23 ± 1.63***
α-Ionone	***-3.38 ± 0.29***	-0.40 ± 0.32	***32.50 ± 0.61***	***119.55 ± 1.32***	***100.23 ± 1.40***
Phenylethyl alcohol	***-2.51 ± 0.27***	***1.07 ± 0.14***	***52.79 ± 0.68***	***124.55 ± 1.80***	***99.25 ± 1.31***
β-Ionone	***-3.30 ± 0.24***	-0.58 ± 0.29	***25.66 ± 0.54***	***115.64 ± 1.13***	***100.42 ± 1.35***
Eugenol	***-3.21 ± 0.37***	0.11 ± 0.23	***41.77 ± 0.63***	***124.04 ± 2.95***	***100.43 ± 1.88***

Previous studies have observed that the magnitude of the ethanol effect is positively correlated with the partition coefficient (Aznar et al., 2004) due to a co-solvent effect of ethanol (Fischer et al., 1996). It is clear from the results presented here and from previous studies that ethanol plays a significant and important role in the headspace partitioning of volatile compounds.

2.3.4. Influence of glucose concentration

Glucose increased the measured headspace peak area for most compounds; however, there was no clear linear trend between 160 and 320 g/L with the exception of ethyl-2-methylbutyrate, ethyl-3-methylbutyrate, isoamyl acetate, 1-hexanol, linalool, and phenylethyl alcohol (Table 2.3). The magnitude of these trends was not as large as was found for ethanol. A previous study observed that increasing solution viscosity using sucrose from 12.7 and 156 mPa/s reduced volatile compound release from solution due to reduced mass transfer of volatile compounds (Roberts et al., 1996). However, sucrose was found to have a larger effect than carboxymethylcellulose and guar gum at similar levels of viscosity indicating that sucrose exhibited both viscosity and binding interactions at the concentrations used.

The viscosity of the glucose solutions used in the current study ranged from 1.5 – 2.3 mPa/s, calculated from Equation 2.1, relative to 1.0 mPa/s for water (Chirife and Buera, 1997). Thus reduced volatile release due to viscosity would not be expected in this study.

Equation 2.1 Viscosity calculation for sugar solutions from Chirife and Buera (Chirife and Buera, 1997)

$$\mu_r = \alpha \times e^{\frac{EM}{55.51+M}}$$

Where μ_r is the relative viscosity, α and E are constants (Glucose at 20 °C: α = 0.954; E = 27.93), and M is the number of moles of glucose.

Other studies have identified that increasing sugar concentration, within the range typical of grape juice, increases the headspace partitioning of volatile compounds with no viscosity effect (Hansson et al., 2001, Rabe et al., 2003). Another study assessed 40 volatiles from different chemical classes and observed that some compounds increased, others decreased, and some remained unchanged with increasing sucrose concentration (Friel et al., 2000). The changes in volatile headspace concentrations were analysed using partial least squares (PLS) regression analysis to find that the square of the log of the partition coefficient ($(\log P)^2$), lowest unoccupied molecular orbital (LUMO) energy, and a first order connectivity index term were the most important descriptors for explaining the change in volatility due to increased sucrose concentration (Friel et al., 2000).

The results of the current study suggest that direct comparisons can be made between different juices using qualitative volatile analysis without taking into account the glucose concentration within the range 160 to 320 g/L.

2.3.5. Influence of ethanol and glucose on SPME linearity

All compounds showed a positive linear trend with respect to relative peak area and solution concentration; however, the slope associated with glucose and ethanol solutions were distinctly different to that in water (Figure 2.5). Slope values for varied volatile concentrations in the 14% vol/vol ethanol solution ranged from 75.09 for limonene to 25.66 for β-ionone. However, the next highest value was 62.86 for ethyl-3-methylbutyrate (Table 2.3). Slope values for varied volatile concentrations in the 240 g/L glucose solution varied from 89.90 for limonene to 139.76 for ethyl hexanoate and were typically higher than 100 with the exception of limonene and ethyl decanoate; however, ethyl decanoate was close to 100 (Table 2.3). This clearly indicates that it is absolutely essential to develop calibration curves in model ethanol or glucose solutions

that reflect the samples to be assessed when conducting quantitative analysis of volatiles in juices or alcoholic beverages using SPME.

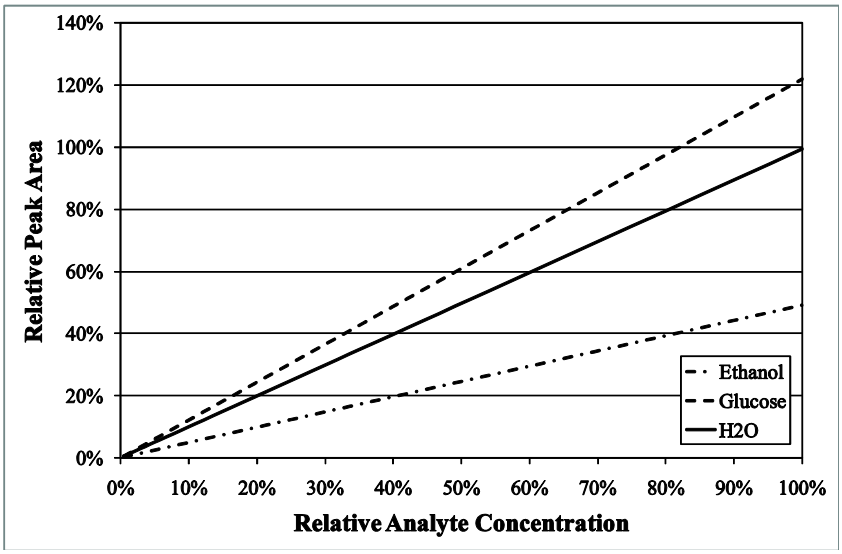

Figure 2.5 Model of the effect of ethanol and glucose on relative peak area. Linear curves reflect the average slope value for compounds listed in Table 2.3 over a 200 fold change in analyte concentration in 14% ethanol, 240 g/L glucose, and Milli-Q water respectively.

2.3.6. Influence of ethanol concentration on wine volatile partitioning

The wine headspace volatiles studied included a large number of compounds; however, a set of 20 compounds common to both the white and red wine were assessed. The SPME methodology was not sensitive enough to detect a number of compounds that were included in the initial synthetic studies, however, ethyl-2-methylbutyrate, ethyl-3-methylbutyrate, isoamyl acetate, ethyl hexanoate, hexyl acetate, 1-hexanol, ethyl octanoate, ethyl decanoate, 2-phenylethyl acetate, and phenylethyl alcohol were common to the previous synthetic studies. Analysis of variance showed that there was a significant difference between different ethanol concentrations for all compounds similar to that observed in the model solutions. Subsequent linear regression analysis showed that all compounds, with the exception of isobutanol, decreased with the addition of ethanol and increased with the addition of water. This is consistent with the observations of Conner and co-workers (Conner et al., 1998) who reported that

increasing the ethanol concentration in aqueous ethanol solutions increases the solubility of esters in solution and reduces the headspace concentration. The results suggest that the matrix is affecting the partitioning of analytes into the headspace of the sample vial. Headspace analysis using SPME can be best understood by using the three-phase system equilibrium as proposed by Zhang and Pawliszyn (Zhang and Pawliszyn, 1993);

Equation 2.2 HS-SPME three phase equilibrium equation (Zhang and Pawliszyn, 1993)

$$n = \frac{C_0 \times V_1 \times V_2 \times K_{h/f} \times K_{s/h}}{(K_{h/f} \times K_{s/h} \times V_1) + (K_{s/h} \times V_3) + V_3}$$

where *n* is the mass of any one analyte absorbed to the fibre, C_O is the initial analyte concentration in solution, V_1 is the volume of SPME phase, V_2 is the liquid volume, V_3 is the headspace volume, $K_{s/h}$ is the sample / headspace partition coefficient, $K_{h/f}$ is the headspace / fibre partition coefficient. Where V_1, V_2, V_3, and $K_{h/f}$ are kept constant this relationship can be simplified to;

Equation 2.3 Relationship between analyte concentration in solution and $K_{s/h}$

$$\Delta C_0 \times K_{s/h} \propto \Delta n$$

The data presented in Table 2.4 clearly demonstrates that increasing the ethanol concentration of either a red or white wine, results in a linear decrease in volatile compound concentration in the headspace. The model proposed in Equation 2.3 suggests that if $K_{s/h}$ for any one analyte remained constant, then a decrease in analyte concentration in solution after dilution would result in a proportional decrease in the mass of compound released into the headspace and consequently absorbed to the SPME fibre. This is not observed, rather dilution with ethanol results in a significant decrease while dilution with water results in a significant increase for all analytes with the

Table 2.4 Volatile compounds identified in wine used for the characterisation of ethanol effects on volatile partitioning. Linear Fit Slope values reflecting the percentage change (± SE) in peak area (relative to the average peak area measured in the 14% ethanol Red and White wines respectively) per 1.0% change in ethanol (over the range 10 – 18% ethanol). Linear Fit Slope values marked in bold italics are significant to $p \leq 0.05$.

Compound	CAS	MW	LogD[§]	Unique Ion[¥]	RT (min)	RI[₦] (calc)	RI[€] (lit)	Ref RI (lit)	Linear Fit Slope (White)	Linear Fit Slope (Red)
Ethyl 2-methylbutyrate	7452-79-1	130.18	2.12	102	4.378	1046	1056	A	***-2.58 ± 0.50***	***-3.59 ± 0.37***
Ethyl 3-methylbutyrate	108-64-5	130.18	2.12	88	4.644	1061	1068	B	***-2.19 ± 0.49***	***-3.54 ± 0.45***
Isobutanol	78-83-1	74.12	0.69	43	5.351	1102	1097	B	1.16 ± 1.02	-1.91 ± 1.24
Isoamyl acetate	123-92-2	130.18	2.12	43	5.650	1115	1125	B	***-2.01 ± 0.80***	***-3.57 ± 0.55***
Isoamyl alcohol	123-51-3	88.15	1.22	55	7.838	1212	1215	B	***-1.38 ± 0.41***	***-2.70 ± 0.48***
Ethyl hexanoate	123-66-0	144.21	2.83	88	8.292	1230	1238	B	***-3.07 ± 0.41***	***-4.01 ± 0.56***
Hexyl acetate	142-92-7	144.21	2.83	43	9.239	1269	1269	B	***-3.00 ± 0.46***	***-2.75 ± 0.62***
Ethyl lactate	97-64-3	88.15	1.22	45	11.037	1340	1353	C	***-0.73 ± 0.20***	***-0.71 ± 0.14***
1-Hexanol	111-27-3	102.17	1.94	56	11.412	1355	1354	B	***-2.08 ± 0.52***	***-3.59 ± 0.48***
Methyl octanoate	111-11-5	158.24	3.37	74	12.204	1387	1387	B	***-3.03 ± 0.52***	***-4.32 ± 0.57***
Ethyl octanoate	106-32-1	172.26	3.90	88	13.453	1437	1438	B	***-3.61 ± 0.33***	***-4.82 ± 0.45***
Vitispirane	65416-59-3	192.30	3.81	192	15.514	1521	1507	D	***-3.72 ± 0.40***	***-4.33 ± 0.43***
1-Octanol	111-87-5	130.23	3.00	56	16.444	1560	1561	B	***-2.03 ± 0.75***	***-4.64 ± 0.48***
Ethyl decanoate	110-38-3	200.32	4.96	88	18.331	1641	1647	B	***-3.67 ± 0.26***	***-6.40 ± 0.31***
Diethyl succinate	123-25-1	174.19	1.26	101	19.078	1673	1690	C	***-3.04 ± 0.45***	***-3.63 ± 0.48***
Ethyl 9-decenoate	67233-91-4	198.30	4.45	88	19.435	1689	1694	C	***-3.36 ± 0.47***	***-6.82 ± 0.42***
TDN	30364-38-6	172.27	4.92	157	20.430	1734	1719	D	***-4.55 ± 0.34***	***-4.28 ± 0.42***
2-Phenylethyl acetate	103-45-7	164.20	2.30	104	22.017	1807	1809	E	***-3.46 ± 0.46***	***-4.85 ± 0.54***
Isoamyl decanoate	2306-91-4	242.40	6.37	70	23.144	1861	1853	D	***-7.70 ± 0.68***	***-6.66 ± 0.64***
Phenylethyl Alcohol	60-12-8	122.16	1.36	91	24.024	1903	1903	F	***-4.15 ± 0.65***	***-3.80 ± 0.44***

[§] LogD: Distribution coefficient at pH 3.0 and 25 °C calculated using Advanced Chemistry Development (ACD/Labs) Software V8.14 for Solaris (© 1994-2009 ACD/Labs).
[¥] Unique ion (*m/z*): used for peak area determination; [₦] RI: retention indices calculated from C8-C20 n-alkanes. [€] RI: retention indices reported in the literature for polyethylene glycol (PEG) capillary GC columns or equivalent. Ref RI (lit) are as follows: A (Bianchi et al., 2007), B (Riu-Aumatell et al., 2006), C (Lee and Noble, 2003), D (Selli et al., 2004), E (Stein, 1999), F (Beck et al., 2008).

exception of isobutanol (data not presented). As a consequence the observed change in relative abundance absorbed to the fibre is likely to be dependent on the solubility of each compound in solution.

Table 2.4 shows the relative slope values for the linear fit curve. This is consistent with the results of the model solution studies above; however, it was interesting to note that the slope values were typically larger for the same compounds found in red wine compared to the model ethanol solutions or the white wine (Figure 2.6). This highlights that ethanol may interact with other major wine components that are present in the red wine and not present in the white wine to influence volatile partitioning.

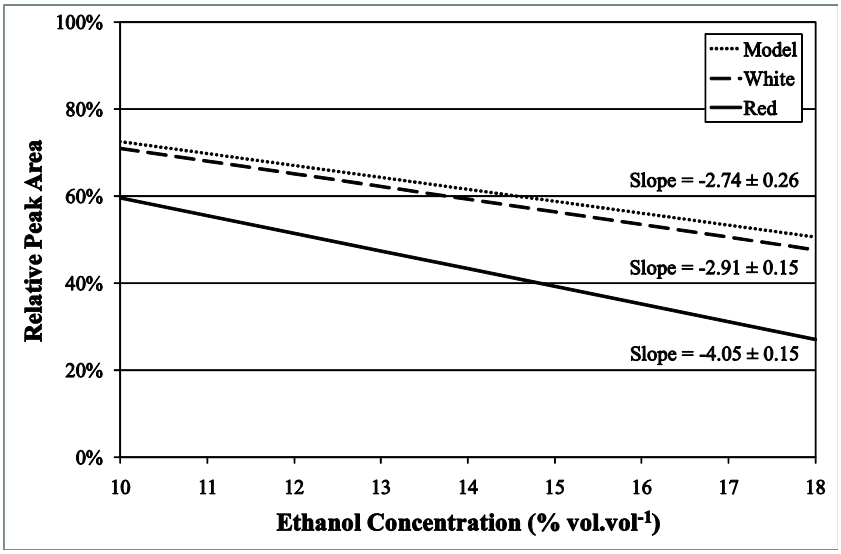

Figure 2.6 Model of the effect of ethanol concentration on relative peak area. Linear curves reflect the average slope value for the compounds assessed in Model solution (Table 2.3), White and Red wines (Table 2.4) (± SE).

A recent study has identified that the odour threshold (OT) for β-damascenone in red wine was 7000 ng/L or 1000 fold higher compared to an OT of 50 ng/L in aqueous ethanol (Pineau et al., 2007). Another recent study suggests that the OT for this compound in water is 13 ng/L, compared to 2 ng/L which is the most frequently referenced OT (Buttery et al., 1990b), with the recognition threshold of 56 ng/L (Czerny et al., 2008). Comparison of both studies highlight that it is difficult to

accurately determine OT for specific compounds and that distinct differences in OT values can be attributed to interactions with the major wine components. Although the results of the current study do not show a reduction in headspace concentration of this magnitude, the results indicate that the wine matrix, in particular the wine ethanol concentration, has a direct impact on the headspace abundance due to changes associated with the compound specific $K_{s/h}$.

2.4. Conclusions

The results presented indicate that the wine matrix, in particular the wine ethanol concentration, has a direct impact on the solubility of wine volatile compounds and subsequently affects the headspace abundance due to changes associated with the compound specific $K_{s/h}$. It is likely that the matrix influence on the compound specific partition coefficient significantly affects the partitioning of aroma compounds into the headspace and therefore changes their aroma impact. These findings help to explain recent observations by other research groups assessing the sensory impact of wine volatiles. A distinction of this study is that it characterised a number of wine matrix interaction effects demonstrating that ethanol plays an important and significant role in volatile partitioning. Further studies into this phenomenon are warranted to better elucidate how the solution matrix changes the aroma perception of complex mixtures.

3. The effect of simulated shipping conditions on the sensory attributes and volatile composition of commercial white and red wines.

*The following is a modified version of the published paper: Robinson, A.L., M. Mueller, H. Heymann, S.E. Ebeler, P.K. Boss, P.S. Solomon, and R.D. Trengove (2010) American Journal of Enology and Viticulture **61**, 337-347.*

3.1. Introduction

The shelf-life of food is defined as the period in which the product will remain safe, is certain to retain desired sensory, chemical, physical, and microbiological, characteristics, and complies with any label declaration of nutritional data (Anon, 1993). Products with a maximum usable lifetime, for instance meats, fruits, vegetables, dairy products etc., are perishable products (Goyal and Giri, 2001) where freight and storage conditions are critical in reducing the growth of microorganisms as well as chemical (including enzymatic) changes in the food.

Risk adverse winemaking practices such as the use of sulphites, lower pH, good winery hygiene, and sterile filtration prior to bottling limit microbiological growth in packaged wines. Wine therefore exhibits a 'random' shelf life as the chemical changes are as much dependent on the initial condition of the product, including packaging, as they are on the storage and freight conditions that the product experiences. However, the storage or freight conditions that the wine experiences prior to consumption may lead to a reduction in the quality of the product due to unintended physical and chemical changes to the wine.

The Arrhenius equation states that the rate constant of a chemical reaction is exponentially related to the temperature of the system. Boulton (Boulton, 1996)

summarised from the research of Ribéreau-Gayon (Ribéreau-Gayon, 1933) and Ough (Ough, 1985) that the relative rates of oxygen uptake, browning, and total SO2 decline in wine would increase 270, 20.7, and 4.8 (red) or 1.7 (white) times faster at 40 °C as compared to 10 °C. Other research has also identified that the low level formation of ethyl carbamate (urethane), primarily from ethanol and urea in wine (Kodama et al., 1994), follows first order kinetics and is accelerated by storage of wine at high temperatures (Hasnip et al., 2004).

The sensory changes associated with elevated storage temperatures is of major concern to both winemakers and consumers, especially, given that little is known about how temperature fluctuates during shipping or how this affects the sensory attributes of wines sold through retail outlets or direct to consumer. Modern wine producers typically store wines in cool cellars or air conditioned storage facilities with the exception of Madeira producers who use a baking process known as 'estufagem' in the production of their unique wines (Campo et al., 2006). Previous work has shown that the temperature variation within a commercial refrigerated shipping container can vary up to 8 °C from the set point using an on-off control system and that the sun exposed roof of a container is usually the warmest area due to a solar effect (Rodríguez-Bermejo et al., 2007). During the summer months in the United States, ambient temperature has been shown to fluctuate up to 20 °C inside wine shipping containers over a two week period and up to 13 °C on a daily basis (Butzke, personal communication 2009).

Eric Vogt of eProvenance, a company providing wine authentication and monitoring services, monitored the temperature of fine wine sent from France to the US, UK, China, Brazil, and Japan. The results showed a wide range of temperature variations noting that during the ocean voyage temperatures were typically stable, but wide fluctuations appeared both before and after the ocean voyage. Vogt indicated that

more than six percent of wines shipped from Europe to the USA experience temperatures above 30 °C (Vogt, personal communication 2009). Although this is a relatively low percentage of products it predominantly reflects the fine wine product category and thus it is feasible, by including products from the commodity wine category, that the percentage of products experiencing these elevated temperatures could be significantly larger.

Partial Least Squares (PLS) regression is a powerful multivariate data analysis technique that can be used to relate a number of response (Y) variables to multiple explanatory (X) variables. The method models the underlying factors or linear combinations of independent variables which best describe the dependent variables. That is, PLS analysis can demonstrate the underlying associations between compositional data and sensory attributes. However, an association does not necessarily indicate that the specific compounds are responsible for any one sensory attribute instead, these compounds should become the focus of future sensory research to confirm their role (Noble and Ebeler, 2002). PLS has been extensively used in the grape and wine field (Aznar et al., 2003, Lee and Noble, 2003, 2006, Jensen et al., 2008) with recent examples including Skogerson and co-workers (Skogerson et al., 2009) who compared wine mouth feel and metabolomic data and Cortell and co-workers (Cortell et al., 2008) who compared vine vigour status with tannin and sensory data.

This study aims to better understand the effect of elevated temperatures, typical of US transcontinental shipping conditions, on wine sensory attributes and volatile composition. The objective of this work was to characterise the relationship between changes in wine volatile composition and sensory attributes associated with wines that experienced elevated storage conditions using PLS regression.

3.2. Materials and Methods

3.2.1. Wines and analytical supplies.

Four commercially available white wines were purchased from Navarro Vineyards (Philo, CA) and four commercially available red wines were donated by Beringer Vineyards (St. Helena, CA). Details of the wines and the codes that identify them are listed in Table 3.1. To minimise temperature effects, the wines were obtained from the wineries shortly after bottling and transported directly to Davis during the fall of 2008 and stored together at a constant 20 °C.

Table 3.1 Details of wines used in this study.

Wine Code	Variety	Vintage	Alcohol
W1	Riesling	2006	13.1%
W2	Gewürztraminer	2006	13.5%
W3	Sauvignon blanc	2006	13.3%
W4	Chardonnay	2006	13.4%
R1	Merlot	2005	13.9%
R2	Cabernet Sauvignon	2006	13.3%
R3	Cabernet Sauvignon	2006	13.7%
R4	Cabernet Sauvignon	2005	13.6%

Polydimethylsiloxane (PDMS) solid phase microextraction (SPME) fibers, 100 µm 23 ga, were purchased from Supelco (Bellefonte, PA, USA). Prior to initial use, all new fibres were conditioned for 30 minutes at 250 °C as per the manufacturer's recommendations. Amber glass, screw threaded, 20 mL headspace vials with magnetic screw caps and white PTFE / blue silicone (thickness 1.3 mm) septa were purchased from Alltech (Alltech Corp, Deerfield, IL, USA). A C8–C20 alkane standard mixture, used for determination of Kovats retention indices (RI) was obtained from Fluka (Sigma–Aldrich, St. Louis, MO, USA).

3.2.2. Experimental design.

Twelve bottles of each wine were stored for 21 days under each of four different temperature conditions. These treatments were constant 20 °C to reflect room temperature, constant 40 °C to reflect a hot environment, diurnal temperature cycle (20

/ 40 °C alternating every 12 hours) to simulate transcontinental shipping conditions, and a treatment where wine was stored in the trunk of a private motor vehicle to simulate wine shipment with movement. The constant 20 °C and 20 / 40 °C cycled treatment wines were stored in Percival growth chambers (Boone, IA, USA), chosen because they could be so programmed, while the constant 40 °C treatment wines were stored in a Steris Reliance 1044 Glassware dryer (Beauport, QC, Canada). The wines stored in the trunk of a vehicle were driven around Davis, California from December 11th to the 31st 2008.

3.2.3. Temperature monitoring.

Ambient temperature was monitored using Tinytag data loggers, model TG-3080 with a 10K NTC Thermistor sensor type purchased from Omni Instruments (Arroyo Grande, CA, USA), able to record temperatures from -40 °C to 85 °C ±1 °C within the temperature range studied with a total reading capacity of 8,000 readings. The loggers were set to start recording ambient temperature in synchronized time every ten minutes over the three week period at which point the logged information was downloaded with Tinytag Explorer Software (SWD-0040) using an ACS-3030 USB inductive pad.

3.2.4. Sensory analysis.

White wines were evaluated by a trained panel of eleven volunteers (five men and six women) and the red wines were evaluated by a second trained panel of thirteen volunteers (six men and seven women). All panelists were between the ages of 21 and 35, had previous wine tasting experience, and were selected due to interest and availability. During initial sessions, panelists developed their own descriptive terminology through consensus to describe and differentiate the wines. Panelists were trained with the reference standards over eight subsequent training sessions to align

panelist terminology. These reference standards were presented in black wine glasses and are listed in Table 3.2 and Table 3.3.

Table 3.2 Composition of sensory reference standards used to define aroma and taste attributes for white wine study.

Attribute	Description	Composition [W]
[A]	Apple/Pear	⅛ x medium Granny Smith apple, chopped ⅛ x medium Bosc pear, chopped
[A]	Burnt Rubber	1 x rubber band lit on fire and immediately extinguished and placed into wine
[A]	Canned Vegetable	¼ x teaspoon canned corn juice (Del Monte) ¼ x teaspoon asparagus juice (Raleys)
[A]	Cardboard	4 x 1 inch squares of corrugated cardboard
[A]	Citrus	⅛ x medium grapefruit with skin ⅛ x medium lemon with skin
[A]	Diesel	2007 Werner Riesling Kabinett ^
[A]	Floral	1 x lemon blossom torn in pieces (no wine)
[A]	Herbaceous	1 x leaf of Agapanthus (Lily of the Nile), ripped 2 x fresh French green beans, chopped
[A]	Tropical Fruit	2 x 1 inch cubes of fresh pineapple ⅙ x fresh mango ¼ x fresh apricot ¼ x teaspoon dried sweetened coconut
[A]	Oak/Fresh Wood	3 x 1 cm cubes of Oak chips, soaked overnight and removed
[A]	Oxidized	Domecq Manzanilla Sherry
[T]	Bitter	800 mg caffeine in 500 mL water
[T]	Sour	200 mg citric acid in 500 mL water
[T]	Sweet	20 g sucrose in 500 mL water

[W] All Standards were prepared in 60 mL Franzia White Chablis unless otherwise noted. [A]: denotes aroma attribute. [T]: denotes taste attribute. ^ Presented during training only.

3.2.5. Quantitative Descriptive Analysis.

Panelists in the red and white wine groups were asked to evaluate each of the 16 wine treatments each in triplicate over the course of six sessions, equating to 8 wines per session presented in a randomised block design. Prior to each formal evaluation session, the reference standards described above were assessed to refresh each panelist's memory. All wine samples were presented in ISO wine tasting glasses (ISO 3591:1977), covered with a plastic lid, labelled with a unique three digit code, under red lighting (to mask differences in colour), in separate booths equipped with a computer screen and mouse for data collection. Ambient temperature was 20 ºC. Wines were assessed monadically and panelists were asked to rate attributes using a continuous unstructured scale (10 cm). A thirty second rest was included between each sample

during which the panelist was asked to refresh his or her palate with water and an unsalted water cracker.

Table 3.3 Composition of sensory reference standards used to define aroma and taste attributes for red wine study.

Attribute	Description	Composition [R]
[A]	Candy	8 x Jelly Belly® beans, squashed
[A]	Canned Veggie	1 x teaspoon canned corn juice (Del Monte) 1 x teaspoon asparagus juice (Raleys) 1 x teaspoon green bean juice (Del Monte) 1 x teaspoon olive juice
[A]	Cardboard	5 x 1 inch squares of corrugated cardboard
[A]	Citrus/Orange Peel	1 x teaspoon orange marmalade 2 x 1 cm squares of orange peel
[A]	Dark Fruit	10 x frozen blackberries (Best Yet) 20 x blueberries (Cascadian Organic)
[A]	Dried Fruit	2 x dried figs (Sunmaid) 3 x prunes (Sunmaid) 20 x raisins (Sunmaid)
[A]	Fresh Vegetable	2 x fresh green beans, chopped 0.5 oz fresh green bell pepper
[A]	Herbal	¼ x teaspoon oregano (McCormick) ¼ x teaspoon basil (McCormick)
[A]	Jam Fruit	2 x teaspoon blueberry jam (Smuckers) 2 x teaspoon blackberry jam (Smuckers) 2 x teaspoon raspberry jam (Smuckers)
[A]	Leather	6 x 1 inch lengths of leather shoe laces (Kiwi Outdoor)
[A]	Menthol	4 x drops Nature's alchemy Eucalyptus 100% pure essential oil into 200 mL water – 10 mL solution in 40 mL wine
[A]	Oak/Fresh Wood	3 x 1 cm cubes of Oak chips, soaked overnight and removed
[A]	Oxidized	20 mL Domecq Manzanilla Sherry
[A]	Pungent	30 mL Popov vodka plus acetone (Nail polish remover)
[A]	Red Berries	2 x strawberries (California grown, purchased fresh and frozen) 8 x frozen raspberries (Best Yet)
[A]	Spicy	¼ x teaspoon of freshly ground black pepper
[A]	Toast	¼ x teaspoon Coffee in 300 mL red base wine with ⅛ x teaspoon liquid smoke
[A]	Tobacco	1 x cigarette (Camel Lights) ⅛ x teaspoon pencil shavings
[A]	Vanilla/Caramel/Cocoa	¼ teaspoon Vanilla-caramel Coffee-mate© nondairy coffee creamer ¼ teaspoon Natural cocoa powder (Scharffen Berger) 1 x piece of milk chocolate (Euphoria Chocolate Company)
[T]	Sweet	20 g sucrose in 500 mL water
[T]	Sour	200 mg citric acid in 500 mL water
[T]	Bitter	800 mg caffeine in 500 mL water
[T]	Astringent	312 mg alum in 500 mL water

[R] All Standards were prepared in 60 mL Franzia Vitners Select Cabernet Sauvignon unless otherwise noted. [A]: denotes aroma attribute. [T]: denotes taste attribute.

FIZZ Software Ver. 2.31G (Biosystèmes, Couternon, France) was used for data acquisition and for generating a randomized presentation order using a modified Williams Latin Square design.

3.2.6. GC-MS Instrumentation.

All experimentation was conducted using a Gerstel MPS2 autosampler with agitator (Baltimore, MD, USA) coupled to an Agilent 6890N gas chromatograph with an Agilent 5975 inert mass selective detector (Little Falls, DE, USA). The GC oven was equipped with a 30 m DB-WAX capillary column with an ID of 0.25 mm and a film thickness of 0.25 µm (J&W Scientific, Folsom, CA, USA) with a 0.75 mm ID SPME inlet liner (Supelco, Bellefonte, PA, USA).

3.2.7. Chromatographic conditions.

The injector was held at 250 °C in the splitless mode with a purge-off time of 1 minute, a 50 mL/min split vent flow at 1 minute and a 20 mL/min gas saver flow at 5 minutes. Ultra high purity (UHP) Helium (Praxair, Danbury, CT, USA) was used as the carrier gas at a constant flow rate of 1.2 mL/min. The temperature program was 40 °C for 1 minute, 5 °C/min to 185 °C, then 40 °C/min to 240 °C, held for 3.62 minutes with a total run time of 35 minutes. The transfer line and ion source were maintained at 240 and 230 °C respectively. The detector collected masses between 40 and 240 amu with a scan rate of 6.61 scans/sec. All samples were analysed in triplicate and the sample sequence order was randomised within replicate blocks using a random number generator (http://www.random.org).

3.2.8. HS-SPME extraction conditions.

Samples were incubated at 30 °C with agitation at 500 rpm for 5 minutes and allowed to rest for an additional 5 minutes prior to extraction. The headspace was sampled for a 15 minute period with the vial at ambient temperature (25 °C ± 2 °C). The PDMS fibre

was desorbed in the inlet at 250 °C for 1 minute. The fibre was then re-conditioned in the inlet for a further 4 minutes to prevent analyte carry over between samples.

3.2.9. GC-MS Data analysis software.

GC-MS interrogation and spectral deconvolution was conducted using AMDIS Ver. 2.65 (Build 116.66) (National Institute of Standards and Technology, Gaithersburg, MD, USA) (Stein, 1999) using a component width of 32 scans, two adjacent peak subtraction, high sensitivity, resolution, and shape requirements. Compound mass spectral data were compared against the NIST 2005 Mass Spectral Library and calculated retention indices were compared to published retention indices (Stein, 2010) for identity confirmation. Peak area integration of unique masses was conducted using MSD Chemstation (G1701-90057, Agilent).

3.2.10. Statistical analysis.

All statistical analysis was conducted using JMP version 8.0.2 (SAS Institute Inc., Cary, NC, USA). A four-way analysis of variance (ANOVA) was conducted using the Restricted Maximum Likelihood (REML) method to test the effects of Treatment, Wine, Judge, Replicate and all two-way interactions for each sensory attribute using a pseudo-mixed model with the Judge by Treatment and Judge by Wine interactions as denominators. A two-way ANOVA was used to analyse effects of Treatment and Wine and their two-way interaction for all volatile compounds measured. Where Treatment had a significant effect for both the analytical and sensory results Partial Least Squares (PLS) regression analysis was used to combine the normalised mean values for significant volatile components (X-variables) and sensory attributes (Y-variables). Mean values were normalised against the maximum value for any one Treatment by Wine combination so that each variable had an equivalent influence on the PLS model. Cross validation was used to determine the lowest number of extracted factors required

to minimise the Root Mean Square Error of Prediction (RMSEP). The PLS output scores and loadings were normalised and plotted, for the significant factors, using JMP. The Variable Influence on Projection (VIP) values and regression coefficients were used to determine which predictive (X) variables were important in modelling the response (Y) variables. VIP values provide weighted sums of squares of the PLS-weights calculated from the Y-variance of each PLS component (Wold et al., 2001). The regression coefficients for each X-attribute were assessed in relation to the Y-attributes through two-way hierarchical cluster analysis using a minimal variance algorithm (Ward, 1963). The cluster membership was then analysed using a one-way analysis of variance (ANOVA) to determine whether X-attribute clusters responded differently for each Y-attribute. Where Treatment did not have a significant effect for both the analytical and sensory results Principal Component Analysis (PCA) was used to explore the inter-relationships between the attributes and the samples.

3.3. Results

3.3.1. Temperature results.

The 20 °C treatment experienced a relatively constant temperature with a mean of 20.8 (± 0.4) °C over the 21 day period. The 40 °C treatment experienced a ~ 24 hour time lag before reaching the intended 40 °C temperature (Figure 3.1). This resulted in the 40 °C treatment wines experiencing a mean temperature of 35.0 (± 7.2) °C over the 21 day period. The 20 / 40 °C cycled treatment also experienced a time lag oscillating between ~26 and ~35 °C over a 24 hour period with approximately 2 hours spent at the ~35 °C temperature each cycle. This resulted in a mean temperature of 28.7 (± 4.3) °C over the 21 day period. The treatment where wine was stored in the trunk of a vehicle experienced the lowest temperatures of all the treatments with a mean of 14.3 (± 3.4) °C over the 21 day period.

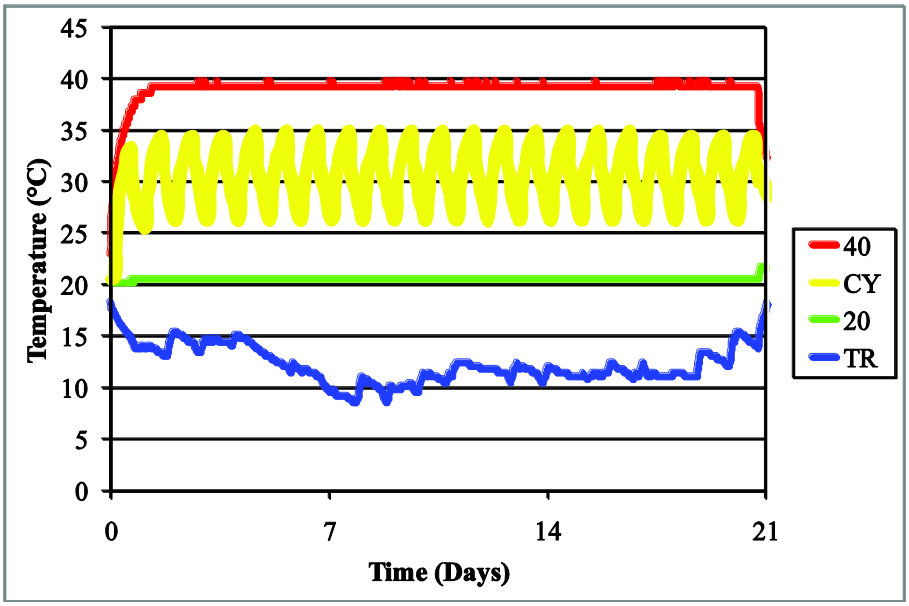

Figure 3.1 Ambient temperature monitored using Tinytag data loggers for each temperature treatment. Treatment is indicated as: car trunk (TR), constant 20 °C (20), cycled 20/40 °C (CY), and constant 40 °C (40).

3.3.2. Analysis of the white wine study.

The four-way ANOVA, using a pseudo-mixed model, showed that the apple, canned veg, citrus, diesel, floral, oxidised, rubber, and tropical fruit sensory attributes were significantly different across the treatments in the white wine study (Table 3.4). A two-way ANOVA of the 48 identified volatile compounds showed that 26 compounds were significantly different due to treatment (Table 3.5).

3.3.3. PLS analysis of the white wine study.

PLS analysis with cross validation, using all significant volatile components to predict the significant sensory attributes, determined that the PLS model with the lowest root mean square error of prediction (RMSEP = 0.791) used four latent vectors. However, the fourth latent vector provided little additional information compared to the first three latent vectors (RMSEP = 0.881) and therefore only the first three latent vectors will be presented. Linalool, propyl octanoate, nerol oxide, hexyl acetate, p-cymene, and 2-phenylethyl acetate were considered important variables in defining the final PLS

model with variable influence on projection (VIP) values above the 75% quartile (Table 3.6).

Table 3.4 Sensory attributes found to be significant due to Treatment for white and red wine products. Treatment is indicated as: car trunk (TR), constant 20 °C (20), cycled 20/40 °C (CY), and constant 40 °C (40). Values represent Least Square Means (LSM) (± SE) for four-way ANOVA. A pseudo-mixed model using the Judge by Treatment and Judge by Wine interactions as denominators was used in all cases.

	Attribute	TR	20	CY	40
White	Apple	3.1 ±0.2	3.1 ±0.2	3.1 ±0.2	2.5 ±0.2
	Canned Vegetable	1.3 ±0.2	1.1 ±0.2	1.2 ±0.2	2.1 ±0.2
	Citrus	2.4 ±0.2	2.6 ±0.2	2.6 ±0.2	2.0 ±0.2
	Diesel	1.0 ±0.2	1.1 ±0.2	1.3 ±0.2	1.6 ±0.2
	Floral	3.2 ±0.2	2.8 ±0.2	2.8 ±0.2	2.2 ±0.2
	Oxidized	0.9 ±0.1	0.7 ±0.1	0.8 ±0.1	1.0 ±0.1
	Rubber	1.0 ±0.2	1.0 ±0.2	1.2 ±0.2	2.0 ±0.2
	Tropical Fruit	2.8 ±0.2	2.5 ±0.2	2.6 ±0.2	2.1 ±0.2
Red	Canned Vegetable	0.9 ±0.1	0.8 ±0.1	0.8 ±0.1	1.2 ±0.1
	Dry Fruit	2.2 ±0.1	2.2 ±0.1	2.0 ±0.1	2.6 ±0.1

The PLS model differentiated among the wines in the first two latent vectors, grouping similar wines due to variety, and accounting for 55.3% and 49.8% of the variance for the X and Y variables respectively (Figure 3.2). The third latent vector accounted for an additional 20.3% and 12.4% of the variance for the X and Y variables respectively, differentiating products primarily due to the heat treatment. Products that experienced either the constant 20 °C or vehicle trunk treatment tended to group in the first, second and third vectors compared to similar wines that experienced the 20 / 40 °C cycled treatment or the constant 40 °C treatment (Figure 3.3).

The tropical fruit and apple sensory attributes were negatively correlated with the rubber and diesel sensory attributes (Figure 3.2). The opposition of these sensory attributes characterised the first latent vector which accounted for the greatest percentage of variance for the sensory attributes in the PLS model.

The tropical fruit sensory attribute was positively correlated with compounds from clusters 1 and 4 with and negatively correlated with compounds in cluster 8. Hexyl acetate had the strongest positive correlation with the tropical fruit sensory attribute with linalool, propyl octanoate and isoamyl acetate having the next strongest association (Table 3.6).

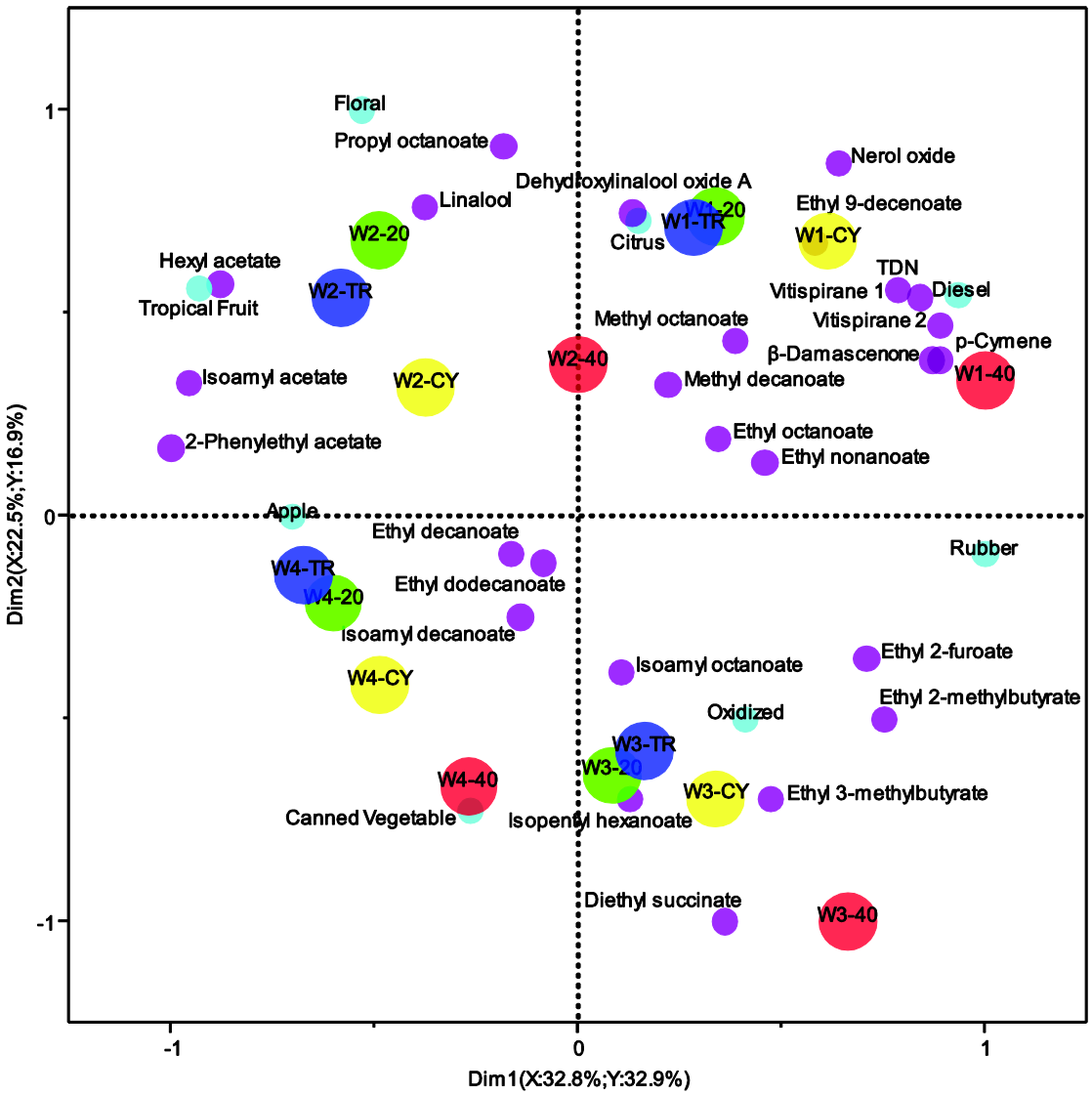

Figure 3.2 Partial Least Squares analysis of white wine products. Light blue circles represent the volatile composition loadings (X matrix), the purple circles represent the sensory attribute loadings (Y matrix), and the large circles represent the sample scores for factor 1 (Dim1) and factor 2 (Dim2). Samples are labelled as per Table 3.1 with the treatment indicated as: car trunk (TR), constant 20 °C (20), cycled 20/40 °C (CY), and constant 40 °C (40).

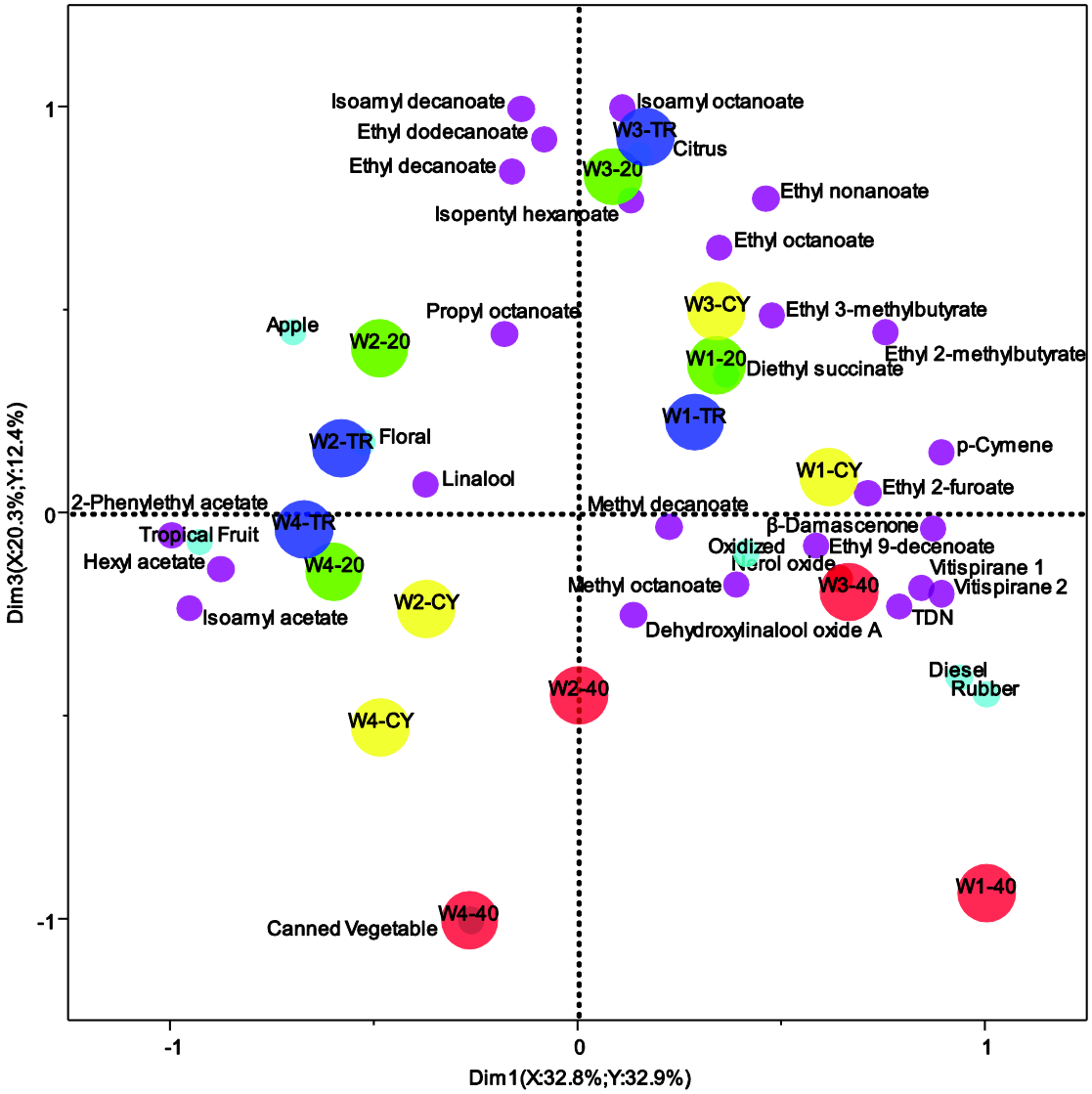

Figure 3.3 Partial Least Squares analysis of white wine products. Light blue circles represent the volatile composition loadings (X matrix), the purple circles represent the sensory attribute loadings (Y matrix), and the large circles represent the sample scores for factor 1 (Dim1) and factor 3 (Dim3). Samples are labelled as per Table 3.1 with the treatment indicated as: car trunk (TR), constant 20 °C (20), cycled 20/40 °C (CY), and constant 40 °C (40).

The apple sensory attribute was not well described by any one cluster of compounds it was, however, positively correlated with ethyl decanoate and negatively correlated with ethyl 2-furoate. Compounds in cluster 2, including the norisoprenoids 1,1,6-trimethyl-l,2-dihydronaphthalene (TDN), vitispirane 1 and 2, and β-damascenone, were strongly correlated with the diesel sensory attribute (Table 3.6). The rubber sensory attribute was

negatively correlated with 2-phenylethyl acetate, propyl octanoate, and hexyl acetate and positively correlated with ethyl 2-furoate and vitispirane 2; however, there was no specific compound cluster that correlated well with the rubber attribute.

The second latent vector was characterised by the separation of the citrus and floral from the canned veg and oxidized sensory attributes. The citrus and floral attributes were associated with the aromatic white wine varieties Riesling (W1) and Gewürztraminer (W2) while the canned veg and oxidized sensory attributes were associated with the Sauvignon blanc (W3) and Chardonnay (W4) wines (Figure 3.2). It was noted that linalool and propyl octanoate, compounds in cluster 4, were important in defining the second latent vector being strongly positively correlated with the citrus and floral sensory attributes and strongly negatively correlated with the canned veg and oxidized sensory attributes (Table 3.6).The citrus attribute was positively correlated with the X-attributes belonging to clusters 3, 4 and 6 while the floral attribute was positively correlated with compounds from clusters 1, 3, and 4. The canned veg attribute was negatively correlated to compounds in clusters 3, 4, and 6 (Table 3.6). Compounds in cluster 4 were also negatively correlated with the oxidized attribute.

3.3.4. Analysis of the red wine study.

In the red wine study it was found that 30 of the 47 volatile compounds changed with respect to the temperature treatment (Table 3.5). However, only two sensory attributes, dry fruit and canned vegetable, were significantly different due to the temperature treatment, both being higher for wines that experienced the 40 °C treatment (Table 3.4). Consequently, only the volatile compounds that were significantly different due to treatment were included in the Principal Component Analysis (PCA).

Table 3.5 Treatment Significance Values for two-way ANOVA for white and red wine products.

Values marked in bold italics are significant at p ≤ 0.05.

CAS	Compound	Unique Ion[¥]	RT (min)	RI[₦] (calc)	RI[€] (lit)	White	Red
105-54-4	Ethyl butanoate	71	4.143	1029	1031	0.993	0.222
71-23-8	1-Propanol	59	4.244	1035	1030	0.950	0.745
7452-79-1	Ethyl 2-methylbutyrate	102	4.426	1046	1036	***<0.001***	***<0.001***
108-64-5	Ethyl 3-methylbutyrate	88	4.693	1062	1053	***0.002***	***<0.001***
78-83-1	Isobutanol	43	5.329	1100	1097	0.876	0.644
7392-19-0	Dehydroxylinalool oxide A	139	5.420	1104	1096	***<0.001***	***<0.001***
123-92-2	Isoamyl acetate	43	5.737	1118	1117	***<0.001***	0.427
99-86-5	α-Terpinene	121	6.888	1170	1175	0.237	***<0.001***
106-70-7	Methyl hexanoate	74	7.188	1184	1190	0.268	0.674
123-51-3	Isoamyl alcohol	55	7.859	1213	1215	0.584	0.185
123-66-0	Ethyl hexanoate	88	8.362	1233	1230	0.750	***0.007***
99-87-6	p-Cymene	119	9.125	1264	1253	***<0.001***	***<0.001***
142-92-7	Hexyl acetate	43	9.301	1271	1269	***<0.001***	***0.002***
586-62-9	Terpinolene	121	9.460	1278	1276	0.363	***<0.001***
97-64-3	Ethyl lactate	45	11.083	1342	1342	0.311	***<0.001***
876-17-5	(Z)-Rose oxide [W]	139	11.287	1350	1338	0.183	ND
111-27-3	1-Hexanol	56	11.451	1357	1354	0.761	0.682
111-11-5	Methyl octanoate	74	12.249	1389	1387	***0.045***	***<0.001***
106-32-1	Ethyl octanoate	88	13.493	1439	1438	***0.012***	***<0.001***
64-19-7	Acetic acid	45	13.811	1452	1449	0.075	0.742
2198-61-0	Isoamyl hexanoate	70	13.986	1459	1464	***0.019***	***<0.001***
1786-08-9	Nerol oxide	68	14.228	1468	1473	***<0.001***	***0.016***
624-13-5	Propyl octanoate	145	15.477	1520	1514	***<0.001***	***<0.001***
65416-59-3	Vitispirane 1	192	15.549	1523	1526	***<0.001***	***<0.001***
65416-59-3	Vitispirane 2	192	15.595	1525	1529	***<0.001***	***<0.001***
123-29-5	Ethyl nonanoate	88	15.879	1530	1528	***0.005***	***<0.001***
78-70-6	Linalool	71	16.204	1550	1554	***<0.001***	***<0.001***
111-87-5	1-Octanol	56	16.488	1562	1561	0.428	0.715
110-42-9	Methyl decanoate	74	17.266	1595	1590	***<0.001***	***<0.001***
614-99-3	Ethyl 2-furoate	95	17.808	1618	1621	***<0.001***	***<0.001***
110-38-3	Ethyl decanoate	88	18.334	1641	1647	***<0.001***	***<0.001***
2035-99-6	Isoamyl octanoate	70	18.729	1658	1652	***<0.001***	***<0.001***
123-25-1	Diethyl succinate	101	19.118	1675	1677	***<0.001***	***<0.001***
67233-91-4	Ethyl 9-decenoate	88	19.454	1690	1689	***<0.001***	***<0.001***
98-55-5	α-Terpineol	59	19.610	1697	1687	0.529	0.061
30364-38-6	TDN	157	20.472	1736	1731	***<0.001***	***<0.001***
101-97-3	Ethyl phenylacetate	91	21.445	1781	1783	0.804	***<0.001***
103-45-7	2-Phenylethyl acetate	104	22.072	1810	1809	***<0.001***	0.787
23726-93-4	β-Damascenone	69	22.191	1816	1813	***0.046***	0.564
106-33-2	Ethyl dodecanoate	88	22.764	1843	1840	***<0.001***	***<0.001***
142-62-1	Hexanoic acid	60	22.919	1851	1840	0.430	0.178
2306-91-4	Isoamyl decanoate	70	23.141	1861	1859	***<0.001***	***<0.001***
100-51-6	Benzyl Alcohol	71	23.396	1873	1869	0.055	***0.004***
55013-32-6	(Z)-Oak-lactone	99	23.497	1878	1886	0.301	0.516
60-12-8	Phenylethyl Alcohol	91	24.097	1907	1910	0.574	0.145
39638-67-0	(E)-Oak-lactone	99	24.900	1948	1957	0.456	0.192
2785-89-9	4-Ethylguaiacol [R]	137	26.401	2020	2024	ND	***0.050***
124-07-2	Octanoic Acid	60	27.180	2062	2060	0.507	0.090

[¥] Unique ion (*m/z*): used for peak area determination; [₦] RI: retention indices calculated from C8-C20 n-alkanes. [€] RI: retention indices reported in the literature for polyethylene glycol (PEG) capillary GC columns (Stein, 2010). [W] Compound only detected in white wines. [R] Compound only detected in red wines.

Table 3.6 Regression coefficients of centred and scaled X-variables for each Y-variable from the four-component PLS model of the white wines. X-Variables are ordered by descending VIP value. Compound cluster membership was determined using hierarchal cluster analysis as described in the Statistical section of the Materials and Methods.

X-Variables	VIP	Cluster	Apple	Canned Veg	Citrus	Diesel	Floral	Oxidized	Rubber	Tropical Fruit
Linalool	1.810	4	-0.02	-0.24	0.27	-0.02	0.26	-0.14	-0.06	0.11
Propyl octanoate	1.550	4	0.07	-0.20	0.19	-0.01	0.18	-0.10	-0.10	0.11
Nerol oxide	1.290	3	-0.07	-0.14	0.15	0.09	0.11	-0.05	0.05	0.00
Hexyl acetate	1.286	1	0.06	-0.05	0.06	-0.05	0.13	-0.08	-0.10	0.12
p-Cymene	1.269	3	-0.08	-0.14	0.15	0.08	0.07	-0.03	0.07	-0.04
2-Phenylethyl acetate	1.209	1	0.09	0.03	-0.02	-0.07	0.05	-0.04	-0.10	0.10
Isoamyl acetate	1.166	1	0.05	0.00	0.01	-0.06	0.09	-0.06	-0.09	0.11
Ethyl succinate	1.144	5	-0.03	0.08	-0.09	-0.02	-0.14	0.08	0.05	-0.10
Dehydroxylinalool oxide A	1.126	3	-0.06	-0.14	0.16	0.04	0.15	-0.08	0.02	0.04
Ethyl dodecanoate	1.123	6	0.07	-0.16	0.14	-0.06	0.07	-0.04	-0.08	0.03
Vitispirane 2	1.115	2	-0.07	-0.03	0.02	0.11	-0.01	0.02	0.09	-0.06
Ethyl 2-furoate	1.092	8	-0.11	-0.04	0.05	0.05	-0.02	0.02	0.10	-0.09
Isoamyl decanoate	1.088	6	0.07	-0.14	0.13	-0.07	0.06	-0.04	-0.09	0.03
Vitispirane 1	1.053	2	-0.06	-0.03	0.02	0.11	0.00	0.01	0.08	-0.05
β-Damascenone	1.006	2	-0.03	-0.01	-0.01	0.09	-0.05	0.04	0.07	-0.06
TDN	1.002	2	-0.05	-0.02	0.01	0.11	-0.01	0.02	0.08	-0.04
Ethyl 2-methylbutyrate	0.978	8	-0.06	-0.05	0.05	0.03	-0.04	0.03	0.07	-0.09
Isoamyl octanoate	0.916	6	0.05	-0.12	0.11	-0.05	0.03	-0.02	-0.06	0.00
Ethyl decanoate	0.878	6	0.10	-0.07	0.05	-0.05	0.01	-0.01	-0.09	0.03
Ethyl 3-methylbutyrate	0.830	8	-0.05	-0.05	0.05	-0.01	-0.03	0.02	0.05	-0.07
Methyl octanoate	0.794	5	0.03	0.08	-0.11	0.06	-0.08	0.05	0.02	-0.02
Methyl decanoate	0.776	5	0.06	0.08	-0.12	0.04	-0.09	0.05	0.00	-0.01
Ethyl nonanoate	0.767	7	0.02	-0.10	0.08	0.02	0.02	0.00	-0.01	-0.02
Ethyl 9-decenoate	0.761	2	0.00	-0.03	0.01	0.08	0.00	0.01	0.03	-0.01
Isopentyl hexanoate	0.629	7	0.00	-0.05	0.05	-0.04	-0.02	0.01	-0.01	-0.04
Ethyl octanoate	0.619	7	0.05	-0.05	0.03	0.02	-0.01	0.01	-0.03	0.00

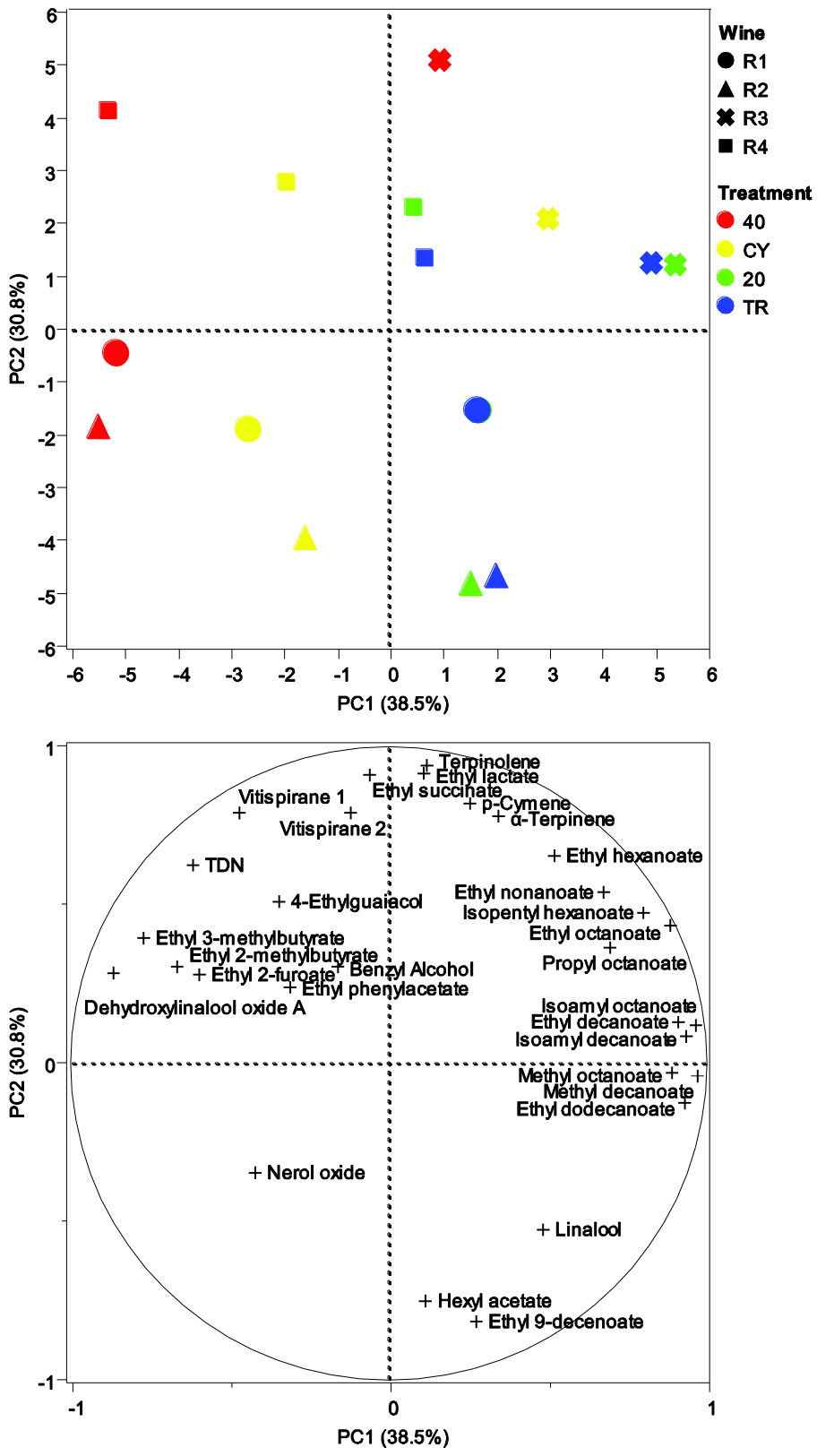

Figure 3.4 Principal Component analysis of red wine products. Samples are labelled as per Table 3.1 with the treatment indicated as: car trunk (TR), constant 20 °C (20), cycled 20/40 °C (CY), and constant 40 °C (40).

The first two principal components accounted for 38.5% and 30.8% of the variance in the first and second dimension respectively (Figure 3.4). Products tended to separate due to heat treatment mostly in the first dimension. As with the white wine study, products that experienced the constant 20 °C or vehicle trunk treatments tended to group and were clearly different to wines that experienced the 20 / 40 °C cycled treatment or the constant 40 °C treatment. There was little separation of the wines themselves with Merlot (R1) and Cabernet (R2) wines being separated from Cabernet (R3) and Cabernet (R4) wines in the second dimension.

Wines that experienced heat were positively correlated with TDN, vitispirane 1 and 2, dehydroxylinalool oxide A and p-cymene and negatively correlated with methyl decanoate, ethyl decanoate, ethyl dodecanoate, isoamyl octanoate and decanoate, propyl octanoate, and linalool.

The separation of the wines in the second dimension was driven by one of the Cabernet (R2) wines which had significantly higher levels of hexyl acetate and lower levels of diethyl succinate and terpinolene than the other wines (data not presented). Both Merlot (R1) and Cabernet (R2) wines were lower in α-terpinene and p-cymene and higher in ethyl 9-decanoate than Cabernet (R3) and Cabernet (R4) wines.

3.4. Discussion

3.4.1. White wines.

The white wines showed significant differences due to variety and heat treatment. The sensory attributes which were found to show significance across the products included canned vegetable, citrus, diesel, floral, oxidised, rubber, and tropical fruit. Data presented in Figure 3.3 showed a greater impact on the aroma and volatile composition of the wines for the constant 40 °C heat treatment compared to the 20 / 40 °C cycled treatment and also compared to the constant 20 °C and trunk treatments which were not

significantly different. The two heated treatments tended toward the diesel, oxidized, and rubber aroma attributes and away from the citrus, floral and tropical fruit aromas. These observations are in line with previous research that has shown elevated storage temperatures decrease the floral character and enhance characters such as honey, butter/vanilla, oak, tea/tobacco, rubber, and smoky in white wines which are typical of aged wines (Francis et al., 1994, De La Presa-Owens and Noble, 1997).

Linalool played an important role in defining both aromatic varieties, Riesling (R1) and Gewürztraminer (R2) wines. Monoterpenes are important to the aroma of white wine wines made from Muscat varieties and aromatic non-muscat varieties (Ribéreau-Gayon et al., 1975, Rapp, 1998, Mateo and Jimeńez, 2000) with correlations between floral sensory attributes and high levels of linalool being well documented (De La Presa-Owens and Noble, 1997, Lee and Noble, 2003, Campo et al., 2005, Lee and Noble, 2006). Linalool was closely associated with the Gewürztraminer wines whereas 1,1,6-trimethyl-l,2-dihydronaphthalene (TDN), vitispirane 1 and 2, and p-cymene were closely associated with the Riesling wines. It is well understood that TDN and vitispirane are typically found in Riesling wines that have been bottle aged (Simpson, 1979) and or heated (Simpson, 1978).

The Sauvignon blanc wines (W3) were characterised by higher levels of diethyl succinate (Figure 3.2) which has been shown to increase with wine age in Airen white wines (Gonzalez-Viñas et al., 1996) and Spanish Cava (Francioli et al., 2003, Riu-Aumatell et al., 2006). However, other research has indicated that this increase in white wines does not occur at cooled storage temperatures of 0-5 °C over a 12 month period (Marais and Pool, 1980, Pérez-Coello et al., 2003). It was clear that for all white wines diethyl succinate was higher in the 40 °C heat treated products; however, the Sauvignon blanc wines had substantially higher initial levels compared to the other varieties.

The Chardonnay (W4) wines were positively correlated with the canned vegetable sensory attribute and negatively correlated with compounds that were significantly different due to temperature treatment including linalool, TDN, and vitispirane 1 & 2. The Chardonnay wines were the only white wines to spend time in new oak barrels and underwent partial malo-lactic fermentation. It was noted that these wines were significantly higher in ethyl lactate, produced through malolactic fermentation (MLF) (Boido et al., 2009), and both (E)- and (Z)-oak lactones, found in wines fermented in oak (Ibern-Gómez et al., 2001) (data not presented). It is possible that the canned vegetable sensory attribute was associated with the oxidative formation of methional (Silva Ferreira and Guedes De Pinho, 2004) which can produce a cooked vegetables character in white wines (Escudero et al., 2000). However, the analytical conditions used within this study may not have been sensitive enough to detect this trace compound.

In the current study, the samples that were exposed to heat tended to have higher levels of TDN, and vitispirane 1 and 2 and lower levels of isoamyl acetate, hexyl acetate, and 2-phenylethyl acetate which are in agreement with previous research investigating wines stored at elevated temperatures (Marais and Pool, 1980, Ramey and Ough, 1980, Leino et al., 1993). Pérez-Coello and colleagues (2003) investigated the influence of storage temperature on the volatile compounds of young white wines. They observed a decrease in ethyl esters and acetates during uncontrolled storage conditions and times (1, 2, 3, and 4 years and recently bottled wines) and as with Marais and Pool (1980), found that wines that were stored chilled (0 and 10 °C) underwent fewer chemical alterations thus retaining their youthful wine aromas.

It is likely that variety, wine style, initial bottled quality of the product and the stage of bottle maturation will determine the degree that elevated temperatures impact wine

sensory characteristics. A previous study by De La Presa-Owens and Noble (1997) observed that noticeable changes in wine aroma of oaked and un-oaked Chardonnays occurred between five and nine days of storage at elevated temperatures, respectively. In contrast, a study by Marais and Pool (1980) of Chenin blanc, Riesling, and Colombard bottled under screw-cap, observed that the young wine bouquet remained unchanged over a 12 month period at storage temperatures of 0 and 10 °C. The same study observed a dramatic loss of young wine bouquet and the development of a maturation bouquet over the same period where the wines were stored at 20 and 30 °C. Samples of Colombard stored for two years at 0 °C showed no deterioration of young wine bouquet, and the 10 °C storage temperature decreased only slightly.

A loss of fruity and floral aromas in young white wine during storage is associated with the hydrolytic loss of acetates and ethyl esters (Marais and Pool, 1980, Ramey and Ough, 1980, Pérez-Coello et al., 2003). The enhancement of aged characters have been correlated with the oxidative formation of methional and phenylacetaldehyde (Silva Ferreira and Guedes De Pinho, 2004) and increases in TDN, and vitispirane (Simpson, 1979) due to acid hydrolysis of aroma precursors (Francis et al., 1994, Versini et al., 2002).

These studies and the results of the current study emphasize the importance of storage temperature for the maintenance of fresh aromas. It is difficult to determine from the current study what minimum length of time is required to cause the observed changes in the white wine sensory attributes. However, this study clearly reinforces the need for cooled storage conditions for white wine in transit and in storage.

3.4.2. Red wines.

Little research has been conducted to assess the sensory changes in red wines stored at different temperatures. In the current study only the dry fruit and canned vegetable

sensory attributes were significantly different due to treatment. This would indicate that the red wines in this study were relatively unchanged due to the treatments imposed which may not be the case for all red wines.

Changes in the volatile composition were similar to the white wine study in that the constant 20 °C and trunk treatment wines were not well differentiated while the constant 40 °C treatment were the most different to the other three treatments. The constant 40 °C treatment wines were characterised by lower levels of linalool, ethyl octanoate, nonanoate, decanoate, and dodecanoate, methyl octanoate and decanoate, isoamyl octanoate and decanoate, isopentyl hexanoate and ethyl 9-decanoate with higher levels of ethyl 2-furoate, ethyl phenylacetate, dehydroxylinalool oxide A, p-cymene, TDN, and vitispirane 1 and 2. Thus there were a substantial number of changes to the volatile composition of the wines.

Ough (Ough, 1985) studied the effects of temperature on a red blend of Zinfandel, Petite Syrah and Gamay at 28, 32, 38, 43 and 47 °C over three weeks with high and low levels of SO_2. The concentration of isoamyl acetate decreased with increasing temperature while ethyl hexanoate, octanoate, and decanoate showed no clear relationship to the temperature treatment, with the level of SO_2 having no obvious effect over the period (Ough, 1985).

Increases in TDN and vitispiranes in wines can be attributed to hydrolysis of multiple glycosylated precursors under acidic conditions which can be accelerated by elevated temperatures (Winterhalter et al., 1990b, Winterhalter, 1991, Francis et al., 1994, Versini et al., 2002). Silva Ferreira and co-workers have shown that temperature and pH are particularly important to the formation of both TDN and vitispiranes (Silva Ferreira and Guedes De Pinho, 2004). It has also been observed that p-cymene can be produced

through heated acid hydrolysis of aroma precursor fractions from grapes (Williams et al., 1982b, Schneider et al., 2001).

The loss of linalool due to increased storage temperature is undesirable in citrus juices (Perez-Cacho and Rouseff, 2008) and has been attributed to the coinciding increase in α-terpineol (Pérez-López et al., 2006). The loss of linalool and increase in α-terpineol has also been observed in heated black currant juice (Varming et al., 2004). It is suggested that the transformation of linalool to α-terpineol occurs through the protonation of linalool's hydroxyl group (Haleva-Toledo et al., 1999). Under acid conditions, as is the case in wine, it is generally understood that linalool is produced as an intermediate in the formation of α-terpeniol and other products from the thermal degradation of geraniol (Baxter et al., 1978, Skouroumounis and Sefton, 2000). Silva Ferreira and co-workers have previously observed that the degradation of linalool, and formation of linalool oxides, was significantly greater at 45 °C when compared to 15 °C (Silva Ferreira et al., 2002). In the current study, dehydroxylinalool oxide A and linalool were negatively correlated, with dehydroxylinalool oxide A being positively correlated with the constant 40 °C treatment. Given that there was no significant increase in α-terpineol observed it would suggest that, in wine, linalool predominantly forms linalool oxides due to elevated temperature storage.

The major observation in the red wine study was a clear separation of the constant 40 °C heat treatment wines from the other three treatments due to changes in the volatile composition. However, a change in the volatile composition of the wines was not coupled to differences in the majority of the sensory descriptors used in this study. As the study was conducted using only a small number of commercial products the sensory results may not reflect potential changes in other red wines. This hypothesis is supported by the observation of substantial changes to the volatile composition of the

wines under the conditions used. These compositional changes may have more significant sensory consequences for other red wines and given that little information is currently in the literature, further research is warranted to more clearly understand the influence of temperature on red wine aroma and composition.

3.5. Conclusions

The objective of this study was to explore the sensory repercussions of adverse temperature conditions on white and red wines. The wines were exposed to simulated shipping conditions and then evaluated using sensory descriptive analysis. The data showed a significant impact of the constant 40 °C heat treatment on the aromatic properties of the wines. The 40 °C treatment produced the most significant differences among the white wines by increasing diesel, oxidized, and rubber aromas and decreasing citrus, floral and tropical fruit aromas. The magnitude of the effect was significant; however, less pronounced in the red wines showing increased dried fruit and canned vegetable aromas. PLS analysis of the white wines identified a number of compounds that may be useful markers, including vitispirane 1&2, TDN, p-cymene and a number of esters and acetates, for monitoring wine product development on-shelf or as a confirmation that wines have not experienced any adverse conditions during shipping. The volatile analysis showed a number of compounds were affected by the temperature treatments; however, an untargeted analytical method was employed for the measurement of volatiles thus it is possible other compounds could be altered due to elevated temperatures. Future research should be extended into documenting the changes in other varietal wines under varied temperature conditions to better understand the changes in wine products due to transport and storage.

4. Development of a sensitive non-targeted method for characterizing the wine volatile profile using headspace solid-phase microextraction comprehensive two-dimensional gas chromatography time-of-flight mass spectrometry.

The following is a modified version of the published paper: Robinson, A.L., P.K. Boss, H. Heymann, P.S. Solomon, and R.D. Trengove (2011) Journal of Chromatography A ***1218****, 504-517.*

4.1. Introduction

The fields of separation science and sensory science have advanced our knowledge of how volatile and semi-volatile compounds contribute to wine aroma (Ferreira et al., 2000, Francis and Newton, 2005). With more than 800 aroma compounds reported in the volatile fraction of wine (Rapp, 1990), it is well understood that the wine volatile profile is complex. Some studies have concluded that the vast majority of wine volatile compounds have little or no aroma activity and that specific aroma profiles can be explained by relatively few aroma compounds (Escudero et al., 2007). However, there is conflicting evidence about the complexity of the system given that odour mixtures have masking (modification of the perceived odour), counteraction (reduction of the odour intensity) (Cain and Drexler, 1974), and synergistic (complementation or enhancement of the odour intensity) (Miyazawa et al., 2008) effects which play an important role in defining the perceived aroma of wine (Pineau et al., 2007, Ryan et al., 2008). It is thus important that grape and wine researchers develop the analytical capacity to measure as many volatiles as possible to enable better comparisons of effects of viticultural and winemaking studies and to identify candidate compounds that can be correlated with differences in the perceived aroma of wine.

The development of comprehensive two-dimensional gas chromatography (GC×GC) (Liu and Phillips, 1991) has been followed by numerous reviews discussing the principals and experimental design of GC×GC (Ong and Marriott, 2002, Dallüge et al., 2003, Górecki et al., 2004). These reviews have shown that GC×GC offers enhanced separation efficiency, reliability in qualitative and quantitative analysis, capability to detect low quantities, and information on the whole sample and its components. In more recent years, there has been a shift towards the use of this technique in the analysis of real-life samples including food and beverages, environmental, biological, and petrochemical (Adahchour et al., 2008).

A number of grape and wine profiling studies have used HS-SPME to better understand the role of various compounds in differentiating varieties, regions, and wine vintage (Marengo et al., 2001, Câmara et al., 2007, Setkova et al., 2007c) and the technique has been repeatedly documented as a sensitive, reproducible, automated method for pre-concentration of wine volatiles prior to analysis (Howard et al., 2005, Câmara et al., 2006, Setkova et al., 2007b). The combination of headspace solid-phase microextraction (HS-SPME) and GC×GC-TOFMS techniques has provided a major advantage in analysing complex samples where the number of analytes may be large or the analytes of interest are present at trace levels – as is the case with wine. A number of publications have emerged in the grape and wine field that have utilized HS-SPME and GC×GC as a technique (Ryan et al., 2005, Rocha et al., 2007, Ryona et al., 2008, Ryona et al., 2009, Perestrelo et al., 2010, Ryona et al., 2010, Schmarr et al., 2010). However, the majority of studies have used the method for targeted analysis (Ryan et al., 2005, Ryona et al., 2008, Ryona et al., 2009, Perestrelo et al., 2010, Ryona et al., 2010) with only two publications to date utilizing the technique for volatile profiling (Rocha et al., 2007, Schmarr et al., 2010).

Rocha and co-workers (Rocha et al., 2007) used GC×GC to analyse monoterpenes in grapes and identified 56 monoterpenes in the Fernão-Pires variety, of which 20 were reported for the first time in grapes. This highlighted the advantage that structured chromatographic separation can provide in compound classification and compound identity confirmation. There continues to be new aroma compound discoveries in the grape and wine research field with recent discoveries including (E)-1-(2,3,6-Trimethylphenyl)buta-1,3-diene (TPB) (Cox et al., 2005) and 1(2H)-Azulenone, 3,4,5,6,7,8-hexahydro-3,8-dimethyl-5-(1-methylethenyl)- ((-)-Rotundone) (Wood et al., 2008). It is anticipated that GC×GC will provide significant advantages in the identification of new compounds which were previously unresolved using traditional one-dimensional chromatography.

A recent critical review (Polášková et al., 2008) identified that future developments in understanding differences in the sensory attributes of wines will be due to: (1) development of improved and high throughput analytical methods that will allow monitoring of a large number of volatiles including those present at low concentrations; (2) improved understanding of the relationships between chemical composition and sensory perception, including an emphasis on the mechanisms of how odorants and matrix components interact chemically to impact odorant volatility and overall flavour perception of wines; and (3) multidisciplinary studies using genomic and proteomic techniques to understand flavour and aroma formation in the grape and during fermentation. The current study addresses the first recommendation from this publication and outlines a comprehensive analytical technique for the analysis of the wine volatile profile. The application of this technique to a small number of commercial wines clearly demonstrates that the optimized method can resolve and identify a large

number of compounds and could be used in the future to differentiate wines based on their volatile profile.

4.2. Materials and Methods

4.2.1. Samples

Method development was conducted using a young (<12 months old) commercially available Cabernet Sauvignon wine (~13.0 % Ethanol v/v) from Australia. The wine was dispensed for use from a 2 L boxed wine bladder (cask) to minimize spoilage and oxidation during the course of analysis. Evaluation of the method was carried out using commercially available Cabernet Sauvignon wines with four wines from the 2005 vintage and one wine from the 2006 vintage representing four Western Australian Geographical Indications (GI, being the official delineation for wine regions within Australia). In all analyses 10 mL of wine was pipetted into the vial and sealed.

4.2.2. Analytical reagents and supplies

SPME fibers 1 cm and 2 cm Divinylbenzene/Carboxen/Polydimethylsiloxane (DVB/CAR/PDMS) 50/30 µm 23 ga metal alloy were purchased from Supelco (Bellefonte, PA, USA). Prior to initial use, all new fibers were conditioned for 30 minutes at 270 °C as per the manufacturer's recommendations. Clear and amber glass, screw threaded, 20 mL headspace vials with magnetic screw caps and white PTFE / blue silicone (thickness 1.3 mm) septa were purchased from Alltech (Alltech Corp, Deerfield, IL, USA). Sodium chloride (NaCl) (AR Grade) was purchased from Merck Pty Ltd (Kilsyth, Victoria, Australia) and was oven dried at 110 °C overnight before use. Methyl nonanoate (Quant Grade) was purchased from PolyScience (PolyScience, Niles, Illinois, USA). 2-Isobutyl-3-methoxypyrazine (99% pure) was purchased from Sigma (Sigma-Aldrich Corporation, St. Louis, MO, USA). Straight-chain alkanes (C8-C20) were purchased from Polyscience and Fluka (Sigma-Aldrich Corporation, St.

Louis, MO, USA). HPLC grade n-pentane was purchased from Lab-Scan (Labscan Asia Co. Ltd., Patumwan, Bankok, Thialand) and HPLC grade methanol was purchased from Burdick & Jackson (SK Chemicals, Ulsan, Korea). Inland 45 Vacuum pump fluid (pump oil) was purchased from Inland Vacuum Industries (Inland Vacuum Industries, Churchville, NY). Ultra-pure water was prepared using a Milli-Q water purification system to a resistivity of 18 MΩ cm (Millipore, Bedford, MA, USA).

4.2.3. Instrumentation

A CTC CombiPAL autosampler (CTC Analytics, Zwingen, Switzerland) with an agitator and SPME fiber conditioning station was used to extract the volatiles from the sample vial headspace. A LECO Pegasus® 4D GC×GC-TOFMS (LECO, St. Joseph, MI, USA) was used for all experiments. The GC primary oven was equipped with a 30 m Varian FactorFour™ VF-5MS capillary column, ID of 0.25 mm and a film thickness of 0.25 µm with a 10 m EZ-Guard™ column (Varian Inc., Walnut Creek, CA, USA). This was joined using a SilTite™ mini-union (SGE, Ringwood, Victoria, Australia) to a 1.65 m Varian FactorFour™ VF-17MS capillary column with an ID of 0.10 mm and a film thickness of 0.20 µm of which 1.44 m was coiled in the secondary oven. The non-polar and medium-polar column combination was chosen due to the low bleed characteristics of both the primary and secondary columns thus allowing for additional sensitivity for the analysis of trace analytes. A Supelco 0.75 mm ID SPME straight-through inlet liner (Bellefonte, PA, USA) was used for all injections. A High Pressure Merlin Microseal® (Bellefonte) was used for all 23 ga SPME injections.

4.2.4. HS-SPME Optimization

The following HS-SPME conditions were used during method development unless otherwise stated. Samples for HS-SPME method development were prepared in clear glass 20 mL headspace vials. Samples for GC×GC-TOFMS method development and

evaluation were prepared in equivalent amber glass vials to prevent light degradation of alkyl-methoxypyrazines known to occur in Cabernet Sauvignon wines (Roujou de Boubée et al., 2000). All samples were incubated at 30 °C with agitation at 500 rpm for 10 minutes prior to extraction at 250 rpm. DVB/CAR/PDMS SPME fibers were previously demonstrated to be suitable for non-targeted analysis of trace volatile and semi-volatile compounds in wine and were consequently used during this study (Howard et al., 2005, Setkova et al., 2007b). The headspace was sampled using a 1 cm DVB/CAR/PDMS 50/30 µm metal alloy fiber for 60 minutes at 30 °C and desorbed in the GC inlet at 260 °C for 1 minute. The fiber was then reconditioned using the fiber conditioning station for 5 minutes at 260 °C to prevent analyte carry over between samples. High purity (HP) Nitrogen (Air Liquide, Australia) was passed over the fiber during reconditioning.

4.2.4.1. Desorption conditions

Fiber desorption times of 10, 20, 30, 40, 50, 60, 80, and 120 sec were assessed at 250 °C. A second experiment assessed desorption temperatures of 230, 240, 250, 260, and 270 °C using a 60 sec desorption time. Sample carry over was also assessed to determine the level of analytes not desorbed from the fiber prior to using the fiber conditioning station.

4.2.4.2. Salting out effect.

Sodium chloride was added at concentrations of 0, 50, 100, 150, 200, 250, 300, 350, 400, 450, and 500 g/L to study the salting out effect.

4.2.4.3. Sample agitation

Agitation speeds of 250, 300, 350, 400, 450, 500, 550, 600, 650, 700, and 750 rpm during extraction were examined. A second experiment was conducted to compare the effect of agitation on samples with and without salt. Extraction agitation speeds of 0,

300, 400, 500, 600, and 700 rpm were compared with samples that had been salted (300 g/L) and unsalted (0 g/L). All subsequent method development was conducted using an extraction agitation speed of 600 rpm as a compromise between extraction efficiency and fiber longevity.

4.2.4.4. Headspace extraction time and fiber length

Headspace extraction times of 30, 60, 90, 120, and 150 min were assessed comparing a 1 cm and a 2 cm length DVB/CAR/PDMS fiber.

4.2.4.5. Influence of sample incubation temperature

Samples were incubated at 30, 35, 40, 45, 50, 55 and 60 °C for 90 min and, after cooling to room temperature, were extracted for 90 min at 30 °C. These values were compared to a sample that remained at ambient temperature (20 °C).

4.2.5. Loading of internal standard onto SPME fiber

Methyl nonanoate was chosen as an internal standard as it has not been previously reported in the literature as occurring in Cabernet Sauvignon wines and was not observed in the wine analysed. The standard was loaded into the SPME fiber coating prior to the sample extraction step using methodology as previously described (Wang et al., 2005, Setkova et al., 2007a, Setkova et al., 2007b). A 20 mL headspace vial containing 4 g of vacuum pump fluid and 20 µL of methyl nonanoate (1.1 g/L in HPLC grade methanol) was extracted for 5 min at 30 °C and 600 rpm.

4.2.6. Loading of retention index probes onto SPME fiber

Retention index probes were loaded into the fiber coating after the internal standard as previously described (Wang et al., 2005). A 20 mL headspace vial containing 1 mL MilliQ water and 10 µL of straight chain n-alkanes (C_8-C_{20}) in HPLC grade pentane was extracted under the same conditions as the internal standard (Setkova et al., 2007b). Pentane was used as a solvent as hexane was found to overload the column and

interfere with early eluting compounds. Alkanes were made up individually at varied concentrations to prevent the overloading of highly volatile low molecular weight probes and underloading of low volatility high molecular weight probes.

4.2.7. Chromatographic conditions

The injector was held at 260 °C in the splitless mode with a purge-off time of 1 minute, a 50 mL/min split vent flow at 1 minute and a gas saver flow of 20 mL/min at 3 minutes. Ultra high purity (UHP) Helium (Air Liquide, Australia) was used as the carrier gas at a constant flow rate of 1.3 mL/min. The temperature program was 30 °C for 1 minute, ramped at 3 °C/min to 240 °C, and held at 240 °C for 9 minutes. The secondary oven program was offset by +15 °C from the primary oven program and the modulator was offset by +30 °C from the primary oven. Single dimensional analysis acquired data at a rate of 10 scans/sec as a compromise between sensitivity and facilitating sufficient peak deconvolution. For GC×GC mode, the data was acquired at a rate of 100 scans/sec to accommodate the peak elution rate for modulated analytes. The transfer line and ion source were maintained at 250 °C and 200 °C, respectively, for both 1D and 2D experiments. The TOFMS detector was operated at 1750 volts and collected masses between 35 and 350 amu.

4.2.8. Optimization of GC×GC parameters

Modulation periods were optimized by assessing modulation times of 4, 6, 8, 10, and 20 seconds with a secondary oven temperature offset of 15 °C to the primary oven. The secondary oven temperature offset was also assessed at +5, 10, 15, and 20 °C to the primary oven with a modulation period of 10 seconds.

4.2.9. Instrument control and data analysis software

Automated HS-SPME sample preparation was controlled using the PAL Cycle Composer with Macro Editor software Version 1.5.2. GC temperature programs,

TOFMS data acquisition was controlled through the LECO ChromaTOF® software Version 3.32 optimized for Pegasus. Data analysis was conducted using LECO ChromaTOF® software Version 3.34 and used automated peak find and spectral deconvolution with a baseline offset of 0.5, Auto data smoothing, and a signal to noise of 100. Results were matched against the NIST 2005 Mass Spectral Library using a forward search on all masses collected and calculated retention indices were compared to published retention indices for 5% phenyl polysilphenylene-siloxane capillary GC columns or equivalents (Adams, 2007, Stein, 2009). All compounds tentatively assigned by the ChromaTOF software were manually assessed with respect to the mass spectral match and the assigned Unique mass which was used for quantification.

4.2.10. Statistical analysis

All statistical analysis was conducted using JMP version 7.0.1 (SAS Institute Inc., Cary, NC, USA). Figures and tables were generated using Microsoft Office Excel 2007 (Microsoft Corporation, Redmond, WA, USA).

4.2.11. SPME Method optimization / data analysis

The relative responses of compounds, peak area of the unique ion expressed as a percentage of the maximum value recorded for the optimization parameter, were assessed in relation to the specific optimization parameter through hierarchical cluster analysis using a minimal variance algorithm (Ward, 1963). Hierarchal cluster analysis is an unsupervised multivariate statistical technique which was employed to simplify the data analysis by clustering compounds that behaved in a similar manner. The cluster membership was then analysed using a one-way analysis of variance (ANOVA) using a Tukey-Kramer HSD test to determine whether compound clusters responded differently to the specified optimization parameter. Cluster means ± standard error (SE) were then

plotted against the optimization parameter with a second order line of best fit to depict the relative response of analytes to the optimization parameters.

4.3. Results and Discussion

4.3.1. HS-SPME Optimization.

Although many compounds were identified, a representative selection of 25 target compounds, regarded as important contributors to wine aroma (Ferreira et al., 2000, Francis and Newton, 2005), were used for HS-SPME method optimization. The SPME optimization results are discussed with reference to Cluster membership of compounds listed in Table 4.1.

4.3.2. Desorption conditions

Fiber desorption temperature had a mixed influence on peak response. It was found that the peak area of compounds belonging to Cluster A increased from 48% to 87% of maximum between 230 and 260 °C respectively (Figure 4.1).

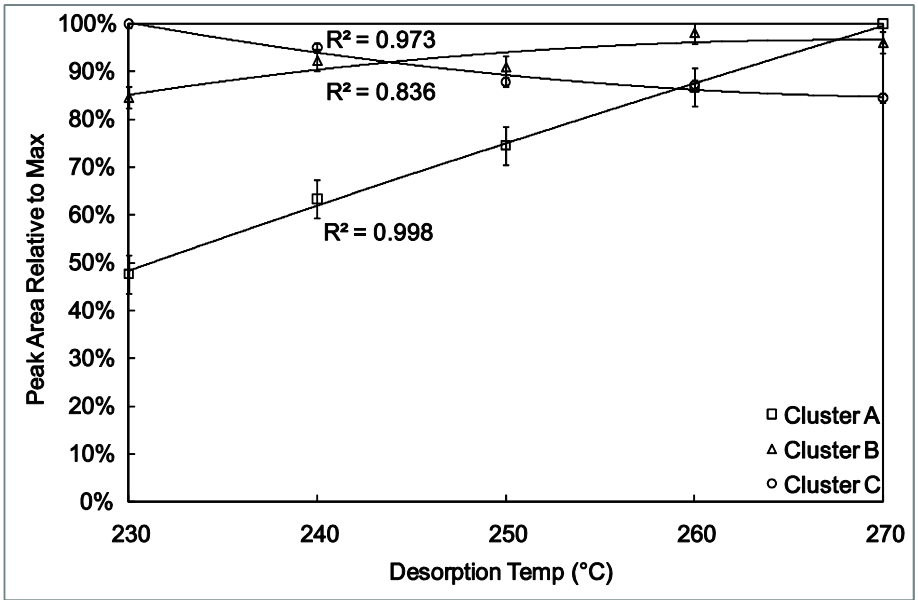

Figure 4.1 Influence of inlet desorption temperature on the relative peak area response. Relative peak area is expressed as a percentage of the maximum value recorded. Data points represent the mean (± SE) of compounds belonging to Clusters A, B and C.

Table 4.1 Target compounds used for HS-SPME method optimization.

Compound	CAS	Unique Ion[¥]	RT (s)	RI[₦] (calc)	RI[€] (lit)	MS Match	% RSD	Desorption Clusters	Salting Clusters	Agitation Clusters	Time Clusters	Incubation Clusters
Ethyl propanoate	105-37-3	102	457.1	732	733	925	4%	C	E	H	J	L
Ethyl isobutyrate	97-62-1	116	557.1	769	756	784	8%	C	E	H	J	L
Ethyl butanoate	105-54-4	89	653.8	804	803	910	7%	C	E	H	J	L
Isohexanol	626-89-1	56	759.7	842	838	891	2%	C	D	H	K	L
Ethyl 2-methylbutyrate	7452-79-1	102	781.3	850	848	944	9%	C	E	H	J	L
Ethyl 3-methylbutyrate	108-64-5	88	794.2	855	852	870	8%	C	E	H	J	L
Ethyl pentanoate	539-82-2	88	929.7	903	898	886	5%	B	E	H	J	L
Methyl hexanoate	106-70-7	74	1000.0	926	923	891	4%	B	E	H	J	L
Hexyl acetate	142-92-7	84	1269.9	1014	1007	898	4%	B	E	H	J	L
p-Cymene	99-87-6	134	1311.1	1028	1026	845	5%	B	E	H	K	M
Eucalyptol	470-82-6	154	1337.3	1036	1033	852	2%	B	D	H	J	L
Benzyl Alcohol	100-51-6	108	1358.0	1043	1041	883	2%	A	D	G	I	L
Phenylacetaldehyde	122-78-1	120	1382.0	1051	1050	890	7%	A	D	G	K	L
Ethyl furoate	614-99-3	95	1396.1	1056	1056	890	6%	A	D	G	I	L
Terpinolene	586-62-9	93	1496.0	1088	1087	895	5%	B	D	G	K	M
Ethyl heptanoate	106-30-9	88	1527.0	1098	1093	905	8%	B	E	H	J	L
Linalool	78-70-6	93	1540.3	1103	1106	873	2%	B	D	H	J	N
α-Terpineol	98-55-5	136	1846.6	1210	1186	823	2%	B	D	F	I	L
2-Phenylethyl acetate	103-45-7	91	1992.5	1262	1256	906	2%	A	D	F	I	L
Vitispirane	65416-59-3	192	2062.6	1288	1272	961	8%	B	D	G	I	M
Methyl decanoate	110-42-9	74	2165.6	1326	1323	790	9%	A	E	G	K	N
(Z)-Oak lactone	55013-32-6	71	2174.3	1330	1340	870	4%	A	D	F	I	L
(Z)-β-Damascenone	23696-85-7	121	2266.7	1365	1367	812	3%	A	D	F	I	L
(E)-β-Damascenone	23726-93-4	121	2322.6	1386	1387	876	3%	B	D	F	I	L
Ethyl decanoate	110-38-3	101	2352.7	1397	1393	912	8%	A	E	H	K	N

[¥] Unique ion (*m/z*): used for peak area determination, identified as the unique ion by ChromaTOF data analysis. [₦] RI: retention indices calculated from C8-C20 n-alkanes. [€] RI: retention indices reported in the literature for 5% phenyl polysilphenylene-siloxane capillary GC columns or equivalent (Adams, 2007, Stein, 2009).

However, compounds belonging to Clusters B and C increased and decreased by ~13% of maximum respectively within the same inlet temperature range. ANOVA indicated that there was no significant difference in the cluster means between 260 and 270 °C for all compound clusters, thus subsequent analysis was conducted at 260 °C.

Analyte carry over declined with increasing desorption temperature, with all trace compounds being below detection threshold and the higher abundant compounds declining to less than 5% of the analysed peak area (data not presented). A 5 minute conditioning step at 270 °C prevented any carry over effects.

4.3.3. Salting out effect

The standard addition of 300 g/L sodium chloride to a wine was selected, given that it covers the saturation range of sodium chloride for the majority of table wines. The resulting salting out, or Setschenow effect (Mazo, 2006), led to an increase in peak area for all compounds analysed. ANOVA indicated that increasing concentrations of salt above 300 and 200 g/L for compounds in clusters D and E respectively did not result in a statistically significant change. Compounds belonging to Cluster D increased from 20 to 88% of maximum at 300 g/L while compounds belonging to Cluster E increased from 53 to 91% of maximum at 200 g/L (Figure 4.2).

Compounds belonging to Cluster D had a range of different functionalities while compounds belonging to Cluster E were typically ethyl and methyl esters with the exception of p-cymene. This is consistent with pharmaceutical research relating the salting out effect in a sodium chloride solution to molar volume, aqueous solubility, and the octanol–water partition coefficient ($K_{o/w}$) (Ni et al., 2000, Ni and Yalkowsky, 2003). Further, Ferreira and co-workers (Ferreira et al., 1998) observed that the ethyl esters had particularly high gas-liquid partition coefficient (GLPC) values and suggested that

their behaviour could be best explained firstly by the functionality, or polarity, and then by their intrinsic volatility.

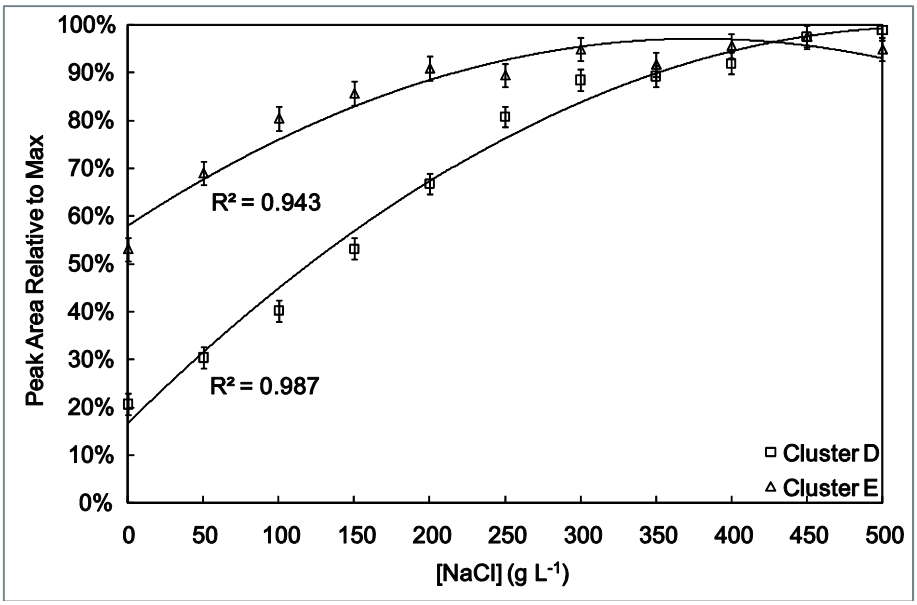

Figure 4.2 Influence of sodium chloride concentration on the relative peak area response. Relative peak area is expressed as a percentage of the maximum value recorded. Data points represent the mean (± SE) of compounds belonging to Clusters D and E.

4.3.4. Sample agitation

ANOVA indicated that there was no significant difference in the cluster means between 600 rpm and subsequent agitation speeds for all three cluster groups. Compounds belonging to Cluster F increased from 20% to 82% of maximum between 250 and 600 rpm respectively (Figure 4.3). Compounds belonging to Cluster G and H increased 46% and 17% of maximum between 250 and 600 rpm respectively.

Compounds tended to cluster according to molecular weight and vapour pressure. That is, compounds belonging to Cluster H had lower molecular weights with higher vapour pressures, whilst compounds belonging to Cluster F were characterized by higher molecular weight and lower vapour pressures and compounds belonging to Cluster G had intermediate molecular weight and vapour pressures compared to compounds

belonging to Clusters F and H. The impact of molecular weight is consistent with the diffusion dependence on this property.

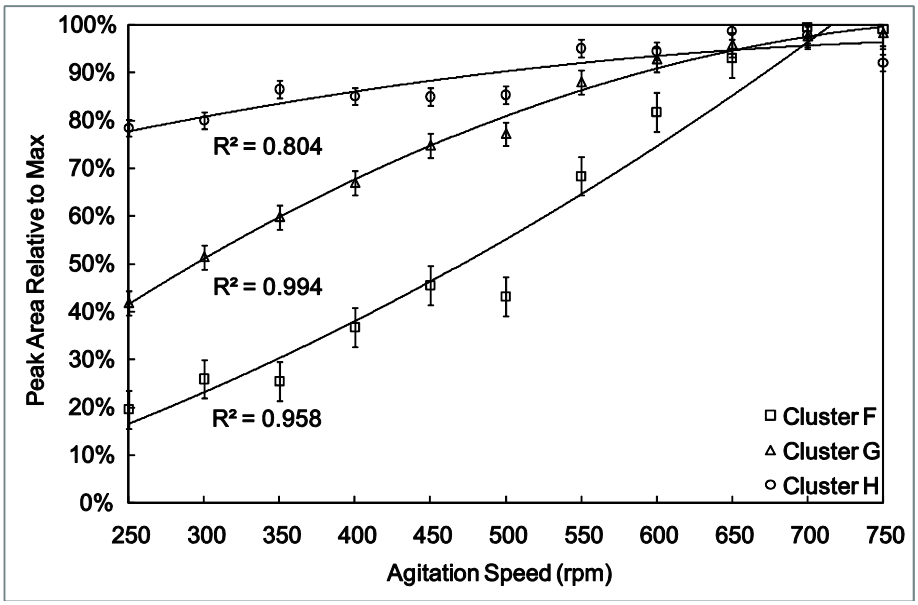

Figure 4.3 Influence of sampling agitation speed on the relative peak area response. Relative peak area is expressed as a percentage of the maximum value recorded. Data points represent the mean (± SE) of compounds belonging to Clusters F, G and H.

4.3.5. Salt and agitation interactions

Previous studies have demonstrated that the new-generation super elastic metal alloy SPME fibers are capable of carrying out several hundred extraction cycles (Setkova et al., 2007a) without showing any significant loss in sensitivity, with one study conducting more than 600 cycles using a single fiber (Setkova et al., 2007c). However, each extraction in the studies by Setkova and co-workers (Setkova et al., 2007a, Setkova et al., 2007c) exposed the SPME fiber to agitation stress for 5 minutes at 500 rpm per extraction which would equate to 50 hours of agitation stress. In this study we found that extreme agitation caused scoring of the SPME needle and eventually damaged the fiber, thus an agitation speed of 600 rpm was selected as a compromise to optimize sensitivity while maintaining the fiber lifetime.

4.3.6. Headspace extraction time and fiber length

The fiber length by extraction time interaction was significant with the 2 cm fiber compared with a 1 cm fiber providing greater peak area values for all compounds (Figure 4.4 (A) and (B)). Compounds belonging to Cluster I and K increased with increasing extraction time while compounds belonging to Cluster J remained constant with respect to extraction time.

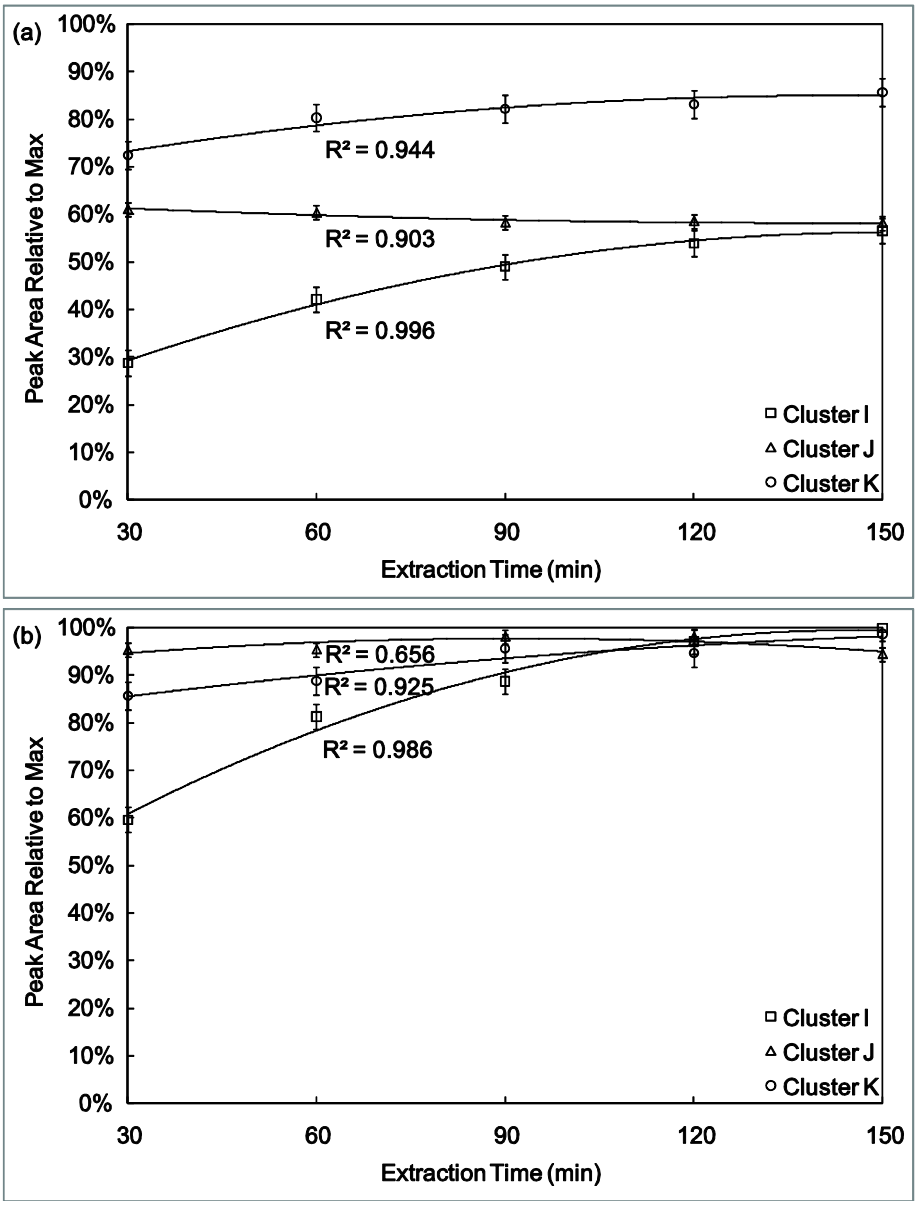

Figure 4.4 Influence of sampling time on the relative peak area response using (a) 1 cm and (b) 2 cm fiber lengths. Relative peak area is expressed as a percentage of the maximum value recorded. Data points represent the mean (± SE) of compounds belonging to Clusters I, J and K.

However, ANOVA indicated that the compounds belonging to Cluster J at 120 minutes increased from 59 to 98% of maximum with the increase in fiber length from 1 to 2 cm. Compounds belonging to Clusters I and K were not significantly different after 120 and 90 minutes respectively. A maximum relative peak area was achieved for all compounds after 120 minutes of extraction using a 2 cm fiber length.

4.3.7. Influence of sample incubation temperature

A previous study correlated the presence of artifacts with HS-SPME extraction temperature in honey samples (Čajka et al., 2007) and this phenomenon was investigated for wines by incubating samples from 30-60 °C for 90 mins as described previously. The results of the analysis are shown in Figure 4.5. ANOVA indicated that the abundance of compounds within Clusters L and N declined significantly at incubation temperatures above 50 °C and 45 °C, respectively, while compounds belonging to Cluster M increased significantly at incubation temperatures above 40 °C.

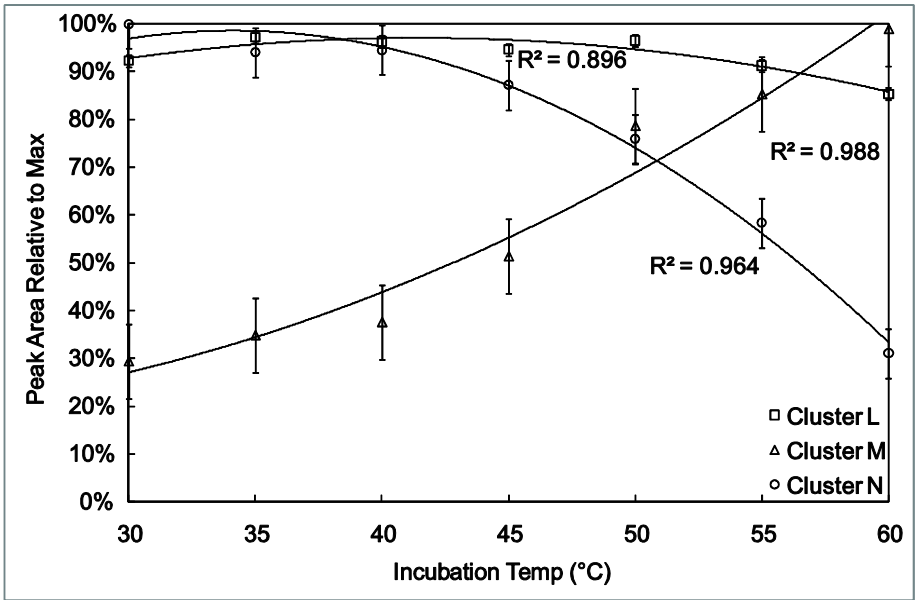

Figure 4.5 Influence of incubation temperature on the relative peak area response. Relative peak area is expressed as a percentage of the maximum value recorded. Data points represent the mean (± SE) of compounds belonging to Clusters L, M and N.

Linalool and ethyl decanoate (Cluster N) showed significant declines in concentration and reflected changes in a number of other compounds including methyl decanoate. Vitispirane, p-cymene and terpinolene represent a much larger set of compounds, including 1,1,6-Trimethyl-1,2-dihydronaphthalene (TDN), and dehydroxylinalool oxide, that changed more dramatically with respect to incubation temperature. Silva Ferreira and co-workers have studied the formation of Vitispirane and TDN with respect to temperature, time, SO_2 concentration, and dissolved oxygen concentration (Silva Ferreira et al., 2002, Silva Ferreira and Guedes De Pinho, 2004). It was shown that temperature and pH were particularly important to the formation of both Vitispirane and TDN (Silva Ferreira and Guedes De Pinho, 2004). Previous research has indicated that both Vitispirane and TDN are generated from multiple glycosylated precursors that are hydrolysed under acidic conditions which can be accelerated by elevated temperature (Winterhalter et al., 1990b, Versini et al., 2002). It also followed that the degradation of linalool and formation of linalool oxides was accelerated at 45 °C compared to 15 °C temperatures (Silva Ferreira et al., 2002).

This is the first study that has documented the formation of artifacts in wine through the use of increased temperature during the SPME incubation step. Given that products were generated and lost under elevated temperature conditions, the lowest controlled temperature available, 30 °C, was chosen as the optimum temperature for incubation and extraction of the sample.

4.3.8. Repeatability of SPME method

Six replicate extractions of the cask wine were analysed with the optimized HS-SPME method (Table 4.2). The internal standard, methyl nonanoate, and retention index probes were loaded onto the fiber prior to sample extraction which made their response

independent of the sample matrix as previously demonstrated (Wang et al., 2005, Setkova et al., 2007a, Setkova et al., 2007b).

Table 4.2 Optimized HS-SPME/GC×GC-TOFMS conditions used for the analysis of five commercial Cabernet Sauvignon Wines from Western Australia

HS-SPME	
HS Vial	20 mL Amber Headspace Vial
Sample Volume	10 mL wine
Salt Addition	300 g/L
SPME Fiber	DVB/CAR/PDMS 50/30 µm, 2 cm, 23 Ga Metal Alloy
Incubation Conditions	30 °C / 600 rpm / 5 min
Extraction Conditions	30 °C / 600 rpm / 120 min
Desorption Conditions	260 °C / 1 min
Fiber bake-out Conditions	270 °C / 5 min
GC×GC	
Injector Mode	Splitless
1° GC Column	VF-5MS (30 m x 0.25 mm I.D. x 0.25 µm & 10 m EZ-Guard)
2° GC Column	VF-17MS (1.65 m x 0.10 mm I.D. x 0.20 µm)
Carrier Gas	UHP Helium
Gas flow	Constant Flow, 1.3 mL/min
GC Oven Program	30 °C (1 min) / 3 °C/min to 240 °C (9 min)
Secondary Oven Offset	+5 °C
Modulation Period	6 sec
Transfer Line Temperature	250 °C
TOFMS	
Detector Voltage	1750 Volts
Data Acquisition Rate	100 scans/sec
Mass Range	35 - 350 amu
Ion Source Temperature	200 °C

RSD values were calculated using the peak area values normalized against the on-fiber internal standard and are presented in Table 4.1. RSD's of the normalized peak area ranged from 2 to 9% which was comparable to previous HS-SPME studies (Howard et al., 2005, Câmara et al., 2006, Setkova et al., 2007b).

4.3.9. Optimization of GC×GC parameters

The objective of coupling HS-SPME to GC×GC-TOFMS was to analyse a substantial number of compounds with gains in sensitivity and resolution from GC × GC modulation coupled to gains in sensitivity and selectivity from HS-SPME. In comprehensive two-dimensional gas chromatography, samples are resolved through two chromatographic separations in series. This process is aided by a modulator which periodically collects, focuses, and reintroduces the eluent at the end of the primary

column into the secondary column where it undergoes an isothermal separation before reaching the detector. The major advantage of this process is that the first dimension separation is maintained while allowing additional separation in the second dimension (Górecki et al., 2004). Parameters controlling the second dimension of chromatography were investigated to determine their influence on resolution.

In order to preserve the primary dimension separation the modulator should sample the first dimension as frequently as possible (Davis et al., 2008). To better accomplish this, it is understood that temperature programming in GC×GC is usually at a lower rate than in one dimensional gas chromatography, i.e. at 2 - 3 °C/min (Adahchour et al., 2008). The resolution of two closely eluting compounds, TDN and (Z)-β-damascenone, were examined at varying modulation times.

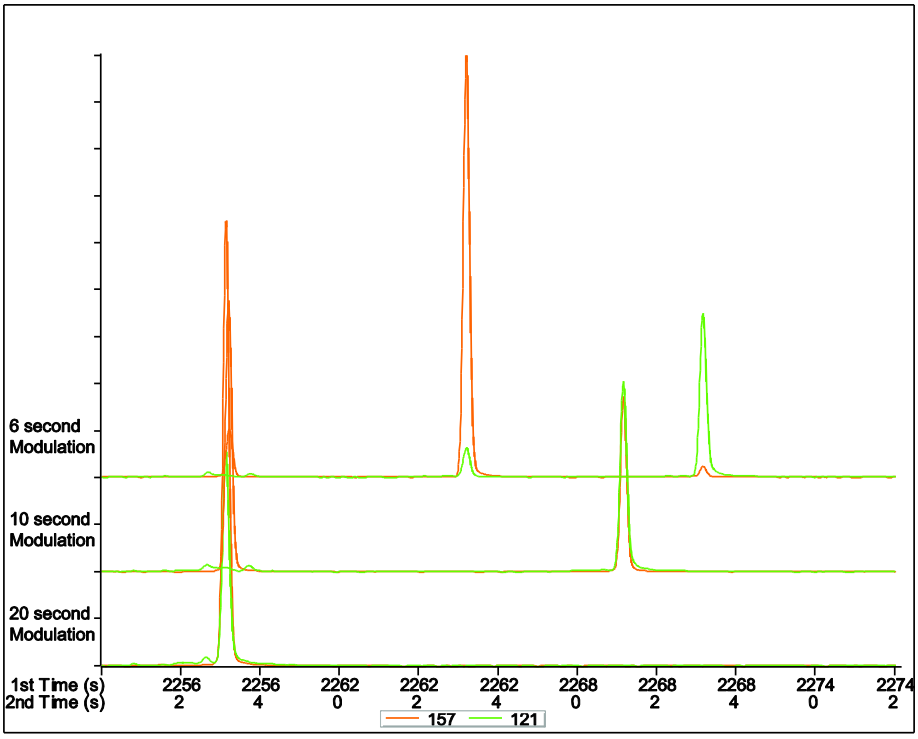

Figure 4.6 Influence of 6, 10 and 20 second modulation times on the second dimension separation of TDN (m/z 157) and (Z)-β-Damascenone (m/z 121). Note with increasing modulation time that the first dimension separation is compromised.

These two compounds were selected as an example as (E)-β-damascenone is well recognized as a potent aroma compound in wine (Pineau et al., 2007) while the (Z)-isomer of β-damascenone, which is present at much lower concentrations, has rarely been identified and reported in wine related studies. Figure 4.6 shows that the shorter modulation time of six seconds resolved TDN and (Z)-β-damascenone, whilst 10 and 20 second modulation times caused a loss in primary dimension separation with both compounds recombined in the modulator (Dallüge et al., 2002). These two compounds were resolved in the first dimension ($R_{S1} \approx 1.1$) but not well resolved in the second dimension ($R_{S2} \approx 0.1$), at the natural concentrations found in the cask wine used. Literature typically suggests that any first dimension peak should be sampled by the modulator at least three times when the sampling is in-phase and four times when the sampling is 180º out-of-phase (Murphy et al., 1998, Ong and Marriott, 2002). With a modulation period of six seconds the majority of peaks were sampled three times or more. Attempting to optimize the modulation phase or peak pulse profiles for all compounds in a real sample is a complex process due to errors associated with the summation of multiple modulated peaks and errors due to shifts in the phase of the primary peak relative to the modulation period (Harynuk et al., 2008).

In practice, the sample rate in the first dimension is limited by the duration of the second dimension separation. To maintain the ordered structure of the chromatogram, compounds should elute within the modulation cycle to prevent compounds from different modulation cycles co-eluting (Dallüge et al., 2003). Decreasing the modulation time to five seconds or less produced a wrap-around effect for a number of substituted benzene compounds and a number of γ- and δ- lactones (data not presented). A comparison of secondary oven temperature offsets showed that higher temperature offsets reduced the second dimension retention time. Increasing the secondary

temperature offset from 5 to 20 ºC resulted in a 15% reduction in secondary dimension retention time with each 5 ºC increment for a number of compounds including the lactones (data not shown). This was accompanied by a reduction in peak width and second dimension resolution. A 6 second modulation time with a 5 ºC secondary oven temperature offset was chosen to be a suitable compromise as it maintained the first dimension separation, maximized the second dimension resolution, and produced a minimal wrap-around effect for compounds that were late to elute from the second dimension. As an example, Figure 4.7 presents a typical contour plot of a HS-SPME/GC×GC-TOFMS chromatogram from a Cabernet Sauvignon wine.

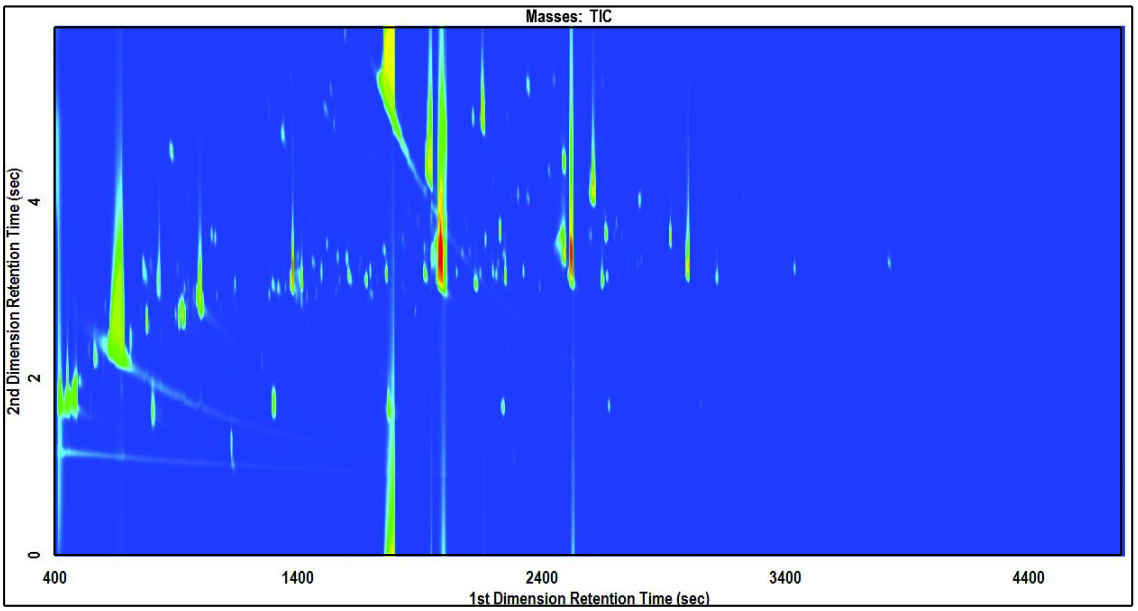

Figure 4.7 Typical contour plot of a HS-SPME/GC×GC-TOFMS chromatogram (TIC) demonstrating the separation of volatile compounds isolated from the headspace of a Cabernet Sauvignon wine. The color gradient reflects the intensity of the TOFMS signal (Z-axis) from low (blue) to high (red). Note that a substantial number of trace volatile compounds are not visible in this chromatogram due to the abundant esters dominating the Z-axis of the plot.

4.3.10. Sensitivity and deconvolution using GC×GC and ChromaTOF

Ryan and co-workers previously demonstrated that GC×GC could be used as a sensitive technique for the analysis of alkyl methoxypyrazines in wines (Ryan et al., 2005). A

2006 vintage Cabernet Sauvignon from Western Australia was anecdotally considered to have a bell-pepper aroma which has previously been associated with the potent aroma compound 2-isobutyl-3-methoxypyrazine (IBMP) (Roujou de Boubée et al., 2000). The 2006 vintage wine was analysed using the optimized method and IBMP was matched to a peak using the deconvoluted mass spectrum and retention index. However, the qualifier ions, 94 and 151 which are 24 and 18% of the base peak respectively, were common to two closely eluting compounds. To confirm the retention time and mass spectral match of the compound the same wine was spiked with approximately 4 ng/L IBMP. The first and second dimension retention times were an exact match with a signal to noise of 209 and 407 for the wine and spiked wine, respectively (Figure 4.8). This confirmed that the optimized methodology was sensitive enough to analyse the potent odour compound IBMP at ppt concentration levels at and below odour threshold for this compound (Roujou de Boubée et al., 2000, Ryan et al., 2005).

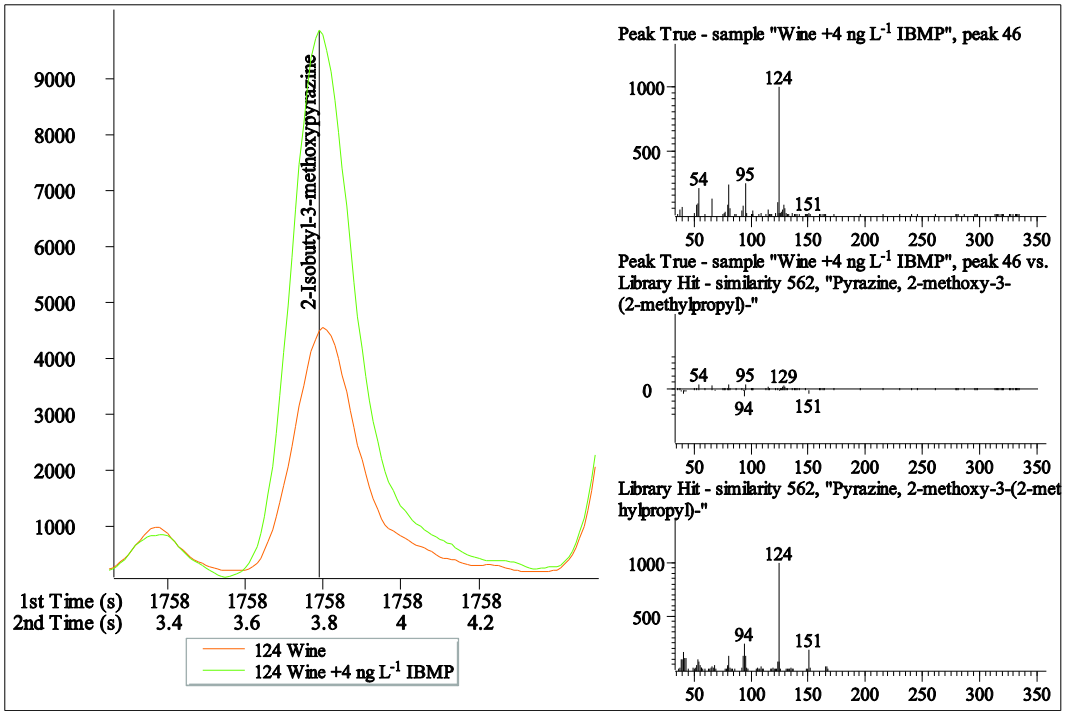

Figure 4.8 Identifies the deconvoluted peak for IBMP in a wine and the same wine spiked with ~4 ng/L of the same compound. Note the deconvoluted Peak True mass spectrum provides additional confirmation on the quality of the spectral match.

Table 4.3 Compound names, CAS numbers, unique masses, mean mass spectral match quality, retention times, and retention indices for compounds analyzed by GC×GC-TOFMS based on MS and RI matches for five commercial Cabernet Sauvignon wines from Western Australia.

Peak#	Compound	CAS	Unique Mass[¥]	MS Match	1° RT(s)	2° RT(s)	RI[₦] (calc)	RI[€] (lit)
1	Isobutyl alcohol	78-83-1	74	845	348	1.703	695	650
2	1-Butanol	71-36-3	56	823	396	1.819	711	662
3	1-Penten-3-ol	616-25-1	57	846	420	1.838	720	684
4	2-Ethylfuran	3208-16-0	81	767	432	1.838	724	720
5	1-Propene, 1-(methylthio)-, (E)-	42848-06-6	73	801	432	1.939	724	726
6	2,3-Pentanedione	600-14-6	57	800	432	2.088	724	697
7	2,5-Dimethylfuran	625-86-5	96	788	444	1.881	729	728
8	Ethyl propanoate	105-37-3	102	918	456	2.034	733	726
9	Propyl acetate	109-60-4	43	917	462	2.031	735	728
10	Acetal	105-57-7	47	812	486	1.786	744	726
11	2,4,5-Trimethyl-1,3-dioxolane	3299-32-9	101	838	486	1.938	744	735
12	Acetoin	513-86-0	88	819	486	2.662	745	743
13	Ethyl isobutyrate	97-62-1	116	841	552	2.147	768	756
14	Isobutyric acid	79-31-2	73	852	567	2.815	773	775
15	Toluene	108-88-3	91	919	570	2.404	774	771
16	2-Methylthiophene	554-14-3	97	831	582	2.676	778	775
17	Isobutyl acetate	110-19-0	56	881	588	2.223	781	780
18	3-Methylthiophene	616-44-4	98	778	600	2.744	785	786
19	Diethyl carbonate	105-58-8	91	854	618	2.762	792	765
20	2,3-Butanediol	513-85-9	47	899	636	3.304	798	789
21	Butanoic acid	107-92-6	60	726	636	3.365	798	789
22	Octane^	111-65-9	85	735	642	1.545	800	800
23	2-Ethyl-5-methylfuran	1703-52-2	95	775	642	2.360	800	802
24	Ethyl butanoate	105-54-4	89	913	648	2.470	803	803
25	Hexanal	66-25-1	82	682	654	2.662	805	804
26	Dibromochloromethane	124-48-1	129	849	654	3.402	806	800
27	Tetrachloroethylene	127-18-4	166	888	660	2.439	807	815
28	Butyl acetate	123-86-4	61	882	684	2.491	816	813
29	Ethyl lactate	97-64-3	75	795	690	3.068	818	815
30	1,3-Octadiene	1002-33-1	54	902	708	1.979	824	827
31	Methyl ethyl disulfide	20333-39-5	108	711	744	3.147	837	846
32	Furfural	98-01-1	96	930	744	4.513	838	835
33	Ethyl crotonate	10544-63-5	69	898	768	3.000	847	834
34	Chlorobenzene	108-90-7	112	836	774	3.190	848	852
35	Ethyl 2-methylbutyrate	7452-79-1	102	927	780	2.493	850	848
36	Isohexanol	626-89-1	56	812	780	2.684	851	838
37	S-Methylmercaptoethanol	5271-38-5	61	834	780	4.121	851	838
38	Isovaleric acid	503-74-2	60	843	786	3.126	853	839
39	Ethyl isovalerate	108-64-5	88	890	792	2.529	855	852
40	3-Hexen-1-ol, (E)-	928-97-2	67	851	792	2.936	855	853
41	3-Hexen-1-ol, (Z)-	928-96-1	67	939	804	2.932	860	860
42	Ethylbenzene	100-41-4	91	931	810	2.859	861	866
43	2-Furanmethanol	98-00-0	98	878	810	4.047	862	866
44	2-Methylbutanoic acid	116-53-0	74	903	816	3.196	864	850
45	2-Ethylthiophene	872-55-9	97	779	822	3.129	866	871
46	m-Xylene	108-38-3	91	907	834	2.842	870	874
47	1-Hexanol	111-27-3	56	893	840	2.821	873	863
48	Isoamyl acetate	123-92-2	70	797	858	2.707	879	876
49	3,4-Dimethylthiophene	632-15-5	111	804	858	3.291	879	887
50	2-Methylbutyl acetate	624-41-9	70	810	864	2.658	880	875
51	2-Butylfuran	4466-24-4	81	710	894	2.593	892	894
52	2-Heptanone	110-43-0	58	894	894	2.960	892	889

Peak#	Compound	CAS	Unique Mass[¥]	MS Match	1° RT(s)	2° RT(s)	RI[₦] (calc)	RI[€] (lit)
53	o-Xylene	95-47-6	91	901	900	3.109	894	894
54	Styrene	100-42-5	104	895	900	3.380	894	897
55	Nonane^	111-84-2	57	897	918	1.737	900	900
56	Propyl butanoate	105-66-8	71	801	918	2.715	900	896
57	Ethyl pentanoate	539-82-2	88	906	924	2.746	903	898
58	2-Heptanol	543-49-7	45	876	936	2.601	906	901
59	Heptanal	111-71-7	86	857	936	2.911	906	900
60	2-Acetylfuran	1192-62-7	95	917	960	4.740	915	914
61	Isobutyl isobutyrate	97-85-8	71	823	966	2.442	916	906
62	Pentyl acetate	628-63-7	70	828	966	2.769	916	916
63	γ-Butyrolactone	96-48-0	86	945	978	1.420	920	915
64	Anisole	100-66-3	108	813	978	3.921	921	920
65	Methyl hexanoate	106-70-7	74	893	996	2.840	926	923
66	Cumene	98-82-8	105	798	996	2.953	925	924
67	Ethyl tiglate	5837-78-5	113	820	1038	3.207	940	939
68	Ethyl 3-hydroxybutanoate	5405-41-4	71	875	1038	3.644	940	945
69	Camphene	79-92-5	93	746	1074	2.458	951	961
70	Propyl isovalerate	557-00-6	85	835	1074	2.634	951	949
71	Propylbenzene	103-65-1	91	884	1086	3.031	955	957
72	Isobutyl butanoate	539-90-2	71	850	1092	2.632	957	955
73	Ethyl 3-methylpentanoate	5870-68-8	88	794	1098	2.717	960	960
74	m-Ethyl toluene	620-14-4	120	883	1110	3.073	964	969
75	Ethyl isohexanoate	25415-67-2	88	883	1122	2.745	967	969
76	Ethyl 2-hydroxyisovalerate	2441-06-7	104	822	1122	3.112	967	987
77	Benzaldehyde	100-52-7	106	903	1122	4.959	968	969
78	5-Methylfurfural	620-02-0	110	893	1122	5.159	968	964
79	Dehydroxylinalool oxide A	7392-19-0	139	840	1134	2.506	971	971
80	Isoamyl propanoate	105-68-0	57	880	1134	2.744	971	969
81	1-Heptanol	111-70-6	56	891	1140	2.949	973	970
82	Dimethyl trisulfide	3658-80-8	126	871	1140	4.615	973	982
83	Methyl furoate	611-13-2	95	915	1158	4.970	979	985
84	o-Ethyltoluene	611-14-3	105	877	1164	3.278	980	988
85	Octen-3-ol	3391-86-4	57	843	1170	2.845	983	986
86	α-Methylstyrene	98-83-9	118	836	1176	3.517	985	988
87	Ethyl (methylthio)acetate	4455-13-4	134	739	1182	4.313	987	990
88	Methionol	505-10-2	106	918	1182	4.733	987	982
89	3-Octanone	106-68-3	99	842	1188	3.019	988	989
90	Methyl heptenone	409-02-9	108	740	1188	3.417	988	987
91	β-Myrcene	123-35-3	93	874	1194	2.461	990	991
92	2-Amylfuran	3777-69-3	81	800	1194	2.773	991	993
93	2-Octanone	111-13-7	58	781	1200	3.099	993	990
94	2-Carene	554-61-0	121	737	1212	2.685	997	1001
95	6-Methyl-5-hepten-2-ol	1569-60-4	95	842	1212	3.022	997	993
96	Pseudocumene	95-63-6	105	933	1212	3.217	997	1000
97	Phenol	108-95-2	94	803	1212	4.474	996	979
98	2-Methylthiolan-3-one	13679-85-1	116	849	1212	5.323	997	994
99	Decane^	124-18-5	43	896	1224	1.899	1000	1000
100	Benzofuran	271-89-6	118	848	1224	4.486	1001	1007
101	(Z)-3-Hexenyl acetate	3681-71-8	67	814	1236	3.120	1004	1006
102	Octanal	124-13-0	84	818	1242	3.080	1006	1003
103	α-Phellandrene	99-83-2	136	682	1248	2.624	1009	1005
104	Ethyl-3-hexanoate	2396-83-0	142	879	1248	3.213	1008	1007
105	α-Thiophenecarboxaldehyde	98-03-3	111	912	1254	0.076	1009	1010
106	m-Dichlorobenzene	541-73-1	146	796	1254	3.840	1010	1022
107	Ethylfurylketone	3194-15-8	95	851	1254	4.794	1011	1008
108	1-Methyl-2-formylpyrrole	1192-58-1	109	814	1254	5.530	1011	1010
109	Isoamyl isobutyrate	2050-01-3	89	844	1266	2.655	1014	1018
110	Hexyl acetate	142-92-7	84	894	1266	2.923	1014	1007

Peak#	Compound	CAS	Unique Mass[¥]	MS Match	1° RT(s)	2° RT(s)	RI[₦] (calc)	RI[¢] (lit)
111	Hexanoic acid	142-62-1	60	910	1266	3.442	1015	978
112	α-Terpinene	99-86-5	93	854	1278	2.671	1019	1018
113	Isocineole	470-67-7	111	828	1278	2.794	1018	1016
114	Benzyl chloride	100-44-7	91	801	1278	4.542	1019	1023
115	p-Dichlorobenzene	106-46-7	146	892	1284	3.957	1020	1015
116	(S)-3-Ethyl-4-methylpentanol	0-00-0	84	883	1296	3.017	1024	1020
117	Hemimellitene	526-73-8	105	932	1296	3.527	1024	1033
118	p-Cymene	99-87-6	134	859	1308	3.100	1027	1026
119	Limonene	5989-27-5	68	884	1320	2.670	1032	1031
120	2-Ethyl hexanol	104-76-7	57	890	1320	2.883	1032	1030
121	Eucalyptol	470-82-6	108	869	1332	2.957	1036	1033
122	(Z)-Ocimene	3338-55-4	92	847	1338	2.661	1038	1040
123	Indane	496-11-7	117	862	1338	3.929	1038	1048
124	2-Acetyl-5-methylfuran	1193-79-9	109	849	1338	5.100	1039	1042
125	2,2,6-Trimethylcyclohexanone	2408-37-9	82	883	1344	3.464	1039	1035
126	Benzyl Alcohol	100-51-6	108	916	1356	5.069	1044	1041
127	Lavander lactone	1073-11-6	111	755	1356	5.691	1045	1041
128	Ocimene quintoxide	7416-35-5	139	712	1362	2.828	1046	1049
129	Ethyl 2-hexenoate	27829-72-7	99	922	1362	3.371	1046	1036
130	(E)-Ocimene	3779-61-1	93	847	1368	2.680	1047	1051
131	3-Nonen-5-one	82456-34-6	83	801	1374	3.095	1050	1051
132	Salicylaldehyde	90-02-8	122	812	1374	5.092	1051	1057
133	Phenylacetaldehyde	122-78-1	120	900	1374	5.231	1051	1050
134	m-Propyltoluene	1074-43-7	105	850	1386	3.122	1053	1052
135	Ethyl furoate	614-99-3	95	908	1392	4.819	1056	1056
136	Isoamyl butyrate	106-27-4	71	892	1398	2.806	1057	1054
137	Butylbenzene	104-51-8	91	835	1398	3.185	1058	1058
138	Ethyl 2-hydroxy-4-methylpentanoate	10348-47-7	69	914	1404	3.224	1059	1060
139	γ-Hexalactone	695-06-7	85	876	1410	0.202	1060	1063
140	γ-Terpinene	99-85-4	93	817	1410	2.855	1061	1062
141	o-Cresol	95-48-7	108	851	1434	4.491	1069	1077
142	Diethyl malonate	105-53-3	115	862	1434	4.382	1070	1069
143	Ethyl 5-methylhexanoate	10236-10-9	88	722	1440	2.899	1071	1072
144	Acetophenone	98-86-2	105	926	1440	5.269	1072	1076
145	1-Octanol	111-87-5	56	904	1452	3.032	1075	1080
146	p-Tolualdehyde	104-87-0	119	835	1452	4.992	1075	1079
147	2-Ethyl-p-Xylene	1758-88-9	119	673	1458	3.320	1078	1077
148	Terpinolene	586-62-9	93	915	1488	2.982	1087	1087
149	4-Ethyl-o-Xylene	934-80-5	119	856	1488	3.348	1087	1093
150	p-Cresol	106-44-5	107	869	1500	4.501	1091	1077
151	Guaiacol	90-05-1	109	896	1500	5.055	1092	1102
152	2-Nonanone	821-55-6	58	793	1506	3.153	1093	1092
153	Dehydro-p-cymene	1195-32-0	117	927	1506	3.585	1093	1091
154	Propyl hexanoate	626-77-7	99	899	1512	2.909	1095	1079
155	Ethyl heptanoate	106-30-9	88	914	1524	2.932	1098	1093
156	Methyl benzoate	93-58-3	105	901	1524	4.768	1099	1100
157	Undecane^	1120-21-4	57	889	1530	1.947	1099	1100
158	Isopentyl 2-methylbutanoate	27625-35-0	85	872	1530	2.703	1100	1100
159	Ethyl sorbate	2396-84-1	140	854	1530	3.825	1101	1103
160	Linalool	78-70-6	93	893	1536	3.031	1103	1106
161	Ethyl methylthiopropanoate	13327-56-5	74	913	1536	4.373	1103	1098
162	2-Nonanol	628-99-9	45	906	1542	2.803	1105	1098
163	Isopentyl isovalerate	659-70-1	85	877	1548	2.707	1107	1105
164	Nonanal	124-19-6	95	893	1548	3.120	1107	1106
165	Heptyl acetate	112-06-1	43	862	1566	2.931	1113	1115
166	(Z)-Rose oxide	16409-43-1	139	830	1566	3.074	1113	1112
167	2-Methylcumarone	4265-25-2	131	887	1566	4.449	1113	1109

Peak#	Compound	CAS	Unique Mass[¥]	MS Match	1° RT(s)	2° RT(s)	RI[₦] (calc)	RI[€] (lit)
168	1,3,8-p-Menthatriene	21195-59-5	134	793	1572	3.406	1115	1111
169	α-Cyclocitral	432-24-6	81	772	1596	3.605	1124	1116
170	Methyl octanoate	111-11-5	127	879	1602	3.002	1126	1129
171	2-Ethylhexanoic acid	149-57-5	88	721	1620	3.300	1132	1128
172	α-Isophoron	78-59-1	82	737	1620	4.553	1132	1118
173	(E)-Rose oxide	876-18-6	139	680	1626	3.149	1133	1127
174	Ethyl 3-hydroxyhexanoate	2305-25-1	71	786	1626	3.617	1134	1133
175	p-Menth-3-en-1-ol	586-82-3	81	691	1650	3.349	1143	1138
176	N-Isopentylacetamide	13434-12-3	72	882	1668	4.786	1149	1150
177	o-Dimethoxybenzene	91-16-7	138	818	1674	5.389	1151	1154
178	Isobutyl hexanoate	105-79-3	99	907	1680	2.798	1152	1144
179	4-Oxoisophorone	1125-21-9	68	839	1680	4.994	1153	1142
180	Prehnitene	488-23-3	119	905	1686	3.753	1155	1120
181	Camphor	464-49-3	95	762	1686	4.207	1155	1151
182	Nerol oxide	1786-08-9	83	820	1692	3.462	1156	1151
183	Pentylbenzene	538-68-1	91	783	1704	3.214	1161	1154
184	(Z)-3-Nonenol	10340-23-5	81	812	1704	3.237	1161	1160
185	γ-Heptalactone	105-21-5	85	802	1704	5.818	1162	1144
186	Menthone	89-80-5	112	756	1710	3.577	1162	1154
187	2-Methylundecane	7045-71-8	85	847	1716	1.936	1165	1165
188	3-Cyclohexene-1-carboxaldehyde, 1,3,4-trimethyl-	40702-26-9	137	752	1722	3.571	1167	1171
189	3-Ethylphenol	620-17-7	107	710	1722	4.408	1168	1184
190	Benzyl acetate	140-11-4	150	880	1728	4.877	1170	1165
191	3-Methylundecane	1002-43-3	57	849	1734	1.968	1171	1169
192	(Z)-6-Nonenol	35854-86-5	67	872	1734	3.206	1171	1172
193	Isomenthone	491-07-6	112	814	1734	3.787	1171	1165
194	m-Dimethoxybenzene	151-10-0	138	864	1740	5.095	1174	1182
195	Ocimenol	5986-38-9	93	738	1746	3.309	1175	1179
196	Ethyl benzoate	93-89-0	105	906	1746	4.527	1177	1180
197	Isobutyl methoxypyrazine	24683-00-9	124	618	1758	3.703	1180	1179
198	m-Methylacetophenone	585-74-0	119	760	1758	5.071	1180	1183
199	1-Nonanol	143-08-8	70	907	1764	2.995	1182	1173
200	(E)-Linalool oxide	14049-11-7	59	797	1764	3.755	1181	1184
201	Phenethyl formate	104-62-1	104	890	1764	4.901	1183	1178
202	Methyl benzeneacetate	101-41-7	150	838	1764	5.175	1183	1194
203	Diethyl succinate	123-25-1	74	890	1770	4.325	1184	1191
204	4-Ethyl phenol	123-07-9	107	930	1776	4.682	1186	1178
205	Terpinen-4-ol	562-74-3	71	859	1782	3.532	1189	1177
206	1-Dodecene	112-41-4	69	903	1794	2.165	1192	1193
207	Octanoic Acid	124-07-2	144	844	1800	3.435	1194	1202
208	Dill ether	74410-10-9	137	751	1800	3.861	1193	1184
209	Naphthalene	91-20-3	128	855	1800	5.179	1194	1191
210	p-Methylacetophenone	122-00-9	119	793	1806	5.064	1196	1179
211	Dodecane^	112-40-3	57	852	1818	2.227	1201	1200
212	Methyl salicylate	119-36-8	120	913	1824	4.894	1202	1201
213	p-Creosol	93-51-6	123	862	1836	4.863	1206	1188
214	α-Terpineol	98-55-5	136	850	1842	3.603	1210	1186
215	Safranal	116-26-7	150	799	1848	4.385	1211	1196
216	Decanal	112-31-2	82	869	1854	3.083	1213	1206
217	Benzofuran, 4,7-dimethyl-	28715-26-6	145	828	1860	4.364	1217	1220
218	4,7-Dimethylbenzofuran	28715-26-6	145	829	1878	4.378	1223	1220
219	Methyl nonanoate*	1731-84-6	141	892	1890	3.003	1226	1229
220	Ethyl nicotinate	614-18-6	106	812	1890	5.045	1226	1218
221	p-Menth-1-en-9-al	29548-14-9	94	764	1896	3.993	1228	1217
222	β-Cyclocitral	432-25-7	137	874	1896	4.196	1229	1220
223	Citronellol	106-22-9	156	899	1908	3.288	1233	1233

Peak#	Compound	CAS	Unique Mass[¥]	MS Match	1° RT(s)	2° RT(s)	RI[₦] (calc)	RI[¢] (lit)
224	2-Hydroxycineol	18679-48-6	108	756	1914	4.201	1236	1227
225	Benzothiazole	95-16-9	135	911	1926	0.497	1239	1244
226	6-Ethyl-o-cresol	1687-64-5	121	859	1926	4.499	1239	1236
227	Benzenepropanol	122-97-4	117	851	1926	5.121	1241	1231
228	Isothiocyanatocyclohexane	1122-82-3	141	860	1932	4.925	1243	1260
229	Ethyl phenylacetate	101-97-3	164	908	1950	4.857	1249	1247
230	Ethyl 2-octenoate	2351-90-8	125	862	1956	3.309	1250	1243
231	2-Methylbutyl hexanoate	2601-13-0	99	874	1962	2.875	1252	1247
232	Isopentyl hexanoate	2198-61-0	99	898	1962	2.875	1252	1250
233	D-Carvone	2244-16-8	82	767	1962	4.509	1253	1254
234	2-Nitro-p-cresol	119-33-5	153	781	1968	5.031	1255	1250
235	Geraniol	106-24-1	69	818	1974	3.596	1257	1255
236	Carvotanacetone	499-71-8	82	764	1974	4.286	1258	1246
237	α-Ionene	475-03-6	159	629	1986	3.320	1261	1256
238	2-Phenylethyl acetate	103-45-7	91	906	1986	4.877	1262	1256
239	γ-Octalactone	104-50-7	85	850	1992	5.575	1264	1262
240	9-Decenol	13019-22-2	68	802	2010	3.258	1270	1267
241	3,5-Dimethoxytoluene	4179-19-5	152	842	2016	4.895	1273	1276
242	Nonanoic acid	112-05-0	60	696	2028	2.336	1277	1280
243	1-Decanol	112-30-1	70	921	2028	3.067	1277	1283
244	Ethyl salicylate	118-61-6	120	858	2028	4.511	1277	1267
245	4-Ethylguaiacol	2785-89-9	137	926	2040	4.755	1281	1282
246	Diethyl glutarate	818-38-2	143	915	2046	4.164	1283	1284
247	Vitispirane	65416-59-3	192	904	2058	3.493	1287	1272
248	Phellandral	21391-98-0	109	814	2058	4.303	1287	1273
249	δ-Octalactone	698-76-0	99	866	2070	0.069	1291	1287
250	p-Ethylacetophenone	937-30-4	133	689	2070	4.963	1292	1281
251	Propyl octanoate	624-13-5	145	895	2076	2.919	1294	1290
252	2-Undecanone	112-12-9	58	885	2082	3.143	1296	1295
253	(E)-Oak Lactone	39638-67-0	99	827	2082	5.011	1297	1304
254	Ethyl nonanoate	123-29-5	88	895	2088	2.931	1298	1295
255	Perilla alcohol	536-59-4	68	760	2088	4.222	1299	1295
256	Thymol	89-83-8	135	831	2088	4.332	1298	1290
257	Tridecane^	629-50-5	57	849	2094	2.083	1300	1300
258	p-Cymen-7-ol	536-60-7	135	850	2094	4.722	1301	1295
259	Theaspirane A	0-00-0	138	844	2106	3.283	1305	1301
260	2-Undecanol	1653-30-1	45	886	2112	2.831	1306	1303
261	p-Menth-1-en-9-ol	18479-68-0	94	797	2112	4.021	1308	1295
262	Carvacrol	499-75-2	135	855	2112	4.433	1307	1304
263	Edulan I	41678-29-9	177	768	2136	3.705	1317	1309
264	4-Hydroxy-3-methylacetophenone	876-02-8	135	839	2136	5.715	1317	1323
265	4-Vinylguaiacol	7786-61-0	150	825	2142	5.287	1319	1317
266	Theaspirane B	0-00-0	138	822	2148	3.395	1322	1319
267	Methyl decanoate	110-42-9	74	873	2160	3.004	1325	1323
268	Methyl geranate	2349-14-6	114	868	2160	3.596	1325	1326
269	(Z)-Oak lactone	55013-32-6	71	920	2166	5.350	1329	1340
270	Isobutyl octanoate	5461-06-3	127	856	2220	2.811	1348	1348
271	Citronellol acetate	150-84-5	81	752	2226	3.191	1350	1352
272	Ethyl dihydrocinnamate	2021-28-5	104	858	2232	4.632	1354	1350
273	Syringol	91-10-1	154	859	2244	0.360	1356	1362
274	Eugenol	97-53-0	164	915	2250	4.933	1360	1359
275	TDN	30364-38-6	157	807	2256	4.137	1361	1364
276	(Z)-β-Damascenone	23696-85-7	121	786	2262	4.101	1364	1367
277	γ-Nonalactone	104-61-0	85	883	2268	5.315	1368	1361
278	Dihydroeugenol	2785-87-7	137	924	2274	4.600	1369	1365
279	Hydroxy citronellol	107-74-4	59	793	2286	2.817	1373	1359
280	1-Undecanol	112-42-5	126	855	2298	3.032	1378	1367

Peak#	Compound	CAS	Unique Mass[¥]	MS Match	1° RT(s)	2° RT(s)	RI[₦] (calc)	RI[€] (lit)
281	(E)-α-Ionol	25312-34-9	138	770	2304	3.464	1381	1376
282	(E)-β-Damascenone	23726-93-4	121	886	2316	4.263	1385	1387
283	Biphenyl	92-52-4	154	894	2322	5.345	1388	1385
284	Ethyl decanoate	110-38-3	101	620	2325	3.225	1388	1393
285	Methyl cinnamate	103-26-4	131	796	2334	5.381	1393	1397
286	2-Phenylethyl isobutyrate	103-48-0	104	771	2346	4.419	1397	1396
287	Tetradecane^	629-59-4	57	869	2358	2.129	1401	1400
288	α-Cedrene	469-61-4	119	685	2391	3.762	1414	1410
289	β-Damascone	85949-43-5	177	760	2394	4.098	1415	1419
290	Dihydro-α-Ionone	31499-72-6	136	699	2406	3.819	1420	1406
291	α-Ionone	127-41-3	136	687	2424	3.931	1428	1426
292	1,7-Dimethylnaphthalene	575-37-1	156	896	2436	5.087	1433	1419
293	Aromadendrene	109119-91-7	161	809	2454	3.077	1439	1443
294	2-Phenylethyl butyrate	103-52-6	104	858	2466	4.506	1445	1439
295	Isoamyl octanoate	2035-99-6	127	859	2472	2.880	1447	1450
296	Dihydropseudoionone	689-67-8	69	838	2481	3.658	1451	1457
297	β-Farnesene	18794-84-8	93	854	2490	2.906	1454	1455
298	DBQ	719-22-2	220	833	2520	3.741	1467	1472
299	γ-Decalactone	706-14-9	85	792	2532	5.134	1472	1470
300	1-Dodecanol	112-53-8	97	874	2544	3.055	1477	1483
301	Cabreuva oxide D	107602-52-8	94	868	2556	3.403	1481	1479
302	dehydro-β-Ionone	1203-08-3	175	914	2556	4.447	1483	1485
303	δ-Decenolactone	54814-64-1	97	841	2556	5.710	1482	1483
304	α-Curcumene	644-30-4	132	795	2562	3.415	1484	1485
305	β-Ionone	79-77-6	177	828	2562	4.174	1485	1486
306	Propyl decanoate	30673-60-0	61	852	2580	2.911	1491	1489
307	Ethyl undecanoate	627-90-7	88	879	2586	2.922	1494	1491
308	(Z)-β-Guaiene	88-84-6	161	737	2586	3.393	1493	1492
309	1,10-Oxidocalamenene	143785-42-6	173	925	2586	4.228	1494	1491
310	Isoamyl phenylacetate	102-19-2	70	844	2586	4.400	1494	1490
311	Phenethyl isovalerate	140-26-1	104	831	2592	4.269	1496	1490
312	δ-Decalactone	705-86-2	99	831	2598	5.550	1500	1505
313	Pentadecane^	629-62-9	57	884	2604	2.159	1499	1500
314	α-Amorphene	483-75-0	105	882	2610	3.335	1504	1505
315	α-Farnesene	502-61-4	189	607	2616	3.755	1506	1511
316	Butylated Hydroxytoluene	128-37-0	205	873	2616	3.806	1506	1533
317	2,4-Di-tert-butylphenol	96-76-4	191	863	2622	3.938	1510	1513
318	β-Bisabolene	495-61-4	204	783	2628	3.087	1512	1509
319	α-Alaskene	28400-12-6	136	632	2628	3.886	1511	1512
320	Methyl dodecanoate	111-82-0	74	846	2658	2.997	1524	1525
321	δ-Cadinene	483-76-1	134	737	2658	3.444	1524	1528
322	α-Panasinsen	56633-28-4	161	610	2658	3.450	1524	1518
323	(E)-Calamene	483-77-2	159	781	2670	3.787	1529	1530
324	Ethyl 4-ethoxybenzoate	23676-09-7	121	827	2670	4.969	1530	1522
325	β-Sesquiphellandrene	20307-83-9	93	668	2676	3.259	1532	1526
326	Isolongifolene, 4,5,9,10-dehydro-	156747-45-4	200	780	2682	4.192	1535	1544
327	Ethyl 3-hydroxytridecanoate	107141-15-1	117	824	2688	3.492	1537	1539
328	Dihydroactinidiolide	17092-92-1	111	860	2706	0.410	1543	1548
329	Isobutyl decanoate	30673-38-2	155	881	2706	2.814	1546	1545
330	α-Calacorene	21391-99-1	157	926	2718	4.085	1550	1549
331	Nerolidol	7212-44-4	93	814	2748	3.343	1563	1566
332	β-Calacorene	50277-34-4	157	862	2766	4.189	1572	1564
333	β-Vetivenene	27840-40-0	187	882	2772	4.728	1575	1554
334	γ-Undecalactone	104-67-6	85	702	2784	4.977	1580	1573
335	Hexyl octanoate	1117-55-1	127	816	2790	2.920	1583	1584
336	Ethyl dodecanoate	106-33-2	101	865	2820	2.965	1595	1593
337	Hexadecane^	544-76-3	57	887	2832	2.194	1600	1600

Peak#	Compound	CAS	Unique Mass[¥]	MS Match	1° RT(s)	2° RT(s)	RI[₦] (calc)	RI[€] (lit)
338	Isopropyl laurate	10233-13-3	60	851	2892	2.759	1627	1618
339	Cubenol	21284-22-0	161	762	2928	4.001	1643	1642
340	Isopentyl decanoate	2306-91-4	70	885	2934	2.863	1646	1647
341	Phenethyl hexanoate	6290-37-5	104	846	2934	4.363	1648	1650
342	Cadalene	483-78-3	183	886	3018	4.763	1684	1684
343	α-Bisabolol	515-69-5	119	893	3036	3.767	1694	1688
344	Ethyl tridecanoate	28267-29-0	88	845	3042	2.915	1695	1687
345	Heptadecane^	629-78-7	57	869	3054	2.222	1700	1700
346	Methyl tetradecanoate	124-10-7	74	720	3108	2.992	1726	1722
347	2,6-Diisopropylnaphthalene	24157-81-1	197	865	3120	4.307	1732	1728
348	(Z)-Farnesol	3790-71-4	69	776	3132	3.173	1737	1718
349	Ethyl 3-hydroxydodecanoate	126679-28-5	117	736	3144	3.412	1743	1743
350	Ethyl tetradecanoate	124-06-1	88	866	3252	2.923	1795	1796
351	Octadecane^	593-45-3	57	864	3264	2.249	1800	1800
352	Isopropyl Myristate	110-27-0	102	791	3312	2.777	1825	1823
353	Isoamyl laurate	6309-51-9	70	826	3354	2.857	1846	1847
354	Phenethyl octanoate	5457-70-5	104	860	3372	4.198	1856	1846
355	Ethyl pentadecanoate	41114-00-5	88	884	3450	2.920	1897	1897
356	Dibutyl phthalate	84-74-2	149	908	3582	5.233	1965	1967
357	Ethyl 9-hexadecenoate	54546-22-4	79	808	3606	3.135	1976	1977
358	Ethyl hexadecanoate	628-97-7	88	889	3642	2.932	1995	1994
359	Eicosane^	112-95-8	57	867	3654	2.300	2000	2000
360	Isopropyl Palmitate	142-91-6	102	710	3696	2.778	2022	2027
361	Ethyl octadecanoate	111-61-5	88	741	4008	2.912	2182	2194
T1	Mercaptoacetone	24653-75-6	90	898	438	2.342	726	
T2	2-(Methoxymethyl)furan	13679-46-4	81	861	720	3.204	829	
T3	Ethyl 3-furoate	614-98-2	95	864	1224	3.957	1000	
T4	Pantolactone	599-04-2	71	874	1404	5.508	1060	
T5	2-Thiopheneacetic acid	1918-77-0	97	758	1410	4.300	1061	
T6	Ethyl levulate	539-88-8	99	777	1422	4.829	1066	
T7	γ-Ethoxybutyrolactone	932-85-4	85	914	1428	5.955	1069	
T8	Isoamyl lactate	19329-89-6	45	843	1440	3.210	1071	
T9	Ethyl methyl succinate	627-73-6	115	903	1554	4.477	1109	
T10	(E)-2-Ethyl heptenoate	54340-72-6	111	758	1680	3.305	1152	
T11	(E)-6-Nonenol	31502-19-9	67	804	1764	3.296	1181	
T12	Ethyl 2-pyrrolecarboxylate	2199-43-1	139	801	1836	5.510	1207	
T13	Diethyl methylsuccinate	4676-51-1	143	799	1842	3.913	1209	
T14	p-tert-Butylcyclohexanone	98-53-3	98	809	1920	4.216	1237	
T15	3,9-epoxy-p-menth-1-ene	70786-44-6	137	774	1932	4.115	1241	
T16	Diethyl malate	626-11-9	117	880	2010	4.667	1270	
T17	Ethyl 5-oxotetrahydro-2-furancarboxylate	1126-51-8	85	930	2112	1.342	1307	
T18	2-Hexanoylfuran	14360-50-0	110	820	2112	4.470	1309	
T19	Isoamyl 2-furoate	615-12-3	95	871	2136	4.389	1317	
T20	3,4-Dihydro-3-oxoedulan	20194-67-6	193	849	2568	4.549	1487	
T21	Megastigmatrienone	38818-55-2	148	782	2796	4.829	1587	
T22	Heptyl ketone	818-23-5	57	870	2994	2.976	1674	

^ Straight chain n-alkanes not present in the wine samples. * Methyl nonanoate internal standard not present in wine samples. [¥] Unique ion (*m/z*): used for peak area determination, identified as the unique ion by ChromaTOF data analysis. [₦] RI: retention indices calculated from C8-C20 n-alkanes. [€] RI: retention indices reported in the literature for 5% phenyl polysilphenylene-siloxane capillary GC columns or equivalents (Adams, 2007, Stein, 2009). NOTE: RI (calc) values for compounds 1-21 are extrapolated using ChromaTOF Software and RI (lit) values could not be found for compounds T1-T22 therefore identification is based on MS match only.

4.3.11. Wine volatile profile compound identification

Five commercial Cabernet Sauvignon wines from Western Australia were analysed using the optimized HS-SPME/GC×GC-TOFMS method described in Table 4.2. Compounds were compared against the NIST 2005 Mass Spectral Library and published retention indices (Adams, 2007, Stein, 2009) for identity confirmation, Table 4.3. Metabolite profiling by GC-MS and subsequent statistical analysis relies on efficient data-processing procedures. The minimum reporting requirements for chemical analysis have recently been suggested by the Metabolomics Standards Initiative (MSI) Chemical Analysis Working Group (CAWG) (Sumner et al., 2007). In the analysis of complex biological samples both MS and RI information are prerequisite for unambiguous compound identification (Sumner et al., 2007).

Data analysis using ChromaTOF identified a total of 375 compounds, plus the 7 alkanes and the 1 internal standard, which had an average mass spectral match of 838 with an upper and lower 95% of the mean at 844 and 831, respectively. The calculated retention index values were also compared to Van Den Dool and Kratz retention indices (van den Dool and Kratz, 1963) reported in the literature with an average difference in the RI values of 5.4 units with an upper and lower 95% of the mean at 6.0 and 4.7, respectively. Bianchi and co-workers commented that differences in retention indices for aroma compounds on comparable stationary phases may vary between 5 and 20 units, however, larger differences have been observed (Bianchi et al., 2007). Babushok and co-workers also noted that in the development of the NIST database of retention indices, 80,427 retention indices representing 9,722 species analysed on dimethylpolysiloxane stationary phases had an average deviation of 10 units but a 99th percentile deviation of 91 units (Babushok et al., 2007). The differences in calculated and reported retention indices reported in this study fall well within these values.

Compounds where retention indices have not been reported in the literature have been listed at the end of Table 4.3 while compounds that were not in good agreement with both mass spectral match and literature RI values were not included.

The majority of current non-targeted GC-MS methodologies tentatively identify ~30-60 analytes in a single analysis (Perestrelo et al., 2008, Tredoux et al., 2008, Gallardo-Chacón et al., 2009) with many other methods developed for targeted and quantitative analysis of fewer but more specific compounds (Siebert et al., 2005, Kotseridis et al., 2008, Mateo-Vivaracho et al., 2008, Canuti et al., 2009). A recent three paper series (Giraudel et al., 2007, Setkova et al., 2007b, 2007c) tentatively identify a total of 201 wine aroma compounds from Ice-wine using a high throughput HS-SPME GC-TOFMS method. However, on review of the data presented in Table 2 of the second paper (Setkova et al., 2007c) tentative identifications included 118 analytes that were not compared to literature retention indices (RI), 26 analytes were >40 RI units different to reported literature RI's, 11 analytes were classified as Unknowns, 71 analytes were quantified using masses that were <10% of the base peak, and 6 analytes were quantified using masses larger than the molecular weight of the assigned analyte. This subsequently reduced the total number of tentatively identified analytes from 201 to a subset of 30 where the calculated RI was within 40 RI units of a literature RI value and where the reported quantification mass was >10% of the base peak. This figure is more in-line with that reported in other single dimensional GC-MS methodologies.

This suggests that most current analytical methods are capable of identifying at most ~10% of the known volatile compounds reported in wines. The current study has demonstrated an optimized analytical method capable of analysing volatile compounds in wine with a number of compounds tentatively identified at an order of magnitude greater than most current single dimensional GC-MS methodologies.

4.3.12. Differentiating commercial wines using volatile profiling

The volatiles in commercial Cabernet Sauvignon wines, from different producers, growing regions and vintages, were run in triplicate and analysed using a one-way analysis of variance for each compound identified in Table 4.3. Of the 375 compounds identified in the commercial wines, 324 compounds were significantly different between the wines to a significance of 0.05 using a Tukey-Kramer HSD test (data not presented). Given that the commercial products were from different producers, growing regions and vintages it is not unexpected that there would be differences among the products. The results of this method evaluation clearly demonstrate that the method developed has the capacity to resolve and identify a large number of compounds and could be used to differentiate wines based on their volatile profile which will be the subject of further work.

4.4. Conclusions

The current study has described the development of a sensitive and comprehensive method for analysing volatile and semi-volatile compounds found in the wine headspace through the use of HS-SPME/GC×GC-TOFMS. This study is the first to clearly show that the use of elevated temperatures during the incubation step of HS-SPME analysis of wine does generate artifacts. It is not intended that this method be used for high throughput or routine analysis of wine volatiles due to the higher costs currently associated with the cryogenic modulation required for GC×GC analysis of low molecular weight volatile compounds. However, further development of consumable-free modulation may extend the application of this analytical technology to production areas of the wine industry for quality assurance and quality control. It is intended that in the immediate future, wine aroma research and wine sensory research

will utilize this non-targeted method to assess compositional changes in the wine volatile profile

5. Influence of yeast strain, canopy management, and site on the volatile composition and sensory attributes of Cabernet Sauvignon wines from Western Australia.

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5.1. Introduction

The characteristics of wine (as a food) that humans are most concerned with are the sensory characteristics of smell, taste and to a lesser extent colour. The wine components that contribute to the sensorial experiences of the consumer are metabolites that can generally be assigned to one of four different origins; they are either produced directly in the grape, transformed from grape substrates through primary and secondary metabolism of micro-flora (yeast and bacteria), are introduced directly from additives used in production (primarily from wood storage or packaging materials), or are by-products of chemical reactions that occur naturally during wine storage and maturation (Ebeler, 2001). However, the interactions between the grapes, micro-flora, wood and chemical environment add complexity to the system which makes it difficult to determine the importance of these various inputs to specific chemical and sensory outcomes. For example, the production of many yeast-derived components can be influenced by juice composition (Hernández-Orte et al., 2002, Keyzers and Boss, 2010). Understanding the source of wine volatile compounds and the mechanisms that influence their formation through production and storage is essential in order to develop strategies to produce wines with specific sensory attributes that appeal to target markets.

In order to better understand the relative contributions these different inputs have on wine composition, a comprehensive analysis in which all wine metabolites are identified and quantified (i.e. metabolomics) is needed. Metabolomic studies have proven useful in characterising the phenotype of an organism of interest (Fiehn, 2002). As wine is a secondary food product and not an organism *per se*, the wine phenotype is a product of multiple genotype and environmental interactions that result in a unique metabolome. Nevertheless, controlled experiments, in which single variables are altered, can reveal how the wine phenotype can be influenced by certain inputs and their interactions. Currently no one analytical method can achieve this objective due to the chemical complexity and heterogeneity of metabolites, the dynamic range that instruments can accommodate, the throughput achievable from many extraction protocols, and the costs associated with the purchase or synthesis of standards especially in the case where the presence of metabolites is not known *a priori* (Goodacre et al., 2004).

The concern of this study is with the volatile composition of wines. With more than 800 aroma compounds reported in the literature it is well accepted that the wine volatile profile is complex (Rapp, 1990). An analytical technique known as comprehensive two-dimensional gas chromatography (GC×GC), developed by Phillips and co-workers in the early 90's (Liu and Phillips, 1991), has been used for the analysis of volatiles in a number of other foods, fats, oils, and fragrances (Adahchour et al., 2008) and is well suited to metabolomic analysis of volatiles in wine. The technique offers enhanced separation efficiency, reliability in qualitative and quantitative analysis and the capability to detect volatile compounds in low quantities (Ong and Marriott, 2002, Dallüge et al., 2003, Górecki et al., 2004). A headspace solid-phase microextraction (HS-SPME) method for the analysis of wine volatiles by GC×GC time-of-flight mass

spectrometry (TOFMS) was recently developed to resolve and identify a substantially larger number of volatile compounds than current single dimensional GC-MS methodologies (Robinson et al., 2011).

The use of sensory evaluation to measure and interpret human responses to wine as perceived through the senses (Stone and Sidel, 1993) can indicate if any relevant changes in the wine metabolome have occurred that correspond to perceived sensory characteristics of the wine. If sensorial differences are noted, the next step is to find patterns within the metabolomic data which give useful biological or sensorial information about the wines. This information about wine composition can then be used to generate hypotheses about the relationship between compounds and sensory attributes or the influence of winemaking inputs on wine composition that can be further tested and refined (Brown et al., 2005). The current study combines descriptive sensory analysis with the compositional results of a recently-developed HS-SPME GC×GC-TOFMS methodology (Robinson et al., 2011). The study takes a systematic approach to investigate the role of yeast, canopy, and site on the composition and sensory characteristics of Western Australian Cabernet Sauvignon wines. As the wines were made solely from Cabernet Sauvignon grapes under controlled winemaking conditions the differences in composition and sensory characteristics should be attributed to the treatments imposed.

5.2. Materials and Methods

5.2.1. Field sites.

Field trials were conducted over the 2007-08 growing season using *Vitis vinifera* L. Cabernet Sauvignon at two commercial vineyards in Western Australia. The first vineyard was located at Gingin in the Swan District Geographical Indication (GI) which has a warm to hot Mediterranean climate with a mean January temperature (MJT) of

24.1 °C. Gingin receives on average 1831 growing degree days (GDD), 1962 sunshine hours, and 168 mm rainfall (865 mm annually) between October and April (Gladstones, 1992). The second vineyard was located at Willyabrup in the Margaret River GI which has a warm Mediterranean maritime climate with a MJT of 20.2 °C. Willyabrup receives on average 1572 GDD, 1661 sunshine hours, and 253 mm rainfall (1132 mm annually) between October and April (Gladstones, 1992). The soils at Gingin are a red clay loam while Willyabrup is a sandy loam (~600 mm) over clay. Vines at Gingin and Willyabrup were planted on own roots in 1968 and 1985 respectively. Both were trained using vertical shoot positioning and rows were planted with an east/west orientation. The Gingin vines were planted at row and vine spacing of 3.6 and 1.8 m respectively while the Willyabrup vine rows were planted more closely at 2.0 m with the same vine spacing. Both sites received supplementary drip irrigation during the season.

5.2.2. Yeast treatments.

Canopy management of Cabernet Sauvignon in the Swan District GI is intended to protect the fruit from sun damage as it is rare for herbaceous characters to be present in the fruit. Thus the fruit from Gingin was used for a yeast trial and not a vineyard trial. Three common commercial *Saccharomyces cerevisiae* strains were selected, Lalvin EC 1118® (EC) and Enoferm QA23® (QA) from Lallemand, and Actiflore® Cerevisiae (also known as Montrachet Strain - Davis 522) (DA) from Laffort.

5.2.3. Canopy treatments.

Canopy management is often employed by viticulturalists in the Margaret River GI to manage herbaceous characters common to Cabernet Sauvignon. Thus the Willyabrup site was used for a fruit light exposure study. The leaves and lateral shoots around the fruiting zone were completely removed at the beginning of flowering between E-L

stages 19 and 20 (Coombe, 1995). A 90% antique green shade cloth was subsequently positioned over the fruiting zone to provide an artificial shade treatment for the fruit. Four treatments were applied to the fruit in a complete randomised block design; shaded from flowering to harvest (SS), light exposed from flowering to harvest (LL), shaded from flowering to veraison then light exposed from veraison to harvest (SL), and light exposed from flowering to veraison then shaded from veraison to harvest (LS).

5.2.4. Micro-scale wine making.

Grape maturity was monitored using a PAL-1 digital refractometer (Atago, Tokyo, Japan) and fruit was harvested between 24 and 25 °Brix. Fruit was crushed and destemmed using a hand operated crusher destemmer into food grade containers blanketed with dry ice. Sulphur dioxide was added to the must at 80 mg/kg as potassium metabisulphite and mixed through before the must was separated evenly into three replicate plastic food grade fermentation vessels (15 L) with lids and fermentation locks. The fermentation vessels were blanketed with dry ice and transferred to a controlled temperature room and allowed to warm to 15 °C before each must was inoculated with *Saccharomyces cerevisiae* at 200 mg/L. Yeast trial strains are listed in the previous section while canopy trials were all inoculated with EC 1118 (Lalvin). A total of 200 mg/L of diammonium phosphate (DAP) was added over the course of fermentation to prevent nitrogen-related fermentation problems. Ferment temperatures were maintained between 17.5 and 18.5 °C through the course of fermentation and were plunged for 2 min every eight hours to submerge and wet the cap. Sugar and temperature were measured using a DMA-35N digital density meter (Anton Paar, Graz, Austria) following cap plunging. Fermentations experienced a 2 day lag phase, while blanketed with dry ice, and then fermented at a rate of 1.0 – 1.5 °Baume per day for 8

days. Fermentations were pressed after reaching 2 °Baume using a hand operated basket press into glass demijohns (10 L) wrapped in aluminium foil with silicone bungs, fermentation locks, and blanketed regularly until bottling using dry ice to prevent oxidation. Wine pH was adjusted to 3.45 – 3.50 using tartaric acid (Australian Tartaric Products, Red Cliffs Victoria). All wines were inoculated with *Oenococcus oeni* (Enoferm Alpha, Lallemand) at 10 mg/L for malolactic fermentation. After malolactic fermentation, wines were racked off lees and potassium metabisulphite was added to obtain similar levels of free sulphur dioxide (20-30 mg/L) which was determined using the Aspiration method (Iland et al., 2000). Copper sulphate ($CuSO_4$) was added after informal sensory assessment at rates of 0.50 – 0.75 mg/L. Wines were sterile filtered prior to bottling through a glass fiber pre-filter, a first stage Sartopure GF2 300 (nominal 0.65 µm), and second stage Sartobran P 300 (nominal 0.65 and absolute 0.45 µm) membrane filter capsule (Sartorius AG, Göttingen, Germany). Wines were bottled in 375 mL, antique green, Bordeaux bottles and sealed with screw cap closures and were stored at room temperature (approximately 20 °C) for 7 months prior to further analysis.

5.2.5. HS-SPME GC×GC-TOFMS volatile compound analysis.

Samples were analysed using a HS-SPME GC×GC-TOFMS methodology previously described (Robinson et al., 2011). Divinylbenzene/Carboxen/Polydimethylsiloxane (DVB/CAR/PDMS) solid phase microextraction (SPME) fibres, 2 cm 50/30 µm, were purchased from Supelco (Bellefonte, PA, USA) and used for all analysis. A LECO Pegasus® 4D GC×GC-TOFMS coupled to a CTC CombiPAL autosampler (CTC Analytics, Zwingen, Switzerland) with an agitator and SPME fibre conditioning station was used for all analysis. Samples were prepared in 20 mL amber glass headspace vials to prevent light degradation of alkyl-methoxypyrazines known to occur in Cabernet

Sauvignon wines (Heymann et al., 1986, Allen et al., 1995). Sodium chloride was added at a rate of 300 g/L to 10 mL of wine pipetted into a 20 mL headspace vial and sealed. An in-fibre internal standard, methyl nonanoate, was loaded into the SPME fibre coating prior to the sample extraction step using methodology previously described (Wang et al., 2005, Setkova et al., 2007a, Setkova et al., 2007b). A commercially available 2008 Cabernet Sauvignon wine (13.0 % Ethanol vol/vol) from Australia was used as a control wine. Retention index probes were loaded into the fibre coating after the internal standard as previously described (Wang et al., 2005, Setkova et al., 2007b) for the analysis of the 2008 Cabernet Sauvignon control wine to monitor for chromatographic drift. TOFMS data was acquired at a rate of 100 scans/sec to accommodate the peak elution rate for modulated analytes and to facilitate peak deconvolution. The TOFMS detector was operated at 1800 V and collected masses between 35 and 350 amu.

5.2.6. Data processing and semi-quantification.

GC×GC-TOFMS interrogation and spectral deconvolution was conducted using ChromaTOF® optimized for Pegasus® 4D software Ver. 4.24 (LECO Corporation, St. Joseph, MI, USA). Chromatograms were processed with baseline offset of 0.5 (computation through the middle of noise), auto peak smoothing, peak find with a S/N of 100, a first dimension peak width of 12 sec, and a second dimension peak width of 0.4 sec. Compound mass spectral data were compared against the NIST 2008 and Wiley 9th ed. Mass Spectral Libraries. Retention index (RI) methods were utilised to calculate RI for each compound identified which was compared to published retention indices for 5% phenyl polysilphenylene-siloxane capillary GC columns or equivalents (Adams, 2007, Stein, 2009) for identity confirmation. Minimum similarity match, with regards to library spectra, was kept at 600 and the first and second dimension RI deviation was set

at 6 and 0.25 respectively to allow for base peak shifts across modulations but not within modulations. Peak area integration was conducted using the unique ion listed in Table 5.1. Peak areas were automatically normalised against the in-fibre internal standard, methyl nonanoate, and exported to a tab delimited file for statistical analysis. Peak assignments, integration and summation of modulations were automatically conducted by the software.

5.2.7. Descriptive sensory analysis.

Red wines were evaluated by a trained panel of twelve volunteers (five men and seven women). All panelists had previous wine tasting experience and were selected due to interest and availability. During three initial sessions, panelists were presented with samples that reflected the range of treatments under study. During these initial sessions the panel developed their own descriptive terminology through consensus to describe and differentiate the wines. Reference standards were developed in consultation with the panel and presented in black wine glasses. Panelists were trained to recognise these standards which are listed in Table 5.2. A subset of the wines were evaluated in duplicate over eight subsequent sessions following the exact procedures that were to be used in the actual testing and the panel performance was assessed using PANELCHEK prior to commencing the study. Panelists were asked to evaluate each of the 21 wine products (7 treatments by 3 replicate fermentations) in triplicate over the course of twelve sessions where wines were presented in a randomised block design. Prior to each formal evaluation session, the reference standards described above were assessed to refresh each panelist's memory.

Table 5.1 Compounds analyzed by GC×GC-TOFMS, based on MS and RI matches, which are significantly different due to treatment at $p \leq 0.05$ using a one-way ANOVA. Compounds are grouped by PLS cluster membership and ordered by descending VIP value within each cluster group. PLS cluster membership was determined using hierarchal cluster analysis of the PLS scores and loadings excluding X-variables with VIP values below 0.80. VIP number represents the importance of the compound as an X-variable in the three-component PLS model. Compound names, CAS numbers, unique masses, mean mass spectral match quality, calculated and literature retention indices are provided for identity confirmation.

Compound	CAS	PLS Cluster	VIP	Treatment Influence	Unique Mass[¥]	MS Match	RI[₦] (calc)	RI[€] (lit)
p-Dichlorobenzene	106-46-7	1	1.161	S	146	948	1024	1015
Ethyl isobutyrate	97-62-1	1	1.149	SY	116	734	770	756
Isobutyl decanoate	30673-38-2	1	1.139	Y	155	839	1549	1545
Ethyl 2-methylbutyrate	7452-79-1	1	1.116	O	102	899	851	848
Ethyl 2-hydroxy-4-methylpentanoate	10348-47-7	1	1.107	Y	69	874	1062	1060
Ethyl undecanoate	627-90-7	1	1.098	Y	88	857	1496	1491
Ethyl 3-methylpentanoate	5870-68-8	1	1.090	Y	88	810	962	960
Ethyl (methylthio)acetate	4455-13-4	1	1.080	Y	134	806	989	990
2-Phenylethyl butyrate	103-52-6	1	1.046	CY	104	855	1451	1439
1-Decanol	112-30-1	1	0.987	Y	70	828	1281	1283
Hexanal	66-25-1	2	1.045	S	82	720	809	804
D-Carvone	2244-16-8	2	1.006	S	82	758	1257	1254
Guaiacol	90-05-1	2	0.999	S	109	868	1095	1102
α-Thiophenecarboxaldehyde	98-03-3	2	0.984	SCY	111	909	1015	1010
Benzenepropanol	122-97-4	2	0.969	S	117	618	1240	1231
1-Dodecanol	112-53-8	2	0.810	CY	97	765	1481	1483
6-Methyl-3,5-heptadiene-2-one	1604-28-0	3	1.220	SC	109	774	1113	1107
4-Oxoisophorone	1125-21-9	3	1.146	SC	68	761	1157	1142
1,3-Octadiene	1002-33-1	3	1.118	SC	110	812	827	827
2-Ethylfuran	3208-16-0	3	1.112	S	81	859	725	720
γ-Nonalactone	104-61-0	3	1.014	SC	85	884	1374	1361
Camphor	464-49-3	3	1.001	S	95	719	1161	1151
2-Amylfuran	3777-69-3	3	1.000	SC	81	838	993	993
2-Undecanone	112-12-9	3	0.990	SY	58	791	1298	1295

Compound	CAS	PLS Cluster	VIP	Treatment Influence	Unique Mass[¥]	MS Match	RI[₹] (calc)	RI[€] (lit)
3,4-Dimethylthiophene	632-15-5	3	0.981	S	111	784	882	887
2-Heptanone	110-43-0	3	0.884	SCY	58	882	894	889
2-Nonanone	821-55-6	3	0.869	SCY	58	794	1095	1092
2-Methylundecane	7045-71-8	3	0.828	S	85	799	1164	1165
2-Isobutyl-3-methoxypyrazine (IBMP)*	24683-00-9	4	1.088	SC	124	517	1183	1179
Naphthalene	91-20-3	5	1.162	SC	128	882	1197	1191
Isomenthone	491-07-6	5	1.156	S	112	676	1175	1165
Prehnitene	488-23-3	5	1.146	SC	119	912	1159	1120
4,7-Dimethylbenzofuran	28715-26-6	5	1.142	S	145	675	1218	1220
Ethyl pentadecanoate	41114-00-5	5	1.135	S	88	890	1897	1897
2-Phenylethyl isobutyrate	103-48-0	5	1.122	SC	104	807	1403	1396
2,4-Dichlorophenol	120-83-2	5	1.118	SCY	162	795	1195	1188
α-Terpineol	98-55-5	5	1.111	S	136	685	1211	1186
Isoamyl propanoate	105-68-0	5	1.106	S	57	893	973	969
Phenethyl isovalerate	140-26-1	5	1.103	SY	104	830	1494	1490
Octen-3-ol	3391-86-4	5	1.092	S	57	836	987	986
1-Nonanol	143-08-8	5	1.092	S	70	691	1182	1173
2-Nitro-p-cresol	119-33-5	5	1.075	SC	153	730	1260	1250
3-Octanone	106-68-3	5	1.072	S	99	755	991	989
Ethyl 4-ethoxybenzoate	23676-09-7	5	0.976	S	121	898	1535	1522
dehydro-β-Ionone	1203-08-3	6	1.183	C	175	908	1487	1485
2,2,6-Trimethylcyclohexanone	2408-37-9	6	1.152	SC	82	904	1043	1035
1,1,6-trimethyl-1,2-dihydronaphthalene (TDN)	30364-38-6	6	1.142	C	157	769	1367	1364
β-Damascone	85949-43-5	6	1.056	C	177	780	1422	1419
Butylated Hydroxytoluene	128-37-0	6	1.023	O	205	841	1511	1533
Ethyl furoate	614-99-3	6	1.021	SC	95	872	1059	1056
Vitispirane	65416-59-3	6	1.018	C	192	855	1292	1272
α-Bisabolol	515-69-5	7	1.000	SC	119	882	1698	1688
2-Methylcumarone	4265-25-2	7	1.000	SC	131	882	1117	1109
Terpinolene	586-62-9	7	0.968	SC	93	898	1091	1087
Theaspirane A	0-00-0	7	0.967	SC	138	823	1312	1301
Theaspirane B	0-00-0	7	0.962	SC	138	822	1328	1319
γ-Terpinene	99-85-4	7	0.950	SC	93	810	1064	1062
Methyl geranate	2349-14-6	7	0.950	SC	114	850	1328	1326

Compound	CAS	PLS Cluster	VIP	Treatment Influence	Unique Mass[¥]	MS Match	RI[₹] (calc)	RI[€] (lit)
p-Menth-3-en-1-ol	586-82-3	7	0.945	C	81	791	1148	1138
Eucalyptol	470-82-6	7	0.940	O	81	833	1041	1033
Dehydro-p-cymene	1195-32-0	7	0.938	SC	132	888	1097	1091
Dehydroxylinalool oxide A	7392-19-0	7	0.936	C	139	808	975	971
Limonene	5989-27-5	7	0.931	SC	68	865	1035	1031
Dibutyl phthalate	84-74-2	7	0.924	SC	149	911	1967	1967
β-Farnesene	18794-84-8	7	0.920	C	93	860	1457	1455
Heptanal	111-71-7	7	0.915	S	86	878	908	900
(Z)-Farnesol	3790-71-4	7	0.913	SC	69	838	1741	1718
Phenethyl octanoate	5457-70-5	7	0.907	SC	104	828	1857	1846
Methyl octanoate	111-11-5	7	0.906	SC	74	882	1128	1129
2-Ethylthiophene	872-55-9	7	0.901	C	97	684	868	871
2-Methylthiolan-3-one	13679-85-1	7	0.901	SC	116	830	1001	994
Benzofuran	271-89-6	7	0.888	C	118	863	1005	1007
p-Cymene	99-87-6	7	0.883	O	119	801	1031	1026
Perilla alcohol	536-59-4	7	0.853	C	68	735	1305	1295
Nerolidol	7212-44-4	7	0.833	SC	93	849	1567	1566
Ethyl 2-hydroxyisovalerate	2441-06-7	8	1.116	Y	73	781	972	987
Propyl isovalerate	557-00-6	8	1.078	SCY	85	808	953	949
Propyl acetate	109-60-4	8	1.061	SY	61	884	737	728
Isobutyl acetate	110-19-0	8	1.041	Y	73	817	784	780
Isobutyl isobutyrate	97-85-8	8	1.028	SY	71	657	918	906
Methyl benzeneacetate	101-41-7	8	0.989	SC	150	686	1185	1194
β-Ionone	79-77-6	9	1.126	S	177	887	1489	1486
Dihydroeugenol	2785-87-7	9	1.087	S	137	674	1374	1365
m-Dimethoxybenzene	151-10-0	9	1.077	S	138	793	1177	1182
(Z)-Rose oxide	16409-43-1	9	1.062	S	139	841	1116	1112
1,10-Oxidocalamenene	143785-42-6	9	1.049	S	173	906	1501	1491
Hemimellitene	526-73-8	10	1.172	S	105	928	1027	1033
2,5-Dimethylfuran	625-86-5	10	1.170	S	96	748	729	728
Dihydroactinidiolide	17092-92-1	10	1.160	S	111	834	1553	1548
Ethyl methylthiopropanoate	13327-56-5	10	1.128	S	148	901	1107	1098
2-Acetylfuran	1192-62-7	10	1.103	S	95	840	918	914
Ethyl pentanoate	539-82-2	10	1.037	SY	88	883	904	898

Compound	CAS	PLS Cluster	VIP	Treatment Influence	Unique Mass[¥]	MS Match	RI[₦] (calc)	RI[€] (lit)
2-tert-Butyl-p-Cresol	2409-55-4	10	0.967	SY	149	741	1360	1355
Acetophenone	98-86-2	10	0.941	SC	105	927	1076	1076
Ethyl propanoate	105-37-3	10	0.924	SCY	102	758	735	726
Methyl heptenone	409-02-9	10	0.861	SY	108	726	991	988
Tetrahydronaphthalene	119-64-2	10	0.841	SC	132	630	1171	1179
Citronellol acetate	150-84-5	11	1.036	SC	81	774	1353	1352
Nerol oxide	1786-08-9	11	1.028	S	83	795	1159	1151
δ-Dodecalactone	713-95-1	11	1.020	S	99	793	1721	1718
Diethyl malonate	105-53-3	11	1.009	S	115	868	1072	1069
Carvacrol	499-75-2	11	1.008	SC	135	695	1306	1304
p-Cymen-7-ol	536-60-7	11	1.008	SC	135	828	1306	1295
α-Terpinene	99-86-5	11	0.992	SC	93	852	1021	1018
Cadalene	483-78-3	11	0.988	SY	183	875	1690	1684
Anisyl formate	122-91-8	11	0.987	S	121	735	1324	1327
Ethyl salicylate	118-61-6	11	0.985	SC	120	889	1279	1267
Methyl decanoate	110-42-9	11	0.974	SC	74	896	1328	1323
δ-Decalactone	705-86-2	11	0.973	S	99	843	1507	1505
2-Hydroxycineol	18679-48-6	11	0.943	SC	108	829	1242	1227
Ethyl 2-octenoate	2351-90-8	11	0.847	SC	125	813	1253	1243
Isobutyl octanoate	5461-06-3	12	0.958	C	127	839	1350	1348
Ethyl isohexanoate	25415-67-2	12	0.958	Y	88	885	970	969
Ethyl crotonate	10544-63-5	12	0.931	CY	99	726	849	834
Phenethyl hexanoate	6290-37-5	12	0.832	C	104	850	1652	1650
1-Octanol	111-87-5	-	0.789	C	84	824	1079	1080
p-Ethylacetophenone	937-30-4	-	0.763	C	133	763	1297	1281
Thymol	89-83-8	-	0.753	SC	135	832	1301	1290
Styrene	100-42-5	-	0.738	C	104	833	897	897
Citronellol	106-22-9	-	0.649	C	95	859	1235	1233
p-Menth-1-en-9-al	29548-14-9	-	0.583	O	94	764	1231	1217

Treatment influence is characterised by Site (S), Canopy (C), and Yeast (Y) treatments. Compounds that were significantly different due to treatment but were not significantly different due to Site, Canopy, or Yeast are designated as Other (O). [¥] Unique ion (*m/z*): used for peak area determination, identified as the unique ion by ChromaTOF data analysis. * Previously confirmed using a wine spiked with isobutyl methoxypyrazine. [₦] RI: retention indices calculated from C8-C20 n-alkanes. [€] RI: retention indices reported in the literature for 5% phenyl polysilphenylene-siloxane (Adams, 2007, Stein, 2009) capillary GC columns or equivalents. NOTE: RI (calc) values below 800 are extrapolated using ChromaTOF Software.

Table 5.2 Composition of sensory reference standards used to define aroma and taste attributes.

Attribute	Description	Composition [R]
[A]	Cherry	10 mL cherry essence (McCormick)
		40 mL water
[A]	Raspberry	30 mL raspberry syrup from canned raspberry's (Oregon fruit products)
		20 mL water
[A]	Strawberry	10 mL strawberry essence (McCormick)
		40 mL water
[A]	Dark Fruit	20 mL blackberry syrup from canned blackberry's (Oregon fruit products)
		10 mL blueberry syrup from canned blueberry's (Oregon fruit products)
		10 mL plum juice (Oregon fruit products)
		10 mL Crème de Cassis (Hiram Walker)
[A]	Dried Fruit	1 x dried figs (Sunmaid)
		1 x prunes (Sunmaid)
		10 x raisins (Sunmaid)
[A]	Jam	4 x tablespoon blueberry spread (Kozlowski Farms)
		50 mL wine
		150 mL water
[A]	Floral	4 x drops India Crafts Violet Essence Oil into 200 mL water – 10 mL solution in 40 mL wine
[A]	Grass	12 x 5 cm blades fresh grass cut finely
		50 mL water
[A]	Bell Pepper	2 cm square frozen green bell pepper cut finely
[A]	Cooked Veg	10 mL asparagus juice (Raleys)
		10 mL green bean juice (Del Monte)
		30 mL wine
[A]	Herbs	⅛ x teaspoon oregano (McCormick)
		⅛ x teaspoon basil (McCormick)
[A]	Black Pepper	⅛ x teaspoon of freshly ground black pepper
[A]	Tobacco/Tea	2 x cigarette (Camel Lights) in 100 mL boiling water (25 mL ea.)
		2 x teabags (Lipton Yellow Label Black Tea) in 100ml boiling water (25 mL ea.)
[A]	Eucalyptus	4 x drops Nature's alchemy Eucalyptus 100% pure essential oil into 200 mL water – 10 mL solution in 40 mL wine
[A]	Leather	2 cm lengths of leather shoe laces (Kiwi Outdoor)
[A]	Butter	½ x teaspoon butter (Challenge Dairy)
		50 mL water
[T]	Sweet	20 g sucrose in 500 mL water
[T]	Sour	200 mg citric acid in 500 mL water
[T]	Bitter	800 mg caffeine in 500 mL water
[T]	Astringent	312 mg alum in 500 mL water

[R] All Standards were prepared in 50 mL Franzia Vitners Select Cabernet Sauvignon unless otherwise noted. [A]: denotes aroma attribute. [T]: denotes taste attribute.

All wine samples were presented in clear ISO wine tasting glasses (ISO 3591:1977), covered with a plastic lid, labelled with a unique three digit code, under red lighting (to mask differences in colour), in separate booths equipped with a computer screen and mouse for data collection. Ambient temperature was 20 ºC. Wines were assessed monadically and panelists were asked to rate attributes using a continuous unstructured scale (10 cm). A 30 sec rest was included between each sample during which the panelist was able to refresh his or her palate with water and an unsalted water cracker. FIZZ Software Ver. 2.31G (Biosystèmes, Couternon, France) was used for data acquisition and for generating a randomized presentation order using a modified Williams Latin Square design.

5.2.8. Statistical analysis.

All statistical analysis was conducted using JMP version 8.0.2 (SAS Institute Inc., Cary, NC, USA). A one-way analysis of variance (ANOVA) of the normalised peak area was used to analyse the volatile composition results. Principal component analysis (PCA) was conducted using mean values for volatile compounds which were significantly different due to treatment. A three-way ANOVA was conducted using the restricted maximum likelihood (REML) method to test the effects of Judge, Treatment, Replicate and all two-way interactions for each sensory attribute using a pseudo-mixed model with the Judge by Treatment interaction as a denominator. Canonical variance analysis (CVA) was conducted using the replicate fermentation mean values for each significant sensory attribute to describe the sensory differences between wine treatments. Bartlett's Chi-square approximation was used to determine the number of significant canonical dimensions (Chatfield and Collins, 1980). Partial least squares (PLS) regression analysis was used to combine the normalised mean values for significant volatile components (X-variables) and sensory attributes (Y-variables). Mean values were

normalised against the maximum value for any one treatment so that each variable had an equivalent influence on the PLS model. Cross validation was used to determine the lowest number of extracted factors required to minimise the root mean square error of prediction (RMSEP). The PLS output scores and loadings were normalised and plotted, for the significant factors, using JMP. The variable influence on projection (VIP) values and regression coefficients were used to determine which predictive (X) variables were important in modelling the response (Y) variables. VIP values provide weighted sums of squares of the PLS-weights calculated from the Y-variance of each PLS component (Wold et al., 2001). The PLS scores and loadings, excluding X-variables with VIP values below 0.80, were assessed through a two-way hierarchical cluster analysis using a minimal variance algorithm (Ward, 1963). Cluster membership, in conjunction with the regression coefficients, was used to interpret the relationship between the X and Y-variables.

5.3. Results

5.3.1. Volatile metabolome profiling of the wines.

The one-way ANOVA showed that the concentration of 123 volatile compounds were significantly different in the wine headspace due to treatment. On further investigation it was found that the relative abundance of 88, 64, and 27 of these compounds were significantly different due to site, canopy treatment (on the Willyabrup site), and yeast treatment (on the Gingin site), respectively. The distribution of compounds between these three influences is depicted in a Venn diagram (Figure 5.1 (a)) and the treatments that significantly affected the concentration of each compound are listed in Table 5.1. Principal component analysis (PCA) of the 88 compounds significantly different due to site accounted for 83% of the variance in the first two principal components. The first component differentiated the treatments due to site whilst the second component

differentiated the LL and LS from the SL and SS canopy treatments on the Willyabrup site (Figure 5.1 (b)). The yeast treatments were not well differentiated. Subsequent analysis of the treatments from each individual site showed similar trends.

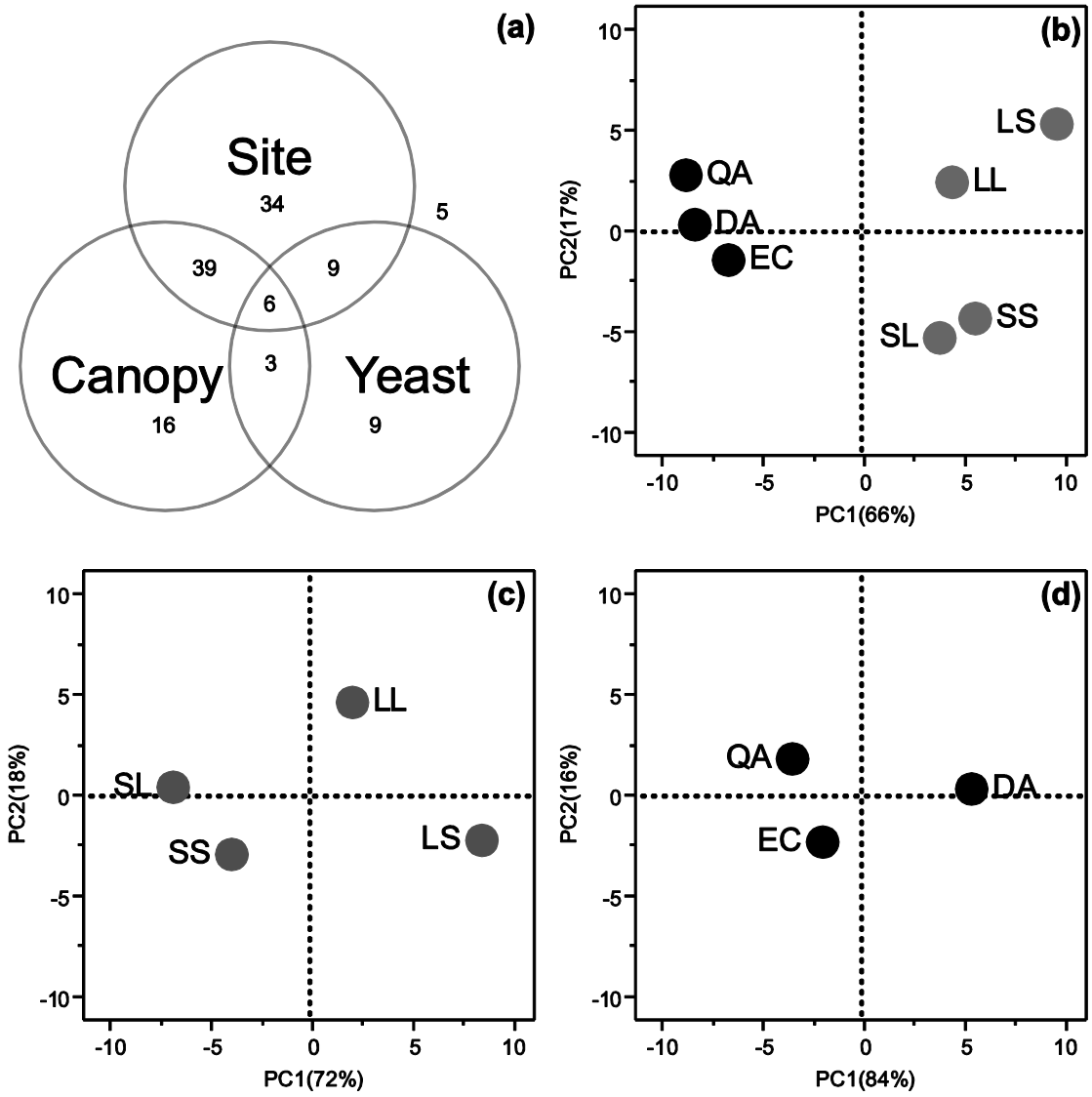

Figure 5.1 Volatile compound analysis for all seven treatments. Venn diagram (a) represents the distribution of the 121 volatile compounds that are significantly different due to treatment, score plot (b) is the PCA of volatile compounds significantly different due to site, score plot (c) is the PCA of volatile compounds significantly different due to canopy treatment at the Willyabrup site, and score plot (d) is the PCA of the volatile compounds significantly different due to yeast treatment from the Gingin site. Treatments DA, EC, QA, LL, LS, SL, and SS are labelled. Black circles are treatments from the Gingin site and grey circles are treatments from the Willyabrup site.

The PCA of the 64 compounds that were significantly different due to canopy treatment at the Willyabrup site accounted for 90% of the variance in the first two principal components (Figure 5.1 (c)) The first component separated the LL and LS treatments from the SL and SS treatments while the second component separated the LL and SL treatments from the SS and LS treatments. The PCA of the 27 compounds that were significantly different due to yeast treatment at the Gingin site accounted for 84% of the variance in the first principal component which separated the EC and QA treatments from the DA treatment whilst the second principal component separated the EC and QA treatments (Figure 5.1 (d)). However, the percentage variance explained in the second dimension of figures 1(c) and 1(d) suggests that both the canopy treatments and the yeast treatments were essentially a one dimensional solution.

5.3.2. Sensory analysis of the wines.

The three-way ANOVA, using a pseudo-mixed model, showed that the bell pepper, cooked vegetable, dried fruit, grass, herbs, astringent, and bitter sensory attributes were significantly different across the treatments (Table 5.3). Bartlett's Chi-square approximation showed that there were four significant dimensions ($p \leq 0.05$); however, the fourth dimension provided little additional information and is not presented. The first three dimensions accounted for 92% of the cumulative variance.

The first dimension accounted for 66% of the variance and differentiated the treatments due to site. The second dimension differentiated the LL and LS from the SL and SS canopy treatments while the third dimension differentiated the LL and LS canopy treatments (Figure 5.2). The SS and SL treatments were not separated in the first three dimensions. It was also observed that the DA, EC and QA yeast treatments were not separated in the first three dimensions. The first dimension of the CVA analysis was

characterised primarily by the bell pepper and herbs aroma attributes which were higher in the Willyabrup treatments (Table 5.3).

Table 5.3 Sensory attributes found to be significantly different due to treatment at $p \leq 0.05$ using a three-way ANOVA. Values represent Least square means (LSM). A pseudo-mixed model using the Judge by Treatment interaction as a denominator was used in all cases. LSM's were compared using a Student's t-Test and differences are denoted by a different lower case letter. Yeast treatments DA, EC, QA and Canopy treatments LL, LS, SL, and SS are labelled.

Treatment	Bell pepper	Cooked vegetable	Dried fruit	Grass	Herbs	Astringent	Bitter
DA	0.7^{d}	1.4^{bc}	2.0^{bc}	1.3^{c}	1.7^{bc}	4.8^{a}	3.1^{a}
EC	0.9^{cd}	1.3^{c}	1.9^{bc}	1.5^{bc}	1.6^{c}	4.7^{a}	2.5^{b}
QA	0.8^{d}	1.6^{bc}	2.4^{abc}	1.4^{bc}	1.6^{c}	4.6^{ab}	2.6^{b}
LL	1.6^{ab}	1.8^{bc}	2.6^{a}	2.0^{a}	1.9^{ab}	4.8^{a}	2.5^{b}
LS	1.4^{bc}	2.6^{a}	2.4^{ab}	2.1^{a}	1.9^{ab}	4.5^{ab}	2.6^{b}
SL	1.9^{ab}	1.6^{bc}	2.3^{abc}	1.8^{ab}	1.7^{abc}	4.1^{b}	2.4^{b}
SS	2.1^{a}	1.9^{b}	1.9^{c}	2.1^{a}	2.0^{a}	4.2^{b}	2.5^{b}

The second dimension was characterised by the astringent and dry fruit sensory attributes which were both notably higher in the LL and LS compared to the SS and SL canopy treatments. The third dimension was characterised primarily by the cooked vegetable aroma which was notably higher in the LS treatment compared to the LL canopy treatment. Primarily the treatments were differentiated by the Willyabrup wines showing "vegetative" and "herbaceous" sensory attributes when compared to the Gingin treatments. The canopy treatments were differentiated from each other, however, this was secondary to the importance of the site.

5.3.3. Partial least squares regression analysis.

PLS analysis with cross validation, using all significant volatile components to predict the significant sensory attributes, determined that the PLS model with the lowest root mean square error of prediction (RMSEP = 0.753) used three latent vectors. The PLS

model differentiated all seven treatments in the first three latent vectors and accounted for 88% and 87% of the variance for the X (composition) and Y (sensory) variables, respectively (Figure 5.3). Treatments were clearly separated by site in the first dimension, which accounted for the greatest percentage of the variance explained, while the different canopy treatments were separated in the second and third dimensions. The first latent vector accounted for ≥75% of the variance explained for 25% of the X-variables while all three latent vectors accounted for ≥74% of the variance explained for 90% of the X-variables indicating that the majority of X-variables were well modelled. Hierarchical cluster analysis was used to simplify the interpretation of the PLS analysis by clustering treatments, X-variables, and Y-variables together that have similar scores or loadings in the first three latent vectors. The first vector was well characterised by compounds from clusters 2, 4, 5, 9 and 11 (Table 5.1) of which 97% were significantly different due to site with 61% and 12% being significantly different due to canopy and yeast treatments, respectively. The second vector was characterised by compounds from clusters 6, 7 and 12 of which 86% of the compounds were significantly different due to canopy treatment and 51% and 6% of the compounds were significantly different due to site and yeast treatment, respectively. The third vector was characterised by compounds from clusters 1 and 3 of which 64%, 50%, and 36% of the 22 variables were significantly different due to site, yeast, and canopy respectively. However, a number of compounds from clusters 1 and 3 were already well explained in the first two vectors. Compounds from cluster 8 were evenly explained across vectors 1 and 2 while compounds from cluster 10 were evenly explained across vectors 1 and 3. The grass, herbs, bell pepper, and cooked vegetable sensory attributes were the major Y variables contributing to the model with 96%, 74%, 71%, and 68% of the cumulative variance explained in the first latent vector respectively.

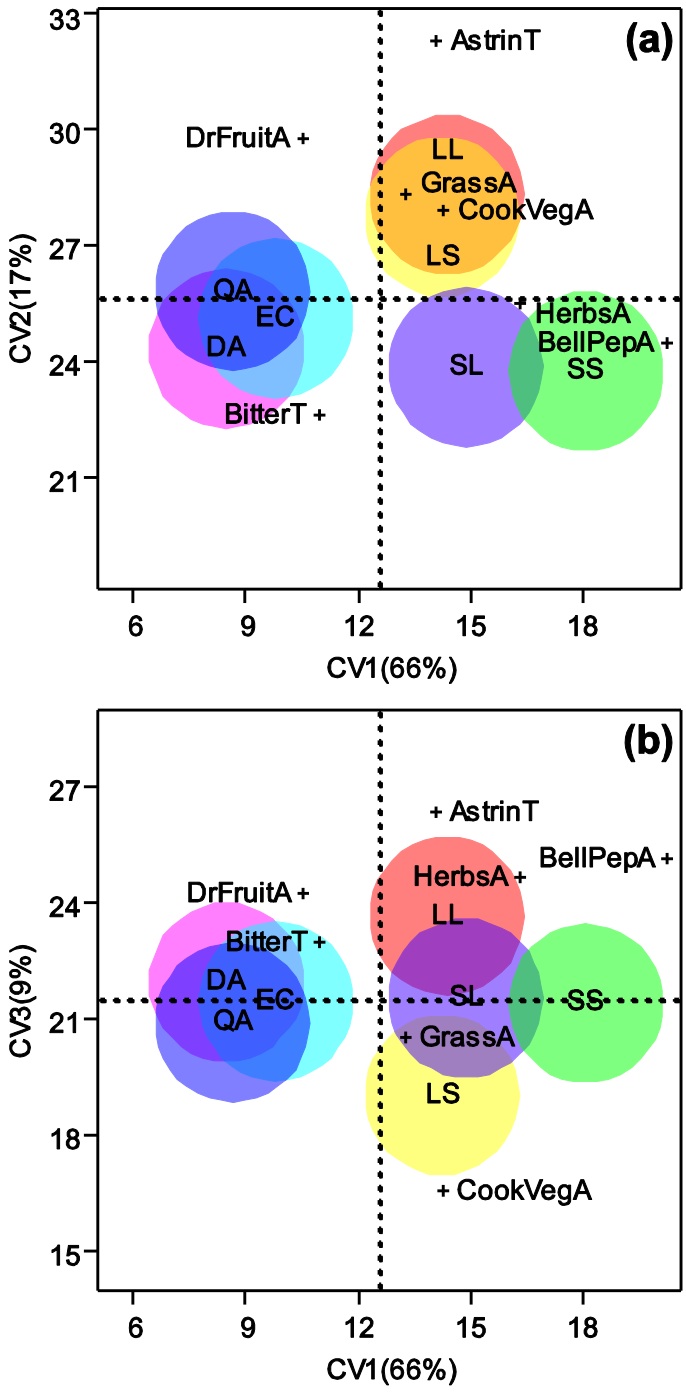

Figure 5.2 Biplot showing the CVA of sensory data for all seven treatments. Circles represent the 95% confidence limits for the mean scores of treatments DA, EC, QA, LL, LS, SL, and SS. Treatments that are significantly different have circles that do not overlap. Loadings for sensory terms are scaled by a factor of 1.5 and are plotted as '+' and labelled. Dimensions 1 and 2 are plotted above (a) and dimensions 1 and 3 are plotted below (b).

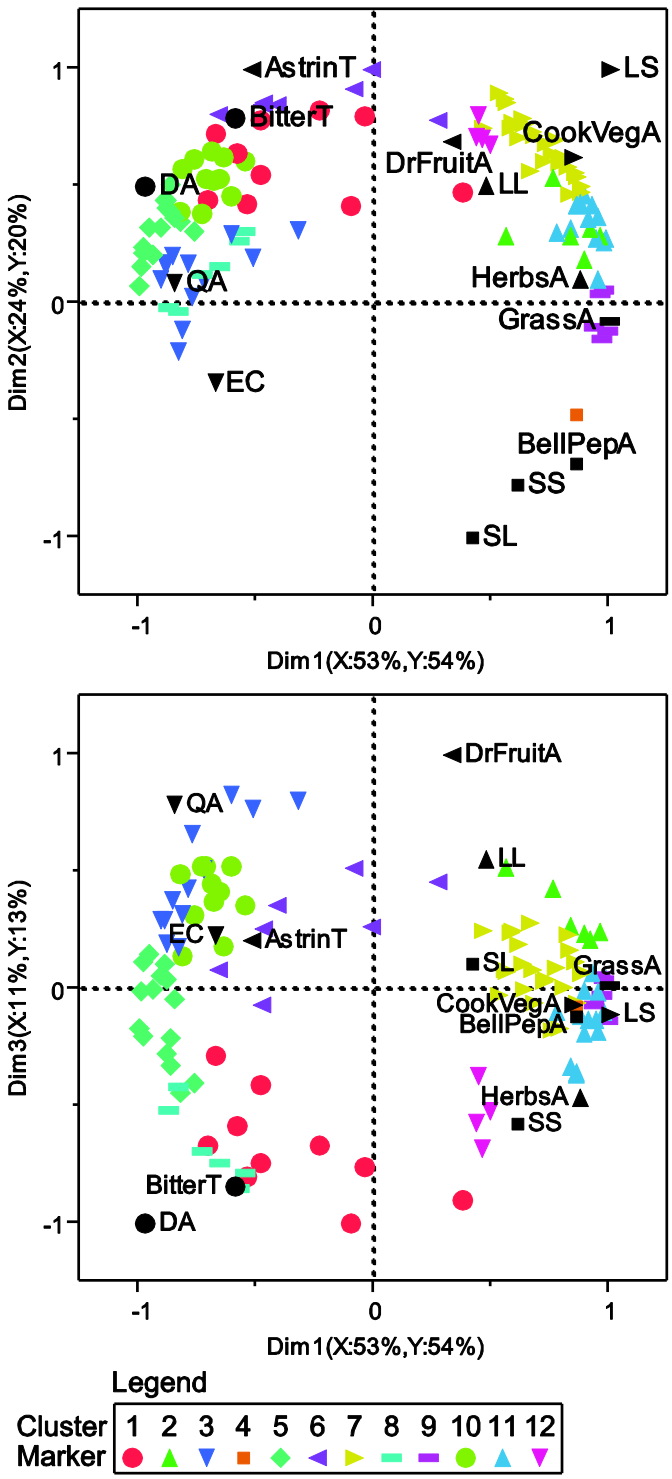

Figure 5.3 PLS analysis of all seven treatments for factor 1 (Dim1) and factor 2 (Dim2) above and factor 1 (Dim1) and factor 3 (Dim3) below. Coloured markers represent the compositional loadings (X matrix), black markers represent the sensory attribute loadings (Y matrix), and the treatment scores. Markers represent different cluster membership as is listed in Table 5.1. Treatments DA, EC, QA, LL, LS, SL, and SS and sensory attributes are labelled as listed in Table 5.3.

The dry fruit, bitter and astringent sensory attributes were not well explained in the first latent vector and better explained by the second and third latent vectors. The second latent vector contributed most to the astringent sensory attribute accounting for 47% of the 75% total cumulative variance explained over the three latent vectors. The second latent vector also accounted for an additional 22% and 19% of the cumulative variance explained for the bell pepper and cooked vegetable sensory terms, respectively.

The third latent vector contributed most to the dry fruit sensory attribute accounting for 45% of the 78% total cumulative variance explained over the three latent vectors. The bitter sensory term was explained evenly across all three latent vectors with 34%, 29%, and 32% of the variance explained in the first, second, and third latent vectors, respectively with a total cumulative variance explained of 95%.

The compounds in clusters 9 and 11 were clustered with the grass and herbs aroma attributes, respectively, which were positively correlated with the Willyabrup site. The bell pepper sensory attribute was clustered with 2-isobutyl-3-methoxypyrazine (IBMP) in cluster 4 and the SS and SL canopy treatments while the cooked vegetable sensory attribute was clustered with compounds in cluster 7 and the LS canopy treatment. Compounds in cluster 2 were clustered with the LL canopy treatment. The dry fruit and astringent sensory attributes were clustered with compounds in cluster 6 which were negatively correlated with SS and SL canopy treatments. Compounds in cluster 1 were clustered with the bitter taste attribute and the DA yeast treatment while the EC and QA yeast treatments were clustered with compounds in cluster 3. Compounds in clusters 5, 8, and 10 were positively correlated with the Gingin site treatments while the compounds in cluster 12 were positively correlated with the Willyabrup site treatments.

5.4. Discussion

5.4.1. Influence of vineyard site.

The objective of this study was to explore wine compositional differences among the treatments (site, yeast strain and bunch shading) using a systematic approach. Compositional analysis indicated that the two field sites used in this study were the major influence on the volatile composition of the wines produced, under the treatments assessed. It was noted that 73% of the compounds that had significantly different abundances amongst the wines were different primarily due to site, which was substantially higher than the number of compounds that were different due to the canopy or yeast treatments. Through the experimental approach taken, we were able to demonstrate that the concentrations of only 28% of the compounds were influenced by the site alone while for the remaining 45% of the compounds, their abundance in the wines were influenced by the site as well as the other treatments imposed.

The effect of site was seen in significant differences in many different classes of volatile compounds, including the grape-derived terpenoids and C13-norisioprenoids, but was also apparent in some esters, which are produced by the yeast during fermentation. This supports previous findings that grape composition can alter the production of fermentation-derived volatile compounds (Keyzers and Boss, 2010). Furthermore, compounds of a similar biochemical origin were differentially affected by the various treatments. For example, it was observed that β-ionone, a norisoprenoid in cluster 9, was significantly more abundant in the wines from the Willyabrup site, but was unaffected by the canopy treatments. In contrast, the norisoprenoids 1,1,6-trimethyl-1,2-dihydronaphthalene (TDN) and vitispirane, which were grouped in cluster 6, were found in significantly lower concentrations in the SS and SL canopy treatments compared to the LL and LS canopy treatments. An increase in the concentration of

TDN and vitispirane with increased grape light exposure has been observed previously in Riesling (Kwasniewski et al., 2010) and Cabernet Sauvignon (Lee et al., 2007). The concentration of TDN and vitispirane were as high in the Gingin wines as they were in the LL and LS canopy treatments from Willyabrup, which suggests the environmental conditions at Gingin resulted in a similar response to the pre-veraison, high light exposure treatment conducted at Willyabrup. All three of these compounds are known to be derived from the degradation of carotenoids (Baumes et al., 2002, Mathieu et al., 2005). However, the results of this study suggest that there are different environmental triggers that regulate the production of these individual compounds and/or their precursors in Cabernet Sauvignon grapes. The yeast treatments had no significant influence on the relative concentrations of these three norisoprenoid compounds, suggesting that the yeast strains studied are not of major importance to the formation of these compounds in wine.

It is well understood that compositional information can provide us with information about what components may be contributing to the sensory perception of a wine. However, it cannot replace the consumer as a variable, in that it is the ability of humans to translate the complex interactions of sight, smell, and taste that defines the sensory experience of consuming wine; flavour is an interaction of consumer and product (Piggott, 1990). The sensory analysis supported the observation that the difference between the sites was the major driver of the variation observed with the Willyabrup treatments showing “vegetative” and “herbaceous” sensory attributes when compared to the Gingin treatments (Figure 5.2). The compounds in clusters 2, 4, 9 and 11 were positively correlated with the Willyabrup treatments and also characterised the bell pepper (cluster 4), grass (cluster 9) and herbs (cluster 11) sensory attributes (Figure 5.3). The bell pepper sensory attribute was positively correlated with IBMP which is

known to be found at higher concentrations in Cabernet Sauvignon wines from regions in Australia and New Zealand with lower MJT's (Allen et al., 1994). A number of terpenes that grouped in clusters 9 and 11 have odour characteristics that have been variously described as citrus, fruity, green, spicy, resinous, floral, caraway, ethereal, and woody (El-Sayed, 2010). It could be proposed that some of these compounds contributed directly to wine sensory characteristics as impact compounds or synergistically through complementation or enhancement effects at sub- and peri-threshold levels (Miyazawa et al., 2008, Ryan et al., 2008). Reconstitution experiments would provide additional information on the role of these compounds in isolation and in combination. However, this was outside the scope of the current study.

This study cannot entirely attribute the differences observed between the Gingin and Willyabrup vineyards to any one characteristic of the sites used. However, it is likely to be a combination of differences in the climate, soils, clonal variation, and management practices that led to the varied composition of the fruit and subsequently the wines produced. An important observation to note from this study is that the sensory and compositional differences due to site were greater than the influence of yeast strain for the wines made from Gingin, and greater than the influence of canopy management at the Willyabrup site.

5.4.2. Influence of yeast treatments.

The compositional analysis indicated that the yeast strains used in this study had little effect in varying the wine volatile composition. The 27 compounds that had significantly different concentrations due to yeast strain were predominantly higher alcohols and esters. However, these only represented 22% of the total number of compounds that were significantly different in abundance due to treatment in this study. The canonical variate analysis of the descriptive sensory data indicated that the yeast

treatments were not significantly different from one another. These strains were used in a commercial manner with a fixed winemaking procedure which suggests that under the conditions used, the changes to the volatile composition did not result in a significant sensory impact. There have been previous studies that have indicated that different yeast strains do influence the volatile composition and subsequently the aroma of wine (Torrens et al., 2008, Callejon et al., 2010). However, the results of this study suggest that site and canopy management, factors that are likely to alter berry composition, have a greater influence on wine composition and sensory scores when compared to yeast.

5.4.3. Influence of canopy treatments.

Compositional analysis indicated that the canopy treatments had a secondary effect, when compared to the site influence, accounting for 53% of the significantly different volatile compounds. The major separation of the canopy treatments was by the light environment experienced prior to veraison with LL and LS treatments being differentiated from the SS and SL treatments (Figure 5.1). This was also observed in the sensory analysis with the LL and LS treatments being lower in bell pepper character, higher in dry fruit and more astringent when compared to the SS and SL treatments (Figure 5.2). These results support previous work that indicates that the pre-veraison stage of berry development is an important time with regards to the production of wine volatile compounds and their precursors (Kalua and Boss, 2009, Dunlevy et al., 2010). The LS treatment was noted as being the highest in cooked vegetable. Compounds from clusters 6, 7, and 12 were negatively correlated with cluster 4 which all tended to characterise the differences in canopy treatments. Norisoprenoid compounds including TDN, vitispirane, and theaspirane A and B, tended to be higher in the LL and LS treatments while IBMP tended to be higher in the SS and SL treatments. It is well understood that IBMP is a potent aroma compound that exhibits a fresh green bell

pepper aroma (Buttery et al., 1969) while norisoprenoids, being ubiquitous to a large number of natural products (Winterhalter and Rouseff, 2002) contribute floral, fruit, kerosene, and camphorous aromas to wine depending on the compound (Mendes-Pinto, 2009). There have been a number of studies that have investigated norisoprenoids and methoxypyrazines in grapes and wines (Allen et al., 1994, Allen et al., 1995, Escudero et al., 2007, Lee et al., 2007, Mendes-Pinto, 2009, Kwasniewski et al., 2010) confirming that they are of particular importance to wine aroma. However, recent research has suggested that the interactions of these compounds together (Pineau et al., 2007) and with other volatiles (Escudero et al., 2007) results in variations in the sensory character of the mixture due to enhancement and suppression effects. For example the combination of β-damascenone, β-ionone, dimethyl sulphide, and fruity esters enhance the perceived berry fruit character (Escudero et al., 2007). Given that the light environment pre-veraison was the major influence on the concentration of these volatiles in the wines produced it can be assumed that the formation of carotenoids (the parent compounds of norisoprenoids) and IBMP was more important than their degradation post-veraison.

The results of the current study identify that whilst yeast treatments influence the composition of the wines produced, the influences of site and canopy were greater. This was reflected in the sensory analysis of the wines where no sensory differences were observed between the yeast treatments applied while there were differences between the two sites and canopy treatments. However, the conclusions made from these observations are limited to the scope of the current study given the treatments applied and the use of only two vineyard sites. The use of metabolomics in this study has highlighted that in many cases the abundances of wine volatile compounds are influenced by multiple factors. PLS analysis of the sensory results has also supported

the concept of volatile compound interactions contributing to the aroma characteristics of Cabernet Sauvignon wine. However, reconstitution studies would be required to provide confirmation of the role that some candidate compounds play. Future advances in the field of wine aroma research should consider the advantages of taking a systematic approach to better understand the variation in wine composition and more importantly those components associated with sensory differences. This should lead to a better understanding of the biological pathways that are important in the formation of volatile compounds in wine and to what degree wine composition can be altered through production management decisions.

6. The relationship between sensory attributes and wine composition for Australian Cabernet Sauvignon wines.

The following is a modified version of the published paper: Robinson, A.L., D.O. Adams, P.K. Boss, H. Heymann, P.S. Solomon, and R.D. Trengove (2011) Australian Journal of Grape and Wine Research, **17**, 327-340.

6.1. Introduction

The wines of Australia are produced from grapes grown in many different climatic and geographically delineated areas which has led to the development of a range of styles and a diverse expression of varietal character. Cabernet Sauvignon, the progeny of Cabernet Franc and Sauvignon Blanc (Bowers and Meredith, 1997), is the third highest planted variety in Australia, accounting for 15% of the total tonnage of wine grapes crushed in 2009 (ABS, 2009). However, to date no research has attempted to explore the diversity of sensory characteristics found in Australian Cabernet Sauvignon wines and how this diversity relates to the chemical composition of these wines.

The volatile components in wine determine the sensations of flavour and aroma experienced by a consumer. The wine volatile profile is complex with more than 800 aroma compounds collectively reported in the literature (Rapp, 1990). These compounds can generally be assigned to one of four different origins; they are either produced directly in the grape, transformed from grape substrates through primary and secondary metabolism of micro-flora (yeast and bacteria), are introduced directly from additives used in production (primarily from wood storage or packaging materials), or are by-products of chemical reactions that occur naturally during wine storage and maturation (Ebeler, 2001). Although there has been interest in the volatile composition of Cabernet Sauvignon wines since initial work by Webb, Kepner and co-workers in the 1960's (Kepner et al., 1969, Webb et al., 1969), there have been few studies that have

attempted to characterise the volatile profile of these wines. In fact the authors are aware of only a few such studies that have attempted to look widely at the volatile composition of Cabernet Sauvignon wines (Slingsby et al., 1980, Shimoda et al., 1993, López et al., 1999, Ferreira et al., 2000, Gürbüz et al., 2006). Research since the 1980's initially focussed on the role of alkyl methoxypyrazines in the vegetative and herbaceous aromas of Cabernet Sauvignon wines (Allen et al., 1994, Hashizume and Samuta, 1997). Most studies have addressed the management of alkyl methoxypyrazines through viticultural practices (Chapman et al., 2004b, Sala et al., 2004, Falcão et al., 2007) with particular emphasis on cluster light interception (Hashizume and Samuta, 1999) as research has indicated that the content of alkyl methoxypyrazines in the wine depended primarily on the composition of the grapes (Roujou de Boubée et al., 2002). Subsequent to this, research into norisoprenoids (Kotseridis et al., 1999a) and aromatic thiols (Bouchilloux et al., 1998) has provided additional information about the potential contribution of impact compounds found in wines made from Cabernet Sauvignon.

Many recent studies have explored the volatile composition of wine almost exclusively using GC-olfactometry (GC-O) and aroma extract dilution analysis (AEDA) (López et al., 1999, Ferreira et al., 2000). Although very useful for initial investigations, GC-O and AEDA may not allow for the extrapolation of the organoleptic contribution of an aromatic compound to the wine sample (Barbe et al., 2008). This can be attributed to interaction effects with the non-volatile matrix (Pineau et al., 2007, Robinson et al., 2009, Sáenz-Navajas et al., 2010) and with other volatile compounds (Atanasova et al., 2005b, Escudero et al., 2007, Pineau et al., 2009) which may result in variations in the sensory character of the mixture due to enhancement and suppression effects. Studies employing these techniques typically report and discuss compounds that are found

above reported odour thresholds (OT) that have been previously determined in synthetic media with less focus on the overall sensory perception of the wine product (López et al., 2003, Cullere et al., 2004). As a consequence little is known about the volatile composition of Cabernet Sauvignon wines, in particular the number of compounds present and the relationships between these compounds and the sensory attributes of Cabernet Sauvignon wines.

In order to better understand the relative contributions that different winemaking inputs have on wine composition and sensory attributes, a comprehensive analysis in which as many wine metabolites as possible are identified and quantified (i.e. metabolomics) is needed. It is important to note that without sensory evaluation, even precise information about the volatile composition of a wine cannot predict the flavour of the system as perceived by humans (Noble and Ebeler, 2002). Subsequently, many studies have been published in the wine research field that focus primarily in relating compositional information to specific sensory attributes (Ebeler, 2001, Noble and Ebeler, 2002, Ebeler and Thorngate, 2009).

A recent critical review (Polášková et al., 2008) identified that future developments in understanding differences in the sensory attributes of wines will be due in part to the development and utilisation of improved and high throughput analytical methods that will allow monitoring of a large number of volatiles including those present at low concentrations. Currently no one analytical method can achieve this objective due to the chemical complexity and heterogeneity of metabolites, the dynamic range that instruments can accommodate, the selectivity and throughput achievable from many extraction protocols, and the costs associated with the purchase or synthesis of standards especially in the case where the presence of metabolites is not known *a priori* (Goodacre et al., 2004).

In turn, a headspace solid-phase microextraction (HS-SPME) method for the analysis of wine volatiles by comprehensive two-dimensional gas chromatography (GC×GC) time-of-flight mass spectrometry (TOFMS) was recently developed in order to resolve and identify a substantially larger number of volatile compounds than current single dimensional GC-MS methodologies (Robinson et al., 2011). The technique offers enhanced separation efficiency, reliability in qualitative and quantitative analysis, capability to detect low quantities, and information on the whole sample and its components (Ong and Marriott, 2002, Dallüge et al., 2003, Górecki et al., 2004). GC×GC-TOFMS is well suited to metabolomic analysis of volatiles in wine and, with commercial instrumentation improving the accessibility of this technology to researchers, the technique has been used for the analysis of volatiles in a number of other foods, fats, oils, and fragrances (Adahchour et al., 2008) but has had limited use in the analysis of wine volatiles.

The current study combines descriptive sensory analysis with the compositional results of a new HS-SPME GC×GC-TOFMS methodology. As the wines were made solely from Cabernet Sauvignon grapes under commercial winemaking conditions the differences in composition and sensory characteristics should reflect the variation of styles available to the consumer. Multivariate statistical techniques including principal component analysis (PCA), hierarchal cluster analysis, and partial least squares (PLS) regression have been utilised to simplify the interpretation of results. The observations from this study are intended to identify candidate compositional components that are correlated with the sensory characteristics of Australian Cabernet Sauvignon wines.

6.2. Materials and Methods

6.2.1. Experimental design

All wines were made from *Vitis vinifera* L. Cabernet Sauvignon produced from the 2009 vintage and represented the Barossa Valley (BV), Clare Valley (CV), Coonawarra (CW), Frankland River (FR), Langhorne Creek (LC), Mount Barker (MB), Margaret River (MR), McLaren Vale (MV), Padthaway (PA), and Wrattonbully (WR) Geographic Indications (GI) which are recognised in Australia as premium Cabernet Sauvignon producing regions. Long term climatic details for each of the GI's are summarised in Table 6.1 from Gladstones (Gladstones, 1992) with comparisons to Bordeaux, France and Napa Valley, California used as international benchmarks for Cabernet Sauvignon production. All wines used in this study were sourced from commercial producers and were considered, in the winemakers' opinion, to be the best regional reflection of Cabernet Sauvignon from that GI. All wines were made entirely from Cabernet Sauvignon grapes using standard commercial winemaking practices.

Table 6.1 Regional characteristics for the Geographical Indications that wine samples were sourced from. Data has been extracted from long term average climatic tables (Gladstones, 1992).

Geographical Indication	GDD (Oct-April)	MJT (°C)	Sunshine Hrs (Oct-April)	Rainfall (Oct-April)	Rainfall (Annual)
Barossa Valley	1525	21.0	1802	204	506
Clare Valley	1594	21.3	1870	245	632
Coonawarra	1337	19.3	1593	257	628
Frankland River	1492	19.8	1611	215	687
Langhorne Creek	1592	19.9	1730	204	495
Margaret River	1529	20.0	1626	274	1192
McLaren Vale	1707	21.5	1765	251	627
Mount Barker	1441	19.0	1518	285	756
Padthaway	1478	20.2	1720	208	509
Wrattonbully *	1337	19.3	1593	257	628
Bordeaux, FR[a]	1392	20.5	1472	427	833
Napa, CA[a]	1499	19.1	2118	-	-

[*] Data used from the Coonawarra climatic table as no data was published. [a] Internationally recognized premium Cabernet Sauvignon producing sites presented for comparison to Australian sites.

Wines were bottled, directly from barrels without fining or filtration, in 750 mL glass bottles and sealed with screw cap closures, shipped to the laboratory in Perth, Western

Australia and then stored at room temperature (about 20 °C), for at most four months prior to analysis. Wine ethanol, pH, titratable acidity, malic acid, glucose and fructose and volatile acidity were determined using a Foss WineScan™ Auto equipped with a CETAC Autosampler (ASX-260). The WineScan™ was calibrated using an extensive commercial selection of wines. The errors associated with these measurements were lower than the differences measured between wine products. Monomeric anthocyanins, small polymeric pigments (SPP), large polymeric pigments (LPP), protein-precipitable tannin, and iron reactive phenolics were analysed using methodology published by Harbertson and Adams (Harbertson et al., 2002, Harbertson et al., 2003). Six bottles of each product were air freighted, over two days, to California in wine bottle foam packaging for sensory analysis at the J. Lohr Sensory Laboratory, University of California, Davis. In Davis, wines were stored at room temperature (about 20 °C) for no more than two months.

6.2.2. HS-SPME GC×GC-TOFMS volatile compound analysis

Samples were analysed using a HS-SPME GC×GC-TOFMS methodology previously described (Robinson et al., 2011). A LECO Pegasus® 4D GC×GC-TOFMS coupled to a CTC CombiPAL autosampler (CTC Analytics, Zwingen, Switzerland) with an agitator and SPME fibre conditioning station was used for all analysis. Samples were prepared in 20 mL amber glass headspace vials to minimise light degradation of alkyl-methoxypyrazines that may occur in Cabernet Sauvignon wines (Heymann et al., 1986, Hashizume and Samuta, 1999). Sodium chloride was added at a rate of 300 g/L to 10 mL of wine pipetted into a 20 mL headspace vial and sealed. An in-fibre internal standard, methyl nonanoate, was loaded into the SPME fibre coating prior to the sample extraction step using methodology previously described (Wang et al., 2005, Setkova et al., 2007a, Setkova et al., 2007b). A commercially available 2008 Cabernet Sauvignon

wine (13.0 % ethanol vol/vol) from Australia was used as a control wine. Retention index probes were loaded into the fibre coating after the internal standard as previously described (Wang et al., 2005, Setkova et al., 2007b) for the analysis of the 2008 Cabernet Sauvignon control wine to monitor for chromatographic drift. TOFMS data was acquired at a rate of 100 scans/sec to accommodate the peak elution rate for modulated analytes and to facilitate peak deconvolution. The TOFMS detector was operated at 1800 V and collected masses between 35 and 350 amu.

6.2.3. Data processing and semi-quantification

GC×GC-TOFMS interrogation and spectral deconvolution was conducted using ChromaTOF® optimized for Pegasus® 4D software Ver. 4.24 (LECO Corporation, St. Joseph, MI, USA). Chromatograms were processed with baseline offset of 0.5 (computation through the middle of noise), auto peak smoothing, peak find with a S/N of 100, a first dimension peak width of 12 sec, and a second dimension peak width of 0.4 sec. Compound mass spectral data were compared against the NIST 2008 and Wiley 9th ed. Mass Spectral Libraries. Retention index (RI) methods were utilised to calculate RI for each compound identified which was compared to published retention indices for 5% phenyl polysilphenylene-siloxane capillary GC columns or equivalents (Adams, 2007, Stein, 2009) for identity confirmation. Minimum similarity match, with regards to library spectra, was kept at 600 and the first and second dimension RI deviation was set at 6 and 0.25 respectively to allow for base peak shifts across modulations but not within modulations. Peak area integration was conducted using the unique ion listed in Table 6.5. Peak areas were automatically normalised against the in-fibre internal standard, methyl nonanoate, and exported to a tab delimited file for statistical analysis. Peak assignments, integration and summation of modulations were automatically conducted by the software.

6.2.4. Sensory panel training

The wines were evaluated by a trained panel of 18 volunteers (five men and thirteen women) between 21 and 43 years of age. All panellists had previous wine tasting experience and were selected due to interest and availability. Descriptive terminology to describe and differentiate the wines was predetermined by assessing terms commonly used in descriptive sensory analysis of red wines from the literature (Heymann and Noble, 1987, Kotseridis et al., 2000, Chapman et al., 2004a, Sivertsen et al., 2005, Madrigal-Galan and Heymann, 2006, Varela and Gámbaro, 2006, Kwiatkowski et al., 2007, Schmid et al., 2007, Cano-López et al., 2008, Pickering et al., 2008, Preston et al., 2008, Hein et al., 2009, Tao et al., 2009, Lattey et al., 2010). Panellists were trained with the reference standards over ten consecutive training sessions to align panellist terminology. Reference standards were presented in black wine glasses and are listed in Table 6.2. Panellists were also asked to evaluate all 30 products broken into blocks of 6 products over 5 consecutive sessions to familiarise the panel with the wine samples that constituted the study.

6.2.5. Descriptive analysis

Following training, panellists were asked to evaluate each of the 30 wine products in triplicate over the course of 18 sessions, equating to 5 wines per session presented in a randomised block design. Prior to each formal evaluation session, the reference standards listed in Table 6.2 were assessed to refresh each panellist's memory. All wine samples were presented in ISO wine tasting glasses (ISO 3591:1977), covered with a plastic lid, labelled with a unique three digit code, under red lighting (to mask differences in colour), in separate booths equipped with a computer screen and mouse for data collection. Ambient temperature was 20 ºC. Wines were assessed monadically and panellists were asked to rate aroma and then taste attributes using a continuous

unstructured scale (10 cm). A thirty second rest was included between each sample during which the panellist was able to refresh his or her palate with water and an unsalted water cracker. FIZZ Software Ver. 2.31G (Biosystèmes, Couternon, France) was used for data acquisition and for generating a randomized presentation order using a modified Williams Latin Square design.

Table 6.2 Composition of sensory reference standards used to define aroma and taste attributes.

Attribute	Description	Composition [R]
[A]	Bell Pepper	2 cm square fresh green bell pepper cut finely
[A]	Black Berry	20 mL black currant syrup (Darbo Inc. Stans, Austria) in 30 mL wine
[A]	Black Pepper	⅛ x teaspoon of freshly ground black pepper
[A]	Butter	½ x teaspoon butter melted (Challenge Dairy)
[A]	Canned Veg	5 mL asparagus juice & 5 mL green bean juice (Green Giant) into 30 mL water 10 mL solution into 40 mL wine
[A]	Chocolate	2 x tbl spoons Double Chocolate Cocoa (Ghirardelli Chocolate) into 200 mL water 10 mL solution into 40 mL wine
[A]	Dried Fruit	1 x dried figs & 1 x prunes & 10 x raisins cut finely (Sunmaid)
[A]	Earthy	2 slices of dried Portobello mushroom ground
[A]	Eucalyptus	2 x drops Nature's alchemy Eucalyptus 100% pure essential oil into 200 mL water 10 mL solution into 40 mL wine
[A]	Floral	1 x drop India Crafts Violet Essence Oil into 1 L water 10 mL solution into 40 mL wine
[A]	Leather	2 cm lengths of leather shoe laces cut into small squares (Kiwi Outdoor)
[A]	Mint	0.25 mL Pure Mint Extract (McCormick) into 200 mL water 10 mL solution into 40 mL wine
[A]	Oak	1 pinch French oak small chips medium toast (ēvOAK)
[A]	Red Berry	20 mL raspberry syrup (Darbo Inc. Stans, Austria) in 30 mL wine
[A]	Smoky	2 mL Wrights All Natural Hickory Seasoning Liquid Smoke into 200 mL water 10 mL solution into 40 mL wine
[A]	Vanilla	10 mL Pure Vanilla Extract (Kirkland Signature) into 200 mL water 10 mL solution into 40 mL wine
[T]	Alcohol	20% v/v ethanol in water
[T]	Astringent	312 mg alum in 500 mL water
[T]	Bitter	800 mg caffeine in 500 mL water
[T]	Sour	200 mg citric acid in 500 mL water

[R] All Standards were prepared in 50 mL Franzia Vitners Select Cabernet Sauvignon unless otherwise noted. [A]: denotes aroma attribute. [T]: denotes taste attribute.

6.2.6. Statistical analysis

All statistical analysis was conducted using JMP version 8.0.2 (SAS Institute Inc., Cary, NC, USA). A one-way analysis of variance (ANOVA) of the normalised peak area was used to analyse the volatile composition results. Differences between the Least Squares (LS) means were tested using a Tukey-Kramer honestly significant difference (HSD) test. Principal component analysis (PCA) was conducted using mean values for volatile compounds which were significantly different due to treatment. A three-way ANOVA was conducted using the restricted maximum likelihood (REML) method to test the effects of Judge, Product, Replicate and all two-way interactions for each sensory attribute using a pseudo-mixed model with the Judge by Product interaction as a denominator. Differences between LS means were again tested using the Tukey-Kramer HSD test. Sensory attributes that were significantly different because of product were analysed through PCA using mean values for each significant sensory attribute. Partial least squares (PLS) regression analysis was used to explain the sensory attributes (Y-variables) using the normalised mean values for significant compositional components (X-variables). X and Y-variables were normalised against the maximum value for any one product so that each variable had an equivalent influence on the PLS model. Univariate response PLS models (PLS1) were used to explore the data (Boulesteix and Strimmer, 2007). The regression coefficients were used to determine which X-variables were important in explaining the Y-variables assessed. The regression coefficients were assessed through a two-way hierarchical cluster analysis using a minimal variance algorithm (Ward, 1963). Cluster membership was used to interpret the relationship between the X and Y-variables by analysing the regression coefficients, averaged within a cluster group, using PCA.

6.3. Results

6.3.1. Chemical analysis of the wines

A one-way ANOVA, using the Tukey-Kramer HSD test to compare LS means, found that ethanol, pH, titratable acidity, malic acid, glucose and fructose, volatile acidity, monomeric anthocyanins, small polymeric pigments (SPP), large polymeric pigments (LPP), protein-precipitable tannin, iron reactive phenolics, and 303 volatile compounds were significantly different among the wines (Table 6.5). The LS means for the major non-volatile components are listed in Table 6.3. Ethanol concentrations ranged from 13.0 to 15.6% vol/vol, pH values ranged between 3.1 and 3.7, TA values ranged from 5.8 to 8.3 g/L, and glucose/fructose, malic acid, and volatile acidity concentrations were measured below 2.0 g/L, 0.8 g/L, and 0.7 g/L, respectively. Monomeric anthocyanins ranged from 209 to 688 mg/L malvidin equivalents, the ratio of large polymeric pigments to small polymeric pigments (LPP/SPP) ranged from 0.52 to 1.10, protein-precipitable tannin ranged from 463 to 1139 mg/L catechin equivalents, and iron reactive phenolics ranged from 680 to 2279 mg/L catechin equivalents. Of the 303 volatile compounds that were significantly different among the wines, 232 were measured in all 30 wines analysed with 71, 16 and 7 compounds below the detection limits of the methodology in one, five and ten of the 30 wines respectively. The mean mass spectral matches and calculated retention indices for each compound are reported in Table 6.5. The mean mass spectral match of the 303 compounds was 815 with an upper and lower 95% of the mean at 823 and 807, respectively. The mean difference between the calculated retention index (RI) and literature RI's for the 303 compounds was 5.4 with an upper and lower 95% of the mean at 5.9 and 4.9, respectively.

6.3.2. Sensory analysis of the wines

A three-way ANOVA, using a pseudo-mixed model of the sensory results showed that the bell pepper, black berry, butter, canned vegetable, dried fruit, earthy, eucalyptus, floral, leather, mint, oak, red berry, smoky, vanilla, alcohol, astringent, bitter, and sour sensory attributes were significantly different ($p \leq 0.05$) due to product.

Table 6.3.Concentration values of major non-volatile components found to be significantly different due to Product using a one-way ANOVA and Tukey's HSD ($p \leq 0.05$). Values represent Least Square (LS) means.

Level	Ethanol	pH	Titratable Acidity	Malic Acid	Glucose + Fructose	Volatile Acidity	Anthocyanins	Small Polymeric Pigments (SPP)	Large Polymeric Pigments (LPP)	Total Polymeric Pigments	Protein-Precipitable Tannin	Iron Reactive Phenolics
BV_731	13.5	3.49	7.23	0.0	0.9	0.54	313	2.16	2.22	4.38	1053	1766
BV_802	14.6	3.53	7.22	0.0	1.8	0.36	408	3.57	2.94	6.51	1019	1959
BV_887	14.1	3.53	6.60	0.2	0.0	0.35	379	2.78	2.30	5.08	1097	2047
CV_081	13.8	3.54	6.55	0.0	1.2	0.45	430	2.45	1.95	4.40	945	1478
CV_517	13.7	3.50	6.87	0.0	1.9	0.49	526	2.24	1.17	3.41	624	884
CV_553	14.6	3.53	7.19	0.0	0.3	0.40	486	2.47	1.52	4.00	878	1254
CW_365	14.6	3.40	6.65	0.0	0.7	0.33	571	1.86	1.38	3.24	812	1018
CW_895	13.7	3.50	7.24	0.6	0.8	0.64	292	1.84	2.03	3.87	1115	1945
CW_961	14.8	3.56	6.39	0.0	1.9	0.52	334	2.21	1.70	3.92	801	1196
FR_478	14.3	3.49	6.36	0.0	0.5	0.26	560	2.31	1.52	3.83	654	1029
FR_762	14.7	3.49	5.82	0.0	0.3	0.29	544	2.19	1.20	3.39	664	1016
FR_855	14.9	3.57	6.08	0.5	0.4	0.17	688	1.66	1.07	2.73	582	680
LC_468	15.7	3.62	7.71	0.7	1.0	0.69	405	2.79	2.96	5.75	1110	1798
LC_697	14.3	3.46	7.42	0.0	0.0	0.52	382	2.89	3.12	6.01	1139	2279
LC_949	14.1	3.41	6.83	0.0	1.2	0.37	518	2.29	1.79	4.08	969	1245
MB_219	14.4	3.49	6.08	0.0	0.5	0.52	444	2.63	2.01	4.64	721	1046
MB_457	13.9	3.14	8.06	0.5	1.7	0.56	321	2.42	1.33	3.75	463	760
MB_565	13.0	3.51	5.94	0.0	1.0	0.43	500	1.43	1.00	2.43	651	931
MR_175	14.3	3.55	7.11	0.7	0.8	0.59	521	1.47	1.10	2.57	621	973
MR_483	14.1	3.45	7.23	0.8	0.0	0.57	407	3.01	3.14	6.15	951	1453
MR_871	13.8	3.46	6.31	0.0	0.4	0.37	471	2.12	1.65	3.76	851	1126
MV_649	13.3	3.73	6.39	0.4	1.0	0.51	373	2.47	2.07	4.55	1058	1711
MV_859	14.4	3.53	7.29	0.0	1.6	0.43	448	2.47	2.09	4.56	1048	1447
MV_992	14.1	3.49	7.14	0.0	0.7	0.31	464	2.57	2.75	5.33	1119	1592
PA_660	14.5	3.37	7.60	0.0	1.4	0.44	457	3.07	2.81	5.88	1022	1626
PA_677	14.3	3.47	7.02	0.0	1.2	0.48	597	1.74	1.14	2.88	717	1058
PA_779	14.6	3.47	8.34	0.0	1.3	0.55	334	2.47	2.11	4.57	1006	1414
WR_462	14.4	3.38	6.94	0.0	0.9	0.55	209	2.18	1.76	3.94	962	1264
WR_582	14.8	3.48	6.76	0.0	0.9	0.31	457	1.86	1.44	3.30	696	936
WR_945	14.0	3.49	6.61	0.1	0.8	0.60	446	1.49	1.63	3.12	935	1240

LS means of each product, for each significant sensory attribute, were compared using the Tukey-Kramer HSD test which identified that the LS means for the black berry, leather, and vanilla sensory attributes were not significantly different among the products. LS means for the 11 aroma and 4 taste attributes that were significantly different are listed in Table 6.4. Principal component analysis (PCA), of the significant sensory attributes, was used to illustrate the relationship between the variables and the wines (Figure 6.1). The first five principal components were considered important according to the Kaiser criterion (eigenvalues greater than or equal to one are retained) (Kaiser, 1960) and the scree test (Cattell, 1966). The fifth principal component added little to the interpretation of the results therefore the first four principal components, which accounted for 73% of the total cumulative variance, are presented. The first principal component accounted for 32% of the variance and primarily characterised the FR_762, FR_855, and MR_871 wines which were positively correlated with the canned vegetable, earthy, and smoky aromas and negatively correlated with the red berry and floral attributes. The second principal component accounted for 17% of the variance and was characterised by the astringent, oak, and butter sensory attributes which were negatively correlated with the bell pepper sensory attribute. These sensory attributes characterised wine CV_553 being higher in bell pepper than LC_468 and WR_462, lower in oak than WR_462 and less astringent than wine LC_468. The third principal component accounted for 15% of the variance and differentiated the wines via the astringent, bitter, eucalypt and mint sensory attributes, which were negatively correlated with the red berry aroma. This differentiated wine LC_697, which was significantly higher in mint aroma than all wines except CW_895 and significantly higher in eucalypt than 50% of the wines. Wine LC_697 was also more bitter, astringent, and less

sour than wine MB_457 and was lower in red berry aroma than wines CW_961, BV_731, and PA_779.

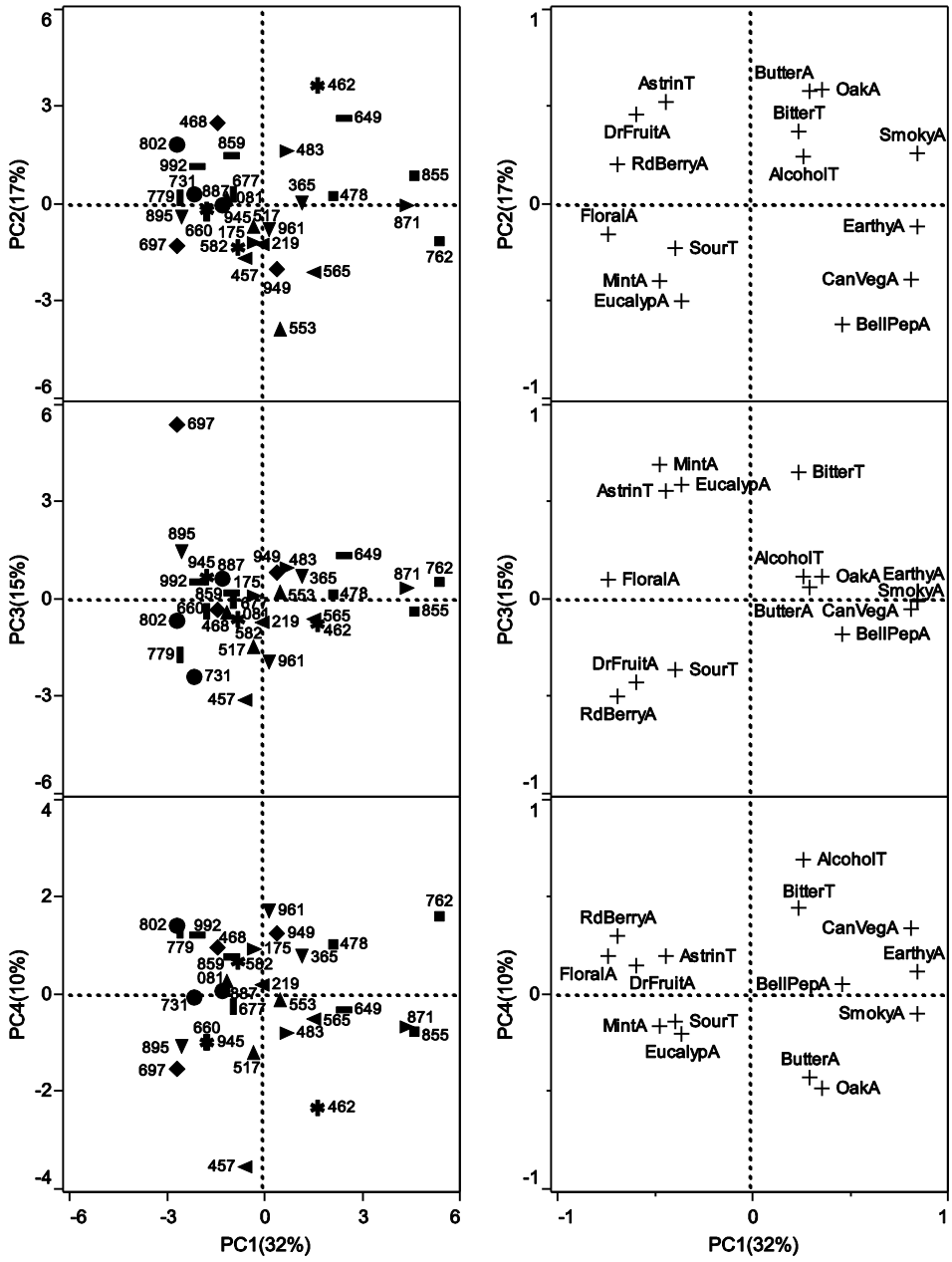

Figure 6.1 Principal component analysis (PCA) of significant sensory attributes for all wine products. Wine scores are plotted on the left and factor loadings are plotted on the right. Sample codes are listed in Table 6.4. Wine symbols are represented as ● Barossa Valley (BV), ▲ Clare Valley (CV), ▼ Coonawarra (CW), ■ Frankland River (FR), ♦ Langhorne Creek (LC), ◄ Mount Barker (MB), ► Margaret River (MR), ▬ McLaren Vale (MV), ▌ Padthaway (PA), and * Wrattonbully (WR).

Table 6.4 Sensory attributes found to be significantly different due to Product using a three-way ANOVA and Tukey's HSD ($p \leq 0.05$). Values represent Least Square (LS) means ± Standard Error (±SE). A pseudo-mixed model using the Judge by Product interaction as a denominator was used in all cases. [A]: denotes aroma attribute. [T]: denotes taste attribute.

Product	AlcoholT	AstrinT	BellPepA	BitterT	ButterA	CanVegA	DrFruitA	EarthyA	EucalypA	FloralA	MintA	OakA	RdBerryA	SmokyA	SourT
BV_731	4.0	5.4	1.3	3.3	1.1	1.4	3.1	1.6	1.4	2.5	1.0	2.5	3.6	1.1	5.3
BV_802	5.1	7.0	1.3	3.4	1.3	1.2	3.5	1.5	1.6	2.7	1.3	2.8	3.6	1.0	4.9
BV_887	4.6	6.2	1.7	3.6	1.3	1.1	2.6	1.8	2.0	2.5	1.4	2.7	2.8	1.0	4.8
CV_081	4.6	5.5	1.6	3.7	1.3	1.4	2.7	1.7	1.7	2.7	1.3	2.9	3.3	1.2	5.2
CV_517	4.1	4.2	1.6	3.3	1.2	1.3	2.7	1.9	1.7	2.0	1.1	2.7	2.8	1.1	4.9
CV_553	4.2	4.7	2.6	3.4	1.3	2.7	2.2	2.3	2.1	2.9	1.7	2.3	2.3	1.0	4.9
CW_365	5.0	5.7	1.4	4.0	1.1	2.0	2.2	2.1	1.6	1.6	1.4	2.9	2.3	1.4	5.2
CW_895	4.4	6.7	1.4	3.6	1.3	1.1	2.5	1.5	2.3	2.5	1.9	3.0	2.9	1.1	5.5
CW_961	4.8	4.8	2.3	3.6	1.5	2.5	2.5	1.5	1.5	2.1	1.1	2.2	4.2	1.1	4.6
FR_478	5.1	4.8	1.3	3.9	1.3	2.2	2.1	1.9	1.7	1.7	0.9	2.8	2.2	1.6	4.7
FR_762	5.4	5.1	2.0	3.9	1.2	3.4	2.1	3.2	2.1	1.3	0.9	2.9	1.5	2.3	5.2
FR_855	4.8	4.4	1.6	3.6	1.8	2.7	2.0	2.6	1.2	1.5	1.1	3.1	1.8	2.0	4.4
LC_468	5.0	6.6	1.1	4.0	1.5	1.2	3.0	1.7	1.6	2.3	1.0	3.0	3.4	1.0	5.4
LC_697	4.3	6.9	1.2	4.1	1.3	1.0	1.9	1.2	3.0	2.5	2.7	2.8	2.0	0.9	4.7
LC_949	4.8	5.5	1.8	3.9	1.0	2.2	1.7	1.6	1.8	2.2	1.4	2.2	2.2	1.2	5.3
MB_219	4.5	4.3	1.7	3.7	1.1	1.8	2.3	1.5	1.9	2.0	1.1	2.6	2.9	1.1	5.0
MB_457	4.0	3.5	1.8	2.5	1.3	1.1	2.4	1.4	1.9	1.8	1.0	3.2	2.6	1.3	6.1
MB_565	4.3	4.8	2.4	3.4	1.1	2.3	2.4	2.3	1.9	2.1	1.3	3.0	2.4	1.4	5.0
MR_175	5.3	4.8	1.6	3.5	1.0	1.9	2.3	1.9	2.1	3.6	1.3	3.1	2.4	1.6	5.8
MR_483	4.6	6.7	1.6	3.9	1.3	1.4	2.6	1.7	1.8	1.8	1.1	3.7	2.1	1.5	5.0
MR_871	4.6	5.3	2.4	4.0	1.5	2.5	2.0	2.5	1.6	1.4	1.0	3.4	1.5	1.9	5.0
MV_649	4.6	6.0	1.6	4.4	1.7	1.7	2.1	2.0	1.4	1.9	0.9	3.6	2.5	1.6	3.9
MV_859	4.7	6.3	1.6	4.1	1.3	1.4	3.2	1.5	1.8	2.3	1.2	3.0	2.9	1.3	4.8
MV_992	4.6	6.4	1.3	4.2	1.2	1.0	2.8	1.4	1.8	2.8	1.1	2.6	3.1	1.1	5.0
PA_660	4.4	5.6	1.4	3.4	1.4	1.2	2.5	1.4	1.9	2.4	1.3	2.7	2.8	1.0	5.5
PA_677	4.7	5.4	1.5	3.8	1.3	1.4	2.3	1.7	1.9	2.5	1.2	3.3	3.1	1.0	5.6
PA_779	4.9	5.9	1.5	3.5	1.0	1.2	2.9	1.6	1.6	2.4	1.2	2.7	4.1	0.8	5.9
WR_462	4.7	5.7	1.3	3.5	2.0	1.3	2.7	1.8	1.6	1.9	0.8	3.9	2.7	2.2	5.2
WR_582	4.9	4.8	1.8	3.5	1.1	1.8	2.6	1.4	2.3	2.1	1.2	2.5	3.2	1.1	5.2
WR_945	4.5	5.8	1.4	3.4	1.6	1.3	2.6	1.5	2.3	2.5	1.4	2.6	2.5	0.9	5.1
± SE	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.3	0.2	0.2

The fourth principal component accounted for 10% of the variance and differentiated wine FR_762 from wine MB_457 which was less bitter and alcoholic tasting and differentiated wine WR_462 from LC_949 which was less oaky and buttery in aroma.

6.3.3. PLS1 analysis relating sensory attributes and chemical composition

The objective of the PLS1 analysis was to optimally explain each sensory attribute (Y-variables) using the compositional variables (X-variables). Interpretation of the PLS1 analysis of each sensory attribute identified that X-variable correlation coefficients became more stable with the subsequent addition of additional latent vectors. Four latent vectors were selected as additional latent vectors did not change the correlation coefficients significantly. The use of four latent vectors explained 97-99% of the cumulative variance for all sensory attributes indicating that the response variables were well modelled using the compositional data obtained. However, the cumulative variance explained for the X-variables was not well explained due to X-variables being poorly modelled for some PLS1 models thus reducing the average cumulative variance for those models. For example eucalyptol had a cumulative variance explained of 76%, 64%, 12%, and 11% in modelling the mint, eucalypt, canned vegetable and floral sensory attributes, respectively. Distribution analysis of the maximum variance explained for each X-variable across all fifteen PLS1 models indicated that the mean percentage variance explained was 52.1%, with an upper and lower 95% of the mean at 53.8 and 50.4%, respectively (Figure 6.2). In contrast the distribution analysis of the minimum variance explained for each X-variable across all fifteen PLS1 models indicated that the mean percentage variance explained was 18.9% with an upper and lower 95% of the mean at 20.7% and 17.1%, respectively (Figure 6.2).

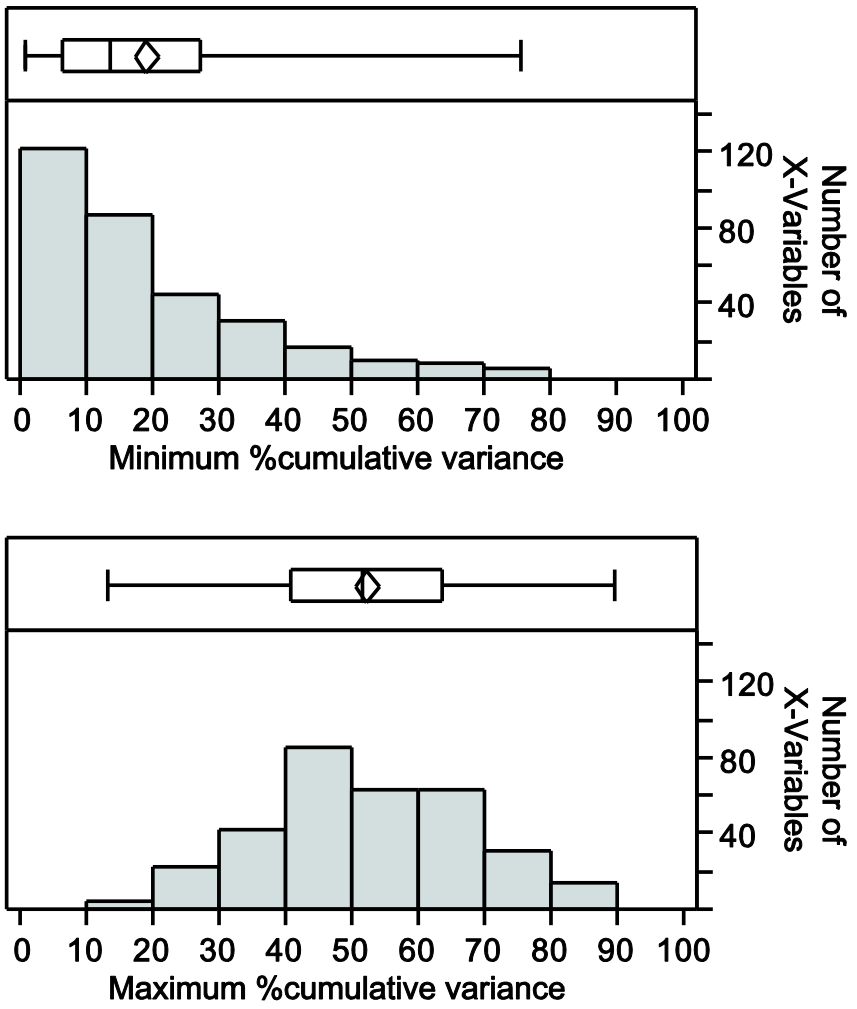

Figure 6.2 Histogram and quantile box plots of the minimum and maximum percentage cumulative variance explained across all 15 PLS1 models for all 315 X-variables. The quantile box plot includes a box that represents the median and the interquartile range with whiskers extending either side representing the minimum and maximum values. The diamond within the box plot represents the sample mean and 95% confidence interval.

The difference between the maximum and minimum cumulative variance explained for the X-variables across all 15 PLS1 models ranged from 8% to 77%. This indicated that a number of variables were well explained in the modelling of some sensory attributes but not all sensory attributes. Two-way hierarchical cluster analysis of the correlation coefficients was conducted to identify which X-variables were unique to the PLS1 models for the Y-variables (Figure 6.3).

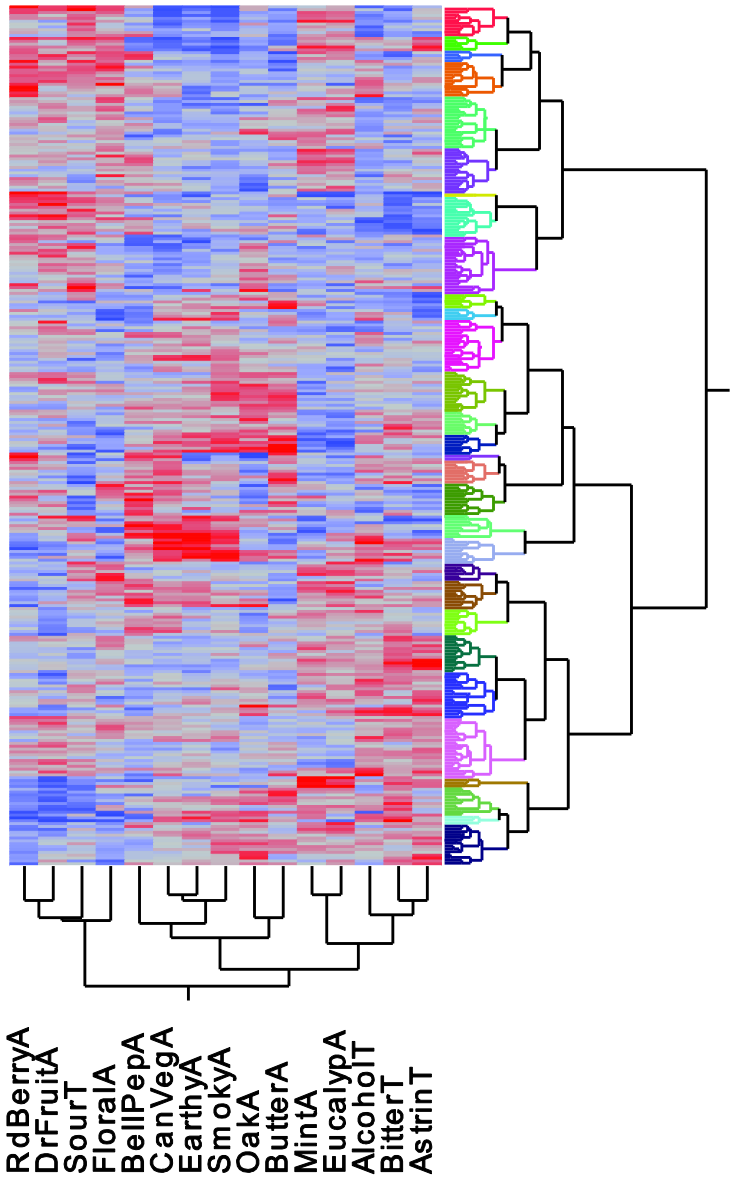

Figure 6.3 Two-way hierarchical cluster analysis of X-variable correlation coefficients from PLS1 analysis of significant sensory attributes. The two-way colour map is scaled from blue (negative) to grey (zero) to red (positive) for each attribute. Dendogram scales are distance scales with X-variable clusters coloured in the dendogram on the right to distinguish different groupings. Cluster groups correspond with clusters, in descending order from top to bottom, numbered 1 to 30 listed in Table 6.5.

The X-variables were grouped into 30 cluster groups which were a mixture of small and large groups ranging from clusters 7 and 16 with 2 X-variables to cluster 26 with 22 X-variables. Principal component analysis of the average correlation coefficient within each cluster group was conducted to visualise which X-variable clusters were positively

or negatively correlated with the Y-variables (Figure 6.4). Ethanol (cluster 26) was important in the PLS1 model of alcohol taste with variables in clusters 20, 21, 25, and 26 being positively correlated and variables in clusters 1, 3, 6, 8, and 10 being negatively correlated. Titratable acidity (cluster 1) and pH (cluster 17) were important in the PLS1 model of sour taste with variables in cluster 1, 2, 8, and 21 being positively correlated and variables in clusters 16, 17, 27, and 28 being negatively correlated. Protein-precipitable tannin, large polymeric pigments, and iron reactive phenolics (cluster 24) were important in the PLS1 model of astringent taste with variables in clusters 2, 24, 25, and 30 being positively correlated and variables in cluster 7, 8, 10, and 11 being negatively correlated. 2-Ethylthiophene (cluster 29), glucose and fructose (cluster 7), and protein-precipitable tannin (cluster 24) were important in the PLS1 model of bitter taste with variables in clusters 24, 27, 28, and 29 being positively correlated and variables in clusters 3, 7, and 8 being negatively correlated. Eucalyptol and hydroxy citronellol (cluster 27) were particularly important in the PLS1 model of eucalypt and mint aroma with variables in clusters 2, 3, 6, and 27 being positively correlated and variables in clusters 7, 8, and 15 being negatively correlated. 2-Isobutyl-3-methoxypyrazine (IBMP) (cluster 19) was particularly important in the PLS1 model of bell pepper aroma with variables in clusters 3, 16, 18, 19, and 22 being positively correlated with variables in clusters 2, 9, 10, 11, and 22 being negatively correlated. The PLS1 models for canned vegetable, earthy and smoky aromas were similar with variables in clusters 19 and 20 being positively correlated and variables in clusters 1, 2 and 9 being negatively correlated. The PLS1 models for butter and oak aroma were defined by similar clusters of variables which included a number of benzene and furan derivatives grouped in clusters 13 and 15.

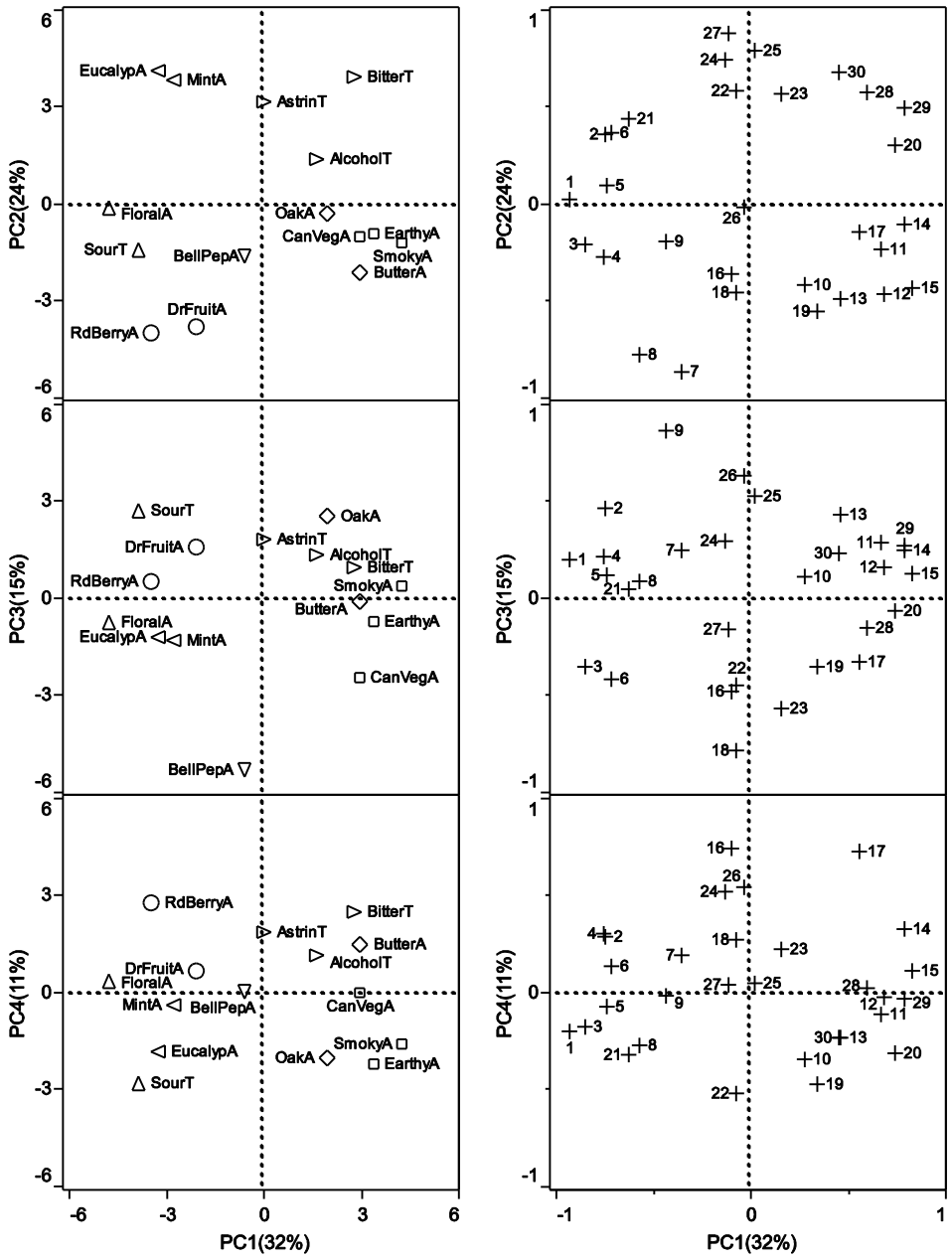

Figure 6.4 Principal component analysis (PCA) of the average PLS1 correlation coefficient values for each cluster of X-variables (compositional attributes). PLS1 Y-variable (sensory attribute) scores are plotted on the left and cluster factor loadings are plotted on the right. Y-variable symbols represent two-way clusters as presented in Figure 6.3 and Table 6.5.

Table 6.5 X-variables used to explain Y-variables (sensory attributes) using PLS1 regression. [A]: denotes aroma attribute. [T]: denotes taste attribute. Compounds analyzed by GC×GC-TOFMS, based on MS and RI matches, which are significantly different due to product using a one-way ANOVA and Tukey's HSD ($p \leq 0.05$). PLS model correlation coefficients for each sensory attribute are distinguished as positive (+) or negative (-) and are coloured using a continuous gradient from blue (negative) to white (zero) to red (positive) where the intensity of the colour distinguishes the importance of the compound in predicting the sensory attribute. X-variables and Y-variables are grouped by two-way hierarchical cluster analysis of all PLS model correlation coefficients and are listed in the order determined by the hierarchical cluster analysis within each cluster group as depicted in Figure 6.3. Compound names, CAS numbers, unique masses, mean mass spectral match quality, calculated and literature retention indices are provided for identity confirmation.

Cluster	X-Variable	CAS	Unique Mass[¥]	MS Match	RI[₦] (calc)	RI[€] (lit)	RdBerryA	DrFruitA	SourT	FloralA	BellPepA	CanVegA	EarthyA	SmokyA	OakA	ButterA	MintA	EucalypA	AlcoholT	BitterT	AstrinT
1	Volatile Acidity						+	+	+	+	+	-	-	-	+	-	-	+	-	-	-
1	Titratable Acidity						+	+	+	+	-	-	-	-	+	-	+	+	-	-	+
1	3-Hydroxy-2,4,4-trimethylpentyl 2-methylpropanoate	74367-34-3	89	745	1378	1381	+	+	+	+	-	-	-	-	-	-	+	+	-	-	+
1	Phenethyl hexanoate	6290-37-5	104	856	1650	1650	+	+	+	+	-	-	-	-	-	-	+	+	-	-	+
1	Terpinen-4-ol	562-74-3	71	818	1193	1177	+	+	+	+	-	-	-	-	-	-	+	+	-	-	+
1	3-Nonen-5-one	82456-34-6	83	689	1052	1051	-	-	+	+	-	-	-	-	-	-	+	+	-	-	-
1	Anisyl formate	122-91-8	121	736	1321	1327	+	+	+	-	-	-	-	-	+	-	+	+	-	-	+
1	δ-Decalactone	705-86-2	99	842	1507	1505	-	-	+	-	-	-	-	-	-	-	+	+	-	-	-
1	Ethyl 3-hydroxytridecanoate	107141-15-1	117	814	1539	1539	+	+	+	+	-	-	-	-	-	-	+	-	-	-	+
1	δ-Dodecalactone	713-95-1	99	784	1721	1730	+	-	+	+	+	-	-	-	-	-	-	+	-	-	-
1	1,10-Oxidocalamenene	143785-42-6	173	894	1498	1491	-	-	+	+	+	-	-	-	+	-	+	+	-	-	-
2	γ-Octalactone	104-50-7	85	863	1269	1262	+	+	+	+	-	-	-	-	-	-	+	+	+	-	+
2	γ-Decalactone	706-14-9	85	859	1477	1470	+	+	+	+	-	-	-	-	-	-	+	+	+	+	+
2	γ-Undecalactone	104-67-6	85	753	1583	1573	+	-	+	+	-	-	-	-	-	-	+	+	+	+	+

Cluster	X-Variable	CAS	Unique Mass[¥]	MS Match	RI[¥] (calc)	RI[€] (lit)	RdBerryA	DrFruitA	SourT	FloralA	BellPepA	CanVegA	EarthyA	SmokyA	OakA	ButterA	MintA	EucalypA	AlcoholT	BitterT	AstrinT
2	Norinone	38651-65-9	83	699	1153	1152	+	+	+	+	-	-	-	-	-	+	+	+	-	-	+
2	2-Nitro-p-cresol	119-33-5	153	669	1260	1250	+	+	+	+	-	-	-	-	-	+	+	+	+	+	+
3	Cubenol	21284-22-0	161	722	1660	1642	+	+	+	+	+	-	-	-	-	-	+	+	-	-	+
3	Cadalene	483-78-3	183	875	1690	1684	+	+	+	+	+	-	-	-	-	-	+	+	-	-	-
3	Isolongifolene, 4,5,9,10-dehydro-	156747-45-4	200	761	1547	1544	+	+	+	+	+	-	-	-	-	-	+	+	-	-	+
3	α-Calacorene	21391-99-1	157	880	1555	1549	+	+	+	+	+	+	+	-	-	-	+	+	-	-	+
4	Cumene	98-82-8	105	722	927	924	+	+	+	+	-	-	-	-	-	-	+	+	+	-	+
4	Phenethyl isovalerate	140-26-1	104	828	1494	1490	+	+	+	+	-	-	-	-	-	-	-	-	+	+	+
4	p-Menth-1-en-9-ol	18479-68-0	94	696	1312	1295	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-
4	Citronellol	106-22-9	95	894	1233	1233	+	+	+	+	-	-	-	-	-	-	+	+	+	-	-
4	Ethyl pentanoate	539-82-2	88	906	902	898	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-
4	γ-Nonalactone	104-61-0	85	874	1372	1361	+	+	+	+	-	-	-	-	-	-	-	-	+	-	+
4	Dihydropseudoionone	689-67-8	69	842	1453	1457	+	+	+	+	-	-	-	-	-	-	+	-	+	-	+
4	(Z)-Farnesol	3790-71-4	69	844	1738	1718	+	+	+	+	-	-	-	-	-	-	-	-	+	-	+
4	Ethyl dihydrocinnamate	2021-28-5	104	731	1360	1350	+	+	+	+	+	+	-	-	-	-	+	-	+	+	+
4	2-Methylnaphthalene	91-57-6	142	896	1328	1315	+	-	+	+	+	-	-	-	-	-	+	+	+	+	+
4	Biphenyl	92-52-4	154	899	1394	1385	+	-	+	+	+	+	-	-	-	-	+	+	+	+	-
4	Naphthalene	91-20-3	128	892	1197	1191	+	+	+	+	+	+	-	+	-	+	-	+	+	-	-
4	Benzenenitrile	100-47-0	103	903	993	994	+	+	-	+	+	+	-	-	-	-	-	-	+	-	-
5	β-Calacorene	50277-34-4	157	801	1578	1564	+	+	+	+	-	-	-	-	-	+	+	+	-	-	-
5	p-Benzoquinone	719-22-2	220	757	1472	1472	+	+	-	+	-	-	-	-	+	+	+	-	-	-	-
5	Ethyl 2-hydroxy-4-methylpentanoate	10348-47-7	69	889	1060	1060	+	+	-	+	-	-	-	-	-	+	+	+	-	-	-
5	Ethyl 2-hexenoate	27829-72-7	99	902	1049	1036	-	+	+	+	-	-	-	-	-	-	-	+	+	-	-
5	3-Hexen-1-ol, (E)-	928-97-2	67	858	858	853	-	+	+	+	-	-	-	-	+	+	+	+	-	-	+
5	2-Phenylethyl isobutyrate	103-48-0	104	793	1403	1396	-	+	+	+	-	-	-	-	-	-	+	+	-	-	+
5	(Z)-3-Nonenol	10340-23-5	81	743	1159	1160	-	+	-	+	-	-	-	-	-	+	+	+	-	-	+
5	Ethyl 3-hydroxybutanoate	5405-41-4	60	805	943	945	-	+	+	+	-	-	-	-	-	+	+	+	-	-	-
5	Methyl benzeneacetate	101-41-7	150	727	1183	1194	+	+	+	+	+	-	-	-	+	+	+	+	-	-	+

Cluster	X-Variable	CAS	Unique Mass[¥]	MS Match	RI[¥] (calc)	RI[€] (lit)	RdBerryA	DrFruitA	SourT	FloralA	BellPepA	CanVegA	EarthyA	SmokyA	OakA	ButterA	MintA	EucalypA	AlcoholT	BitterT	AstrinT
5	α-Cyclocitral	432-24-6	81	790	1127	1116	+	+	-	+	-	-	-	-	+	+	+	+	-	+	+
5	Benzaldehyde	100-52-7	105	907	972	969	+	+	-	+	+	-	-	-	+	+	+	+	-	-	-
5	2-Heptanol	543-49-7	45	922	908	901	+	+	-	+	+	-	-	-	+	-	-	+	-	+	+
5	Methyl hexanoate	106-70-7	74	888	927	923	-	+	-	+	+	-	-	-	+	+	+	+	-	-	-
5	Phenol	108-95-2	94	811	995	979	-	-	+	+	-	-	-	+	+	+	+	+	-	+	+
5	(Z)-Oak lactone	55013-32-6	99	900	1333	1340	+	+	+	+	-	-	-	-	-	+	+	-	-	-	-
5	Ethyl propanoate	105-37-3	102	804	733	726	+	+	+	+	-	-	-	-	-	+	+	+	+	-	-
5	Toluene	108-88-3	92	900	776	771	+	+	-	+	-	-	+	-	-	-	+	-	-	-	-
5	Methyl hexadecanoate	112-39-0	74	819	1925	1926	+	+	+	+	-	-	+	-	-	+	+	+	+	-	+
5	3-Methylundecane	1002-43-3	57	839	1170	1169	+	+	-	+	-	+	+	-	-	-	+	-	+	-	-
6	δ-Cadinene	483-76-1	161	773	1529	1528	+	-	+	+	-	-	-	-	-	-	+	+	-	-	+
6	Camphene	79-92-5	93	822	954	961	+	+	+	+	+	-	+	-	-	-	+	+	-	-	+
6	Dihydro-α-Ionone	31499-72-6	136	691	1425	1406	+	+	-	+	+	-	-	-	-	-	+	+	-	+	+
6	α-Phellandrene	99-83-2	136	788	1010	1005	+	+	+	+	+	-	+	+	+	-	+	+	-	-	+
6	(E)-Calamene	483-77-2	159	764	1534	1530	-	+	-	+	+	-	-	-	-	+	+	+	-	-	+
6	Methyl dodecanoate	111-82-0	74	863	1526	1525	-	+	-	+	+	-	-	-	-	+	+	+	-	-	+
6	α-Cedrene	469-61-4	119	672	1419	1410	+	+	+	+	+	-	-	-	-	+	+	+	-	+	+
6	Ethyl tetradecanoate	124-06-1	88	864	1795	1796	+	-	-	+	+	-	-	-	-	+	+	+	-	-	-
6	Methyl tetradecanoate	124-10-7	74	773	1726	1722	-	-	-	+	+	-	-	-	-	-	+	+	-	-	+
6	Ethyl undecanoate	627-90-7	88	853	1496	1491	-	-	-	+	-	-	-	-	-	-	+	+	-	+	+
6	Chlorobenzene	108-90-7	112	688	850	852	-	-	-	+	+	-	-	-	-	+	-	+	-	+	+
6	1-Decanol	112-30-1	70	895	1279	1283	+	+	-	-	-	-	-	-	-	-	+	+	-	+	+
6	1-Nonanol	143-08-8	70	873	1180	1173	+	+	-	+	-	-	-	-	-	-	+	+	-	+	-
6	2-Tridecanone	593-08-8	58	829	1498	1497	+	+	-	+	+	+	-	-	-	+	+	+	-	-	+
6	Nonanoic acid	112-05-0	60	765	1279	1280	-	+	-	-	-	-	-	-	-	-	+	+	-	-	+
6	Benzothiazole	95-16-9	135	903	1245	1244	-	-	-	+	-	+	-	-	-	+	+	+	-	-	+
7	Glucose + Fructose						+	+	+	+	+	-	-	-	-	-	-	-	-	-	-
7	Isoamyl butyrate	106-27-4	71	761	1060	1054	+	+	+	-	-	-	-	-	-	+	-	-	-	-	-
8	p-Ethylacetophenone	937-30-4	133	712	1295	1281	+	+	+	+	-	-	-	-	-	-	+	-	-	-	-

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8	Diethyl glutarate	818-38-2	143	909	1283	1284	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-
8	α-Amorphene	483-75-0	161	846	1508	1505	+	+	+	+	+	+	+	-	-	-	-	-	+	-	+
8	Edulan I	41678-29-9	177	741	1321	1309	+	+	+	+	-	-	+	-	-	-	-	-	-	-	-
8	Isopentyl hexanoate	2198-61-0	99	847	1253	1250	+	+	+	+	-	-	-	-	-	-	-	+	-	-	-
8	Propyl butanoate	105-66-8	71	818	900	896	+	+	+	+	-	-	-	-	-	-	-	+	-	-	-
8	2-Tetradecanone	2345-27-9	58	729	1600	1597	+	+	+	+	-	-	-	-	-	+	-	-	-	-	-
8	Propyl acetate	109-60-4	61	903	735	728	+	+	+	+	+	+	+	-	-	+	-	-	-	-	-
8	Propyl hexanoate	626-77-7	117	870	1096	1079	+	+	+	+	+	-	-	+	-	-	-	-	-	-	-
8	β-Ionone	79-77-6	177	881	1489	1486	-	+	+	+	+	-	-	-	-	-	-	+	-	-	-
8	p-Methylacetophenone	122-00-9	119	766	1199	1179	+	+	+	-	+	-	+	-	-	+	-	-	-	-	-
8	Ethyl 3-hydroxyhexanoate	2305-25-1	117	716	1137	1133	-	+	+	+	+	-	+	+	+	+	-	-	-	-	-
8	o-Dimethoxybenzene	91-16-7	138	704	1151	1154	+	+	+	+	+	-	-	+	+	+	-	-	-	-	-
8	m-Dimethoxybenzene	151-10-0	138	793	1175	1182	+	+	+	-	+	-	-	-	+	-	-	-	-	-	-
9	4-Oxoisophorone	1125-21-9	68	715	1157	1142	+	+	+	+	-	-	-	-	-	-	+	-	-	+	+
9	1-Octanol	111-87-5	84	877	1077	1080	+	+	-	+	-	-	-	-	-	-	-	+	-	+	-
9	(Z)-Ocimene	3338-55-4	92	762	1039	1040	+	-	-	-	-	-	-	-	-	+	-	-	+	+	-
9	Vitispirane	65416-59-3	192	891	1292	1272	+	+	+	-	-	-	-	-	+	-	-	-	-	-	-
9	Diethyl malonate	105-53-3	115	858	1072	1069	+	+	+	-	-	-	-	-	+	-	-	-	+	-	-
9	Ethyl (methylthio)acetate	4455-13-4	134	685	989	990	+	+	+	-	-	-	-	-	+	-	-	-	-	-	+
9	β-Damascone	85949-43-5	177	799	1422	1419	+	+	-	-	-	-	-	-	+	-	-	-	-	-	-
9	1,1,6-Trimethyl-1,2-dihydronaphthalene (TDN)	30364-38-6	157	771	1367	1364	+	+	+	-	-	-	-	-	+	-	-	-	-	-	+
9	Dehydro-β-ionone	1203-08-3	175	894	1487	1485	+	+	+	-	-	-	-	-	+	-	-	-	-	-	-
9	Dehydroxylinalool oxide A	7392-19-0	139	787	973	971	-	+	+	-	-	-	-	-	+	-	-	-	-	-	-
9	Ethyl 3-methylpentanoate	5870-68-8	88	694	960	960	+	+	+	+	-	-	-	-	+	-	-	-	-	-	-
9	Ethyl isohexanoate	25415-67-2	88	883	968	969	-	+	+	+	-	-	-	-	+	+	+	+	+	-	-
9	Ethyl benzoate	93-89-0	105	894	1179	1180	-	+	+	+	-	-	-	-	+	-	-	+	-	+	+
9	Ethyl heptanoate	106-30-9	88	887	1098	1093	-	+	+	+	-	-	-	-	+	-	-	+	-	+	-
9	2-Acetyl-5-methylfuran	1193-79-9	109	857	1043	1042	-	+	+	-	-	-	-	+	+	+	+	+	-	-	+

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9	1-Hexanol	111-27-3	56	824	876	863	-	+	-	-	-	-	-	+	+	+	-	-	-	-	-
9	Malic Acid						-	+	+	+	-	-	-	-	+	+	-	-	+	-	+
9	Ethyl 3-methylbutyrate	108-64-5	88	864	855	852	-	+	+	-	-	-	-	+	+	+	-	+	+	+	+
9	Ethyl 9-hexadecenoate	54546-22-4	79	796	1975	1977	+	-	+	+	-	-	-	-	+	-	-	-	+	-	-
9	δ-Octalactone	698-76-0	99	843	1297	1287	+	+	+	+	-	-	-	-	+	-	-	-	+	+	+
9	Ethyl phenylacetate	101-97-3	164	846	1251	1247	+	+	+	-	+	-	+	-	+	-	-	-	-	-	+
10	Dehydro-p-cymene	1195-32-0	132	865	1097	1091	+	-	+	-	-	-	-	-	+	+	-	+	-	-	-
10	Menthone	89-80-5	112	671	1165	1154	-	-	+	-	-	-	-	+	+	+	-	-	-	-	-
10	Ethyl tiglate	5837-78-5	113	857	943	939	-	-	+	-	-	-	+	+	+	+	-	+	-	-	-
10	(E)-Oak lactone	39638-67-0	99	838	1299	1304	+	-	-	+	+	+	-	-	-	+	+	-	-	-	-
10	Ethyl pentadecanoate	41114-00-5	88	893	1894	1897	-	-	-	-	-	-	+	+	+	+	+	-	-	-	-
10	Ocimenol	5986-38-9	93	635	1174	1179	-	-	-	-	-	+	+	+	+	+	-	-	-	-	-
11	(Z)-β-Damascenone	23696-85-7	121	787	1369	1367	+	+	-	-	-	+	+	+	-	-	-	-	-	-	-
11	(E)-β-Damascenone	23726-93-4	121	859	1389	1387	+	-	-	-	-	+	-	+	+	-	-	-	+	+	-
11	Isomenthone	491-07-6	112	695	1175	1165	-	-	-	-	-	+	+	+	-	+	-	-	+	-	-
11	Decanal	112-31-2	82	624	1213	1206	-	-	-	-	-	+	+	+	-	+	-	-	+	+	-
12	3-Hexen-1-ol, (Z)-	928-96-1	67	852	864	860	-	+	-	-	-	-	-	-	-	+	+	-	-	-	+
12	α-Pinene	80-56-8	93	739	937	933	+	+	-	+	-	-	-	-	-	+	-	-	-	-	-
12	β-Cyclocitral	432-25-7	137	868	1231	1220	-	+	-	-	-	-	+	+	+	+	-	-	-	+	-
12	4-Hydroxy-3-methylacetophenone	876-02-8	135	818	1322	1323	+	+	-	-	-	-	+	+	-	-	-	-	-	-	+
12	α-Methylstyrene	98-83-9	118	816	987	988	+	+	+	-	-	-	+	+	+	-	-	-	-	-	-
12	2,4-Di-tert-butylphenol	96-76-4	191	856	1511	1513	+	+	+	+	+	+	+	+	-	-	-	-	+	-	+
12	Undecane	1120-21-4	71	882	1100	1100	-	+	+	+	+	+	+	+	-	-	+	-	+	-	-
12	2,6-Diisopropylnaphthalene	24157-81-1	197	839	1735	1728	-	+	+	-	-	+	+	+	-	+	-	-	+	-	-
12	2-Octanone	111-13-7	58	800	995	990	-	+	-	+	+	-	+	+	-	+	-	-	+	-	+
12	Heptyl acetate	112-06-1	43	820	1114	1115	-	+	-	-	-	+	+	-	+	-	+	-	+	+	+
12	Octane	111-65-9	85	837	800	800	-	+	-	-	-	+	-	+	+	-	+	-	-	+	+
12	Nonane	111-84-2	85	884	900	900	-	+	-	-	+	+	+	+	+	+	+	-	-	-	+
12	α-Terpinene	99-86-5	93	847	1021	1018	-	+	+	-	-	-	+	+	+	+	-	+	+	-	+

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12	Butyl acetate	123-86-4	56	906	819	813	-	+	-	-	+	+	+	+	+	+	+	-	+	-	+
12	S-Methylmercaptoethanol	5271-38-5	61	753	850	838	-	-	-	-	+	+	+	+	+	-	+	-	-	+	-
12	Isoamyl phenylacetate	102-19-2	91	813	1499	1490	-	-	-	-	+	-	-	+	-	+	-	-	-	+	-
12	Nerol oxide	1786-08-9	83	827	1159	1151	-	-	-	-	-	+	-	+	+	-	-	-	+	+	-
12	(Z)-Rose oxide	16409-43-1	139	843	1114	1112	-	-	-	-	-	+	+	+	+	+	-	-	+	-	-
12	(E)-Rose oxide	876-18-6	139	689	1134	1127	-	-	-	-	-	+	+	+	+	+	-	-	+	-	-
13	Dill ether	74410-10-9	137	711	1197	1184	+	+	+	-	-	-	-	+	+	+	-	+	-	-	-
13	m-Ethyl toluene	620-14-4	105	863	964	969	+	+	+	-	-	-	-	+	+	+	-	-	+	-	+
13	Methyl cinnamate	103-26-4	131	761	1399	1397	+	+	+	-	+	-	+	+	+	+	-	-	+	+	+
13	Methyl benzoate	93-58-3	105	857	1103	1100	-	+	+	-	-	-	+	+	+	+	-	-	-	+	+
13	p-Tolualdehyde	104-87-0	119	819	1080	1079	+	+	+	-	-	+	+	+	+	+	-	+	+	-	+
13	Methyl eugenol	93-15-2	178	684	1408	1402	+	-	+	+	-	-	-	+	+	+	-	-	+	+	-
13	Thymol	89-83-8	135	773	1301	1290	-	+	-	+	-	-	+	+	+	+	-	-	+	+	-
13	Syringol	91-10-1	154	807	1361	1362	-	+	+	+	-	-	+	+	+	+	-	+	+	+	+
13	Guaiacol	90-05-1	109	889	1095	1102	-	+	+	+	+	+	+	+	+	+	-	-	-	-	-
13	4-Ethyl-o-xylene	934-80-5	119	838	1089	1093	-	+	-	-	-	-	-	+	+	+	-	-	-	-	-
13	4-Methylindane	824-22-6	117	805	1157	1151	-	+	-	-	-	+	+	+	+	+	-	-	+	-	-
13	Salicylaldehyde	90-02-8	122	797	1055	1057	+	-	+	-	+	+	+	+	+	+	-	-	-	-	-
13	Eugenol	97-53-0	164	885	1365	1359	-	-	-	+	+	+	+	+	+	+	-	-	+	+	-
13	Vanillin	121-33-5	152	754	1416	1415	-	-	+	-	+	+	+	+	+	+	+	-	-	-	-
14	2,4,5-Trimethyl-1,3-dioxolane	3299-32-9	101	767	745	735	+	+	-	-	+	-	+	+	+	+	-	-	+	+	+
14	Acetal	105-57-7	73	822	743	726	+	+	-	-	+	+	+	-	+	+	-	-	+	-	+
14	Tetrachloroethylene	127-18-4	166	838	808	815	-	+	-	-	+	+	+	+	+	+	-	-	+	+	+
14	Dihydroeugenol	2785-87-7	137	820	1374	1365	-	-	-	-	+	-	-	+	+	+	-	-	-	+	+
14	p-Creosol	93-51-6	123	636	1203	1188	-	-	-	-	+	+	+	-	+	-	-	-	+	+	+
14	Butylbenzene	104-51-8	91	686	1064	1058	-	+	-	+	-	-	+	+	+	+	-	-	-	+	+
14	3-Octanone	106-68-3	99	817	989	989	-	-	-	-	-	-	+	+	+	+	-	-	-	+	+
14	2-Ethyl hexanol	104-76-7	57	730	1035	1030	-	+	-	-	-	+	-	+	+	+	-	-	-	+	+
14	2-Methylthiophene	554-14-3	97	804	780	775	-	-	-	-	-	+	+	+	+	+	-	-	+	+	+

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15	2-Methylcumarone	4265-25-2	131	881	1117	1109	+	+	+	+	+	+	+	+	+	+	-	-	+	-	-
15	Furfural	98-01-1	96	938	840	835	+	+	+	-	-	+	+	+	+	+	-	-	+	+	+
15	2-Nonanone	821-55-6	58	784	1095	1092	-	+	-	-	-	+	+	+	+	+	-	-	+	-	+
15	Pentylbenzene	538-68-1	91	822	1162	1154	+	+	-	-	-	+	+	+	+	+	-	-	+	-	+
15	Hexanal	66-25-1	82	790	806	804	-	-	-	-	+	+	+	+	+	+	-	-	-	+	-
15	Anisole	100-66-3	108	848	922	920	-	-	-	-	-	+	+	+	+	+	-	-	-	+	-
15	5-Methylfurfural	620-02-0	110	880	970	964	-	-	-	-	+	+	+	+	+	+	-	-	-	-	-
16	Ethyl 2-hydroxyisovalerate	2441-06-7	73	738	970	987	+	+	-	+	+	+	-	-	-	+	+	-	-	-	-
16	Benzenepropyl acetate	122-72-5	118	834	1378	1373	+	+	-	-	+	+	-	-	-	+	-	-	+	-	-
17	Ethyl isobutyrate	97-62-1	116	782	770	756	+	+	-	-	+	+	-	-	-	+	-	-	+	+	+
17	2-Nonanol	628-99-9	45	858	1106	1098	+	-	-	-	+	+	-	-	-	+	-	-	+	+	+
17	(E)-Ocimene	3779-61-1	93	874	1048	1051	+	+	-	+	+	+	+	+	-	-	-	-	+	+	+
17	β-Myrcene	123-35-3	93	885	991	991	+	+	-	-	+	+	+	+	-	+	-	-	+	+	+
17	2-Undecanone	112-12-9	58	799	1298	1295	-	-	-	-	+	+	+	+	-	+	-	-	+	+	+
17	Isoamyl octanoate	2035-99-6	127	751	1450	1450	+	-	-	-	+	+	+	+	-	+	-	-	-	+	-
17	Tetrahydronaphthalene	119-64-2	132	735	1171	1179	-	-	-	+	+	+	-	+	+	+	-	-	-	+	+
17	2-Heptanone	110-43-0	58	898	894	889	-	+	-	-	+	+	+	+	-	+	-	-	+	+	+
17	pH						+	-	-	+	+	+	+	-	-	+	+	-	+	+	+
18	2,4-Dichlorophenol	120-83-2	162	721	1193	1188	+	-	-	+	+	+	+	-	-	+	+	-	-	-	-
18	Hexyl octanoate	1117-55-1	127	815	1582	1584	-	-	-	+	+	+	+	-	-	+	+	+	-	-	-
18	Propyl octanoate	624-13-5	145	850	1294	1290	-	+	+	+	+	+	+	+	-	+	-	-	-	-	-
18	Isoamyl propanoate	105-68-0	57	889	971	969	+	+	-	+	-	+	+	+	-	+	-	-	+	-	-
18	Isopentyl decanoate	2306-91-4	70	841	1649	1647	+	+	+	+	+	+	-	+	-	-	-	-	+	-	-
18	Ethyl tridecanoate	28267-29-0	88	793	1695	1687	+	-	-	+	+	+	+	-	-	+	+	-	+	-	-
18	2-Ethylhexanoic acid	149-57-5	73	719	1130	1128	+	-	-	+	+	+	+	+	-	+	+	-	+	-	-
18	Isobutyl isobutyrate	97-85-8	71	748	916	906	+	+	-	-	+	+	-	-	-	+	-	-	-	-	-
18	α-Isophoron	78-59-1	82	788	1137	1118	+	+	-	+	+	+	-	+	+	+	-	-	-	-	-
18	6-Ethyl-o-cresol	1687-64-5	121	738	1236	1236	-	+	-	-	+	-	+	-	-	+	-	-	-	-	-
18	p-Cresol	106-44-5	107	779	1093	1077	-	+	-	-	+	-	-	-	+	+	+	+	-	+	-

Cluster	X-Variable	CAS	Unique Mass[¥]	MS Match	RI[¥] (calc)	RI[€] (lit)	RdBerryA	DrFruitA	SourT	FloralA	BellPepA	CanVegA	EarthyA	SmokyA	OakA	ButterA	MintA	EucalypA	AlcoholT	BitterT	AstrinT
18	(E)-α-Ionol	25312-34-9	138	665	1387	1376	+	-	-	-	+	+	-	+	+	+	+	+	-	+	+
19	Propyl isovalerate	557-00-6	85	718	952	949	-	+	+	-	+	+	+	+	-	-	-	+	+	-	-
19	Ethylbenzene	100-41-4	91	775	864	866	-	+	+	-	+	+	+	+	+	+	-	-	-	-	-
19	Dibromochloromethane	124-48-1	129	823	809	800	-	+	+	-	+	+	+	+	+	-	-	-	-	-	-
19	2-Isobutyl-3-methoxypyrazine (IBMP)*	24683-00-9	124	505	1183	1179	+	+	+	+	+	+	+	+	+	+	-	-	+	-	+
19	Isobutyl acetate	110-19-0	73	873	782	780	+	+	-	-	+	+	+	+	-	+	-	-	+	-	-
19	Isobutyl butanoate	539-90-2	71	800	958	955	+	+	-	-	+	+	+	+	-	-	-	-	+	-	-
19	Butanoic acid	107-92-6	60	698	802	789	-	+	+	-	+	+	+	+	-	-	-	+	+	-	-
19	Tridecane	629-50-5	71	873	1300	1300	-	-	+	+	+	+	+	+	-	-	-	+	+	-	-
20	2-Phenylethyl butyrate	103-52-6	104	854	1449	1439	-	-	+	-	-	+	+	+	-	-	-	+	+	+	+
20	Ethyl crotonate	10544-63-5	99	788	848	834	-	-	+	-	+	+	+	+	-	-	-	+	+	+	+
20	Citronellol acetate	150-84-5	81	881	1353	1352	-	-	+	-	+	+	+	+	-	-	+	-	+	+	+
20	Pentyl acetate	628-63-7	70	834	916	916	-	-	-	-	+	+	+	+	-	-	+	-	+	+	+
20	Hexyl acetate	142-92-7	84	858	1016	1007	-	-	-	-	+	+	+	+	-	-	+	+	+	+	+
20	Benzofuran	271-89-6	118	890	1003	1007	-	-	+	-	+	+	+	+	+	+	-	+	+	+	-
20	Ethylfurylketone	3194-15-8	95	830	1014	1008	-	-	-	-	+	+	+	+	+	+	-	+	+	+	+
20	3-Furaldehyde	498-60-2	95	838	823	815	-	+	+	-	+	+	+	+	+	+	-	+	+	+	+
20	Styrene	100-42-5	104	886	896	897	-	-	-	-	-	+	+	+	+	+	-	-	+	+	-
21	Isobutyl hexanoate	105-79-3	99	860	1152	1144	-	-	+	+	+	+	-	-	-	-	-	+	+	-	+
21	(S)-3-Ethyl-4-methylpentanol	0-00-0	84	877	1027	1020	-	-	+	+	-	-	-	-	-	-	+	+	+	-	-
21	γ-Pentalactone	108-29-2	85	813	965	954	-	-	+	+	-	-	-	-	-	-	+	+	+	+	+
21	Angelica lactone	591-12-8	98	780	874	885	-	-	+	+	-	-	+	-	-	-	+	+	+	+	+
21	2,5-Dimethylfuran	625-86-5	96	840	727	728	-	-	+	+	-	-	-	+	+	-	+	+	+	+	-
21	Isopentyl 2-methylbutanoate	27625-35-0	85	794	1102	1100	-	+	+	+	+	-	-	+	-	-	-	+	+	+	-
21	Dimethyl trisulfide	3658-80-8	126	659	976	982	+	-	+	+	+	-	-	+	+	-	-	-	+	-	-
22	Ethyl-3-hexanoate	2396-83-0	142	762	1008	1007	-	-	-	+	+	+	+	-	-	+	+	+	-	-	+
22	p-Cymen-7-ol	536-60-7	135	787	1306	1295	-	-	-	+	+	+	+	-	+	-	+	+	-	-	+
22	(Z)-3-Hexenyl acetate	3681-71-8	67	787	1006	1006	-	-	-	+	+	+	+	-	-	-	+	+	-	+	+

Cluster	X-Variable	CAS	Unique Mass[¥]	MS Match	RI[₦] (calc)	RI[€] (lit)	RdBerryA	DrFruitA	SourT	FloralA	BellPepA	CanVegA	EarthyA	SmokyA	OakA	ButterA	MintA	EucalypA	AlcoholT	BitterT	AstrinT
22	α-Ionene	475-03-6	159	744	1264	1256	-	-	+	+	-	+	+	+	+	-	+	+	+	-	-
22	(E)-Linalool oxide	14049-11-7	68	719	1183	1184	-	-	+	+	+	+	+	+	+	-	+	+	+	+	+
22	3,5-Dimethoxytoluene	4179-19-5	152	760	1275	1276	-	-	+	+	+	+	+	+	+	-	+	+	+	+	-
22	Dodecane	112-40-3	57	879	1200	1200	-	-	+	-	+	+	+	+	-	-	+	+	+	+	+
22	Carvacrol	499-75-2	135	717	1306	1304	-	-	+	+	+	+	+	+	+	-	+	+	+	+	+
22	Benzyl acetate	140-11-4	108	876	1171	1165	-	+	+	-	+	+	+	+	+	-	+	+	+	-	+
22	o-Cresol	95-48-7	108	680	1068	1077	-	-	-	+	+	+	+	+	+	-	+	+	+	+	+
23	Isopropyl laurate	10233-13-3	60	816	1627	1618	+	-	+	-	-	-	-	-	-	-	+	-	+	-	-
23	Ethyl 5-methylhexanoate	10236-10-9	88	788	1064	1072	-	-	-	-	-	-	-	-	-	-	+	+	+	-	-
23	2-Pentadecanone	2345-28-0	58	839	1700	1689	-	-	-	+	-	+	-	-	-	+	+	+	+	+	+
23	Ethyl hexadecanoate	628-97-7	88	879	1994	1994	+	-	-	-	+	+	+	-	-	+	+	+	+	-	-
23	Isoamyl laurate	6309-51-9	70	858	1847	1847	-	-	-	+	+	+	-	-	-	-	+	+	+	-	-
23	Propyl decanoate	30673-60-0	61	799	1491	1489	-	-	-	+	+	+	-	+	-	-	+	-	-	+	+
23	Methyl decanoate	110-42-9	74	914	1326	1323	-	-	-	+	+	+	-	+	-	-	+	+	+	+	+
23	Isobutyl decanoate	30673-38-2	155	872	1546	1545	+	-	-	-	+	+	-	+	-	-	+	-	+	+	-
23	Isobutyl octanoate	5461-06-3	127	869	1350	1348	-	-	-	-	+	+	+	+	-	+	-	+	+	+	-
23	Ethyl 4-ethoxybenzoate	23676-09-7	121	774	1535	1522	-	-	+	-	+	-	-	+	+	-	-	+	-	+	-
24	β-Bisabolene	495-61-4	204	844	1513	1509	+	+	+	+	-	-	-	-	-	+	+	+	-	+	+
24	β-Farnesene	18794-84-8	93	901	1455	1455	+	+	+	+	+	-	-	-	-	+	-	+	+	+	+
24	2-Hydroxycineol	18679-48-6	108	789	1240	1227	-	+	-	+	+	-	-	-	+	-	+	+	+	+	+
24	Nerolidol	7212-44-4	93	853	1567	1566	-	+	-	+	-	-	-	-	+	-	+	+	+	+	+
24	6-Methyl-3,5-heptadiene-2-one	1604-28-0	109	805	1111	1107	-	-	-	+	-	-	-	+	-	-	+	+	+	+	+
24	2-Methyl-2-butenolide	22122-36-7	98	803	986	989	-	-	-	+	-	-	-	-	+	+	+	+	+	+	+
24	Dihydroactinidiolide	17092-92-1	111	843	1550	1548	-	-	-	+	-	-	-	-	-	-	+	-	+	+	+
24	Isopentyl isovalerate	659-70-1	85	811	1108	1105	-	+	-	+	-	-	-	+	-	+	-	+	+	+	+
24	2,2,6-Trimethylcyclohexanone	2408-37-9	82	908	1043	1035	-	-	-	-	-	-	-	-	-	+	+	+	+	+	+
24	Iron Reactive Phenolics						+	-	-	+	-	-	-	-	-	-	+	+	+	+	+
24	Protein-Precipitable Tannin						-	+	-	+	-	-	-	-	-	+	+	+	+	+	+
24	Large Polymeric Pigments (LPP)						-	+	-	-	-	-	-	-	+	+	+	+	+	+	+

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24	Total Polymeric Pigments						-	+	-	-	-	-	-	-	+	+	+	+	+	+	+
25	Ethyl cinnamate	103-36-6	131	854	1480	1480	+	-	+	+	-	-	-	-	-	-	+	+	+	+	+
25	Phenethyl octanoate	5457-70-5	104	832	1857	1846	-	-	+	+	-	-	-	-	-	-	+	+	+	+	+
25	Ethyl 3-hydroxydodecanoate	126679-28-5	117	818	1743	1743	-	-	+	+	-	-	-	-	-	-	+	+	+	+	+
25	Phenylacetaldehyde	122-78-1	120	873	1055	1050	+	-	+	+	-	-	-	+	-	-	+	+	+	+	+
25	2-Ethyl-5-methylfuran	1703-52-2	95	795	800	802	-	-	-	+	-	+	+	+	+	-	+	+	+	+	+
25	p-Menth-1-en-9-al	29548-14-9	94	803	1229	1217	-	+	+	-	-	-	-	+	+	-	-	+	+	+	+
25	Ethyl furoate	614-99-3	95	876	1059	1056	-	-	+	-	-	-	-	+	+	-	+	+	+	+	+
25	Mesitylene	108-67-8	105	879	999	996	+	+	+	+	-	-	-	+	+	-	+	+	+	-	+
25	Hemimellitene	526-73-8	105	912	1027	1033	+	-	+	-	-	-	-	-	+	-	+	+	+	-	+
25	Phenethyl formate	104-62-1	104	865	1185	1178	-	-	+	-	-	-	+	-	+	-	+	+	+	+	+
25	Theaspirane B	0-00-0	138	833	1326	1319	-	+	+	-	-	-	+	+	+	-	+	+	+	-	+
25	Theaspirane A	0-00-0	138	843	1310	1301	+	+	+	-	-	+	+	+	+	-	+	+	+	-	+
25	1-Pentadecene	13360-61-7	83	850	1493	1493	+	-	+	+	+	-	+	-	+	-	+	+	+	+	+
25	D-Carvone	2244-16-8	82	754	1255	1254	+	-	+	+	-	-	-	-	+	+	+	-	-	+	+
25	Methyl salicylate	119-36-8	120	896	1203	1201	-	-	-	+	-	-	-	+	+	-	+	+	+	+	+
25	Ethyl methylthiopropanoate	13327-56-5	148	889	1105	1098	-	-	-	-	-	-	-	+	+	+	-	+	+	+	+
25	Limonene	5989-27-5	68	865	1033	1031	-	-	+	+	-	+	+	+	+	-	+	+	+	+	+
26	2-Carene	554-61-0	121	778	1000	1001	+	+	+	+	+	+	-	-	-	-	-	+	+	+	+
26	α-Curcumene	644-30-4	132	784	1489	1485	+	+	+	+	+	+	-	+	-	-	-	-	+	+	+
26	6-Methyl-5-hepten-2-ol	1569-60-4	95	727	998	993	+	+	-	+	+	+	-	-	-	-	+	-	+	+	+
26	β-Sesquiphellandrene	20307-83-9	93	696	1534	1526	+	+	+	+	+	+	+	-	-	-	-	-	+	+	+
26	α-Farnesene	502-61-4	189	805	1508	1511	+	+	+	+	+	+	-	-	-	-	-	-	+	+	+
26	α-Bisabolol	515-69-5	119	886	1698	1688	-	+	+	+	-	-	+	+	+	-	-	-	+	+	+
26	4,7-Dimethylbenzofuran	28715-26-6	145	804	1218	1220	-	+	-	+	+	-	+	+	-	-	+	+	+	+	+
26	Linalool	78-70-6	93	864	1104	1106	-	+	+	+	-	+	+	+	+	-	-	-	+	+	+
26	Ethyl salicylate	118-61-6	120	881	1279	1267	-	+	+	+	-	+	+	+	-	-	-	+	+	+	+
26	Ethyl 2-methylbutyrate	7452-79-1	102	898	851	848	+	+	+	-	-	-	-	-	+	+	-	+	+	-	+
26	Octen-3-ol	3391-86-4	57	844	985	986	+	+	+	-	-	-	-	-	-	+	-	-	+	-	+

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26	Heptanal	111-71-7	86	897	906	900	+	+	+	+	-	-	-	-	+	+	-	-	+	+	+
26	1,3-Octadiene	1002-33-1	110	912	825	827	+	+	+	-	-	-	-	-	-	-	-	-	+	+	+
26	2-Ethylfuran	3208-16-0	81	893	723	720	+	+	+	-	-	-	+	-	+	+	-	-	+	+	+
26	Safranal	116-26-7	150	794	1214	1196	+	+	-	-	-	-	-	+	+	+	-	-	-	+	+
26	Small Polymeric Pigments (SPP)						-	+	-	-	-	-	-	-	+	+	+	-	+	+	+
26	2-Amylfuran	3777-69-3	81	896	993	993	+	+	+	+	-	-	+	-	-	-	-	-	+	+	+
26	2-Butylfuran	4466-24-4	81	820	892	894	+	+	+	-	-	-	+	+	-	+	-	-	+	+	+
26	1-Heptanol	111-70-6	56	865	975	970	+	+	+	-	-	-	+	+	-	-	-	-	+	+	+
26	Ethyl nonanoate	123-29-5	88	855	1298	1295	+	+	+	+	+	+	-	-	-	-	+	+	+	+	+
26	Methyl geranate	2349-14-6	114	859	1326	1326	-	+	+	+	-	+	-	-	-	+	+	+	+	+	+
26	Ethanol						+	+	+	-	-	+	+	+	-	+	+	+	+	+	+
27	Hydroxy citronellol	107-74-4	59	754	1375	1359	-	-	-	-	-	-	-	-	-	-	+	+	-	+	+
27	2-Ethyl-p-Xylene	1758-88-9	119	699	1083	1077	-	-	-	-	-	-	-	-	-	+	+	+	+	+	+
27	α-Panasinsen	56633-28-4	161	775	1534	1518	-	-	-	-	-	-	-	+	+	+	+	+	-	+	+
27	Eucalyptol	470-82-6	81	890	1039	1033	-	-	-	+	+	-	-	-	-	+	+	+	-	+	+
28	Benzyl Alcohol	100-51-6	108	838	1047	1041	-	-	-	+	-	-	-	-	+	+	+	+	-	+	-
28	2-Formylpyrrole	1003-29-8	95	764	1030	1030	-	-	-	+	-	-	-	+	+	+	+	+	-	+	-
28	Ethyl octanoate	106-32-1	101	770	1203	1198	-	-	-	-	-	-	-	+	+	+	-	+	+	+	-
28	2-Acetylfuran	1192-62-7	95	866	918	914	-	-	-	-	-	+	+	+	+	+	+	+	+	+	+
28	1-Methyl-2-formylpyrrole	1192-58-1	109	804	1015	1010	-	-	-	-	+	+	+	+	+	+	+	+	+	+	-
28	Methyl octanoate	111-11-5	74	875	1126	1129	-	-	-	-	-	-	+	+	+	+	+	+	+	+	+
28	Geraniol	106-24-1	69	803	1259	1255	-	-	-	+	-	-	-	+	-	+	+	+	+	+	+
28	Methionol	505-10-2	106	849	989	982	-	-	-	+	-	+	-	+	-	-	+	+	+	+	-
28	2-Methylthiolan-3-one	13679-85-1	116	849	1001	994	-	-	-	-	+	-	+	+	+	+	+	+	-	+	-
28	m-Dichlorobenzene	541-73-1	146	715	1014	1022	-	-	-	-	+	+	+	+	+	+	+	+	-	+	-
29	α-Thiophenecarboxaldehyde	98-03-3	111	889	1013	1010	-	-	+	-	-	+	+	+	+	-	+	-	+	+	+
29	Anthocyanins						-	-	-	-	-	+	+	+	+	+	+	+	+	+	+
29	2-Ethylthiophene	872-55-9	97	710	868	871	-	-	-	-	-	+	+	+	+	+	-	-	+	+	+
30	p-Dichlorobenzene	106-46-7	146	962	1024	1015	-	-	-	-	+	+	-	+	+	-	+	+	+	+	+

Cluster	X-Variable	CAS	Unique Mass[¥]	MS Match	RI[₦] (calc)	RI[€] (lit)	RdBerryA	DrFruitA	SourT	FloralA	BellPepA	CanVegA	EarthyA	SmokyA	OakA	ButterA	MintA	EucalypA	AlcoholT	BitterT	AstrinT
30	γ-Terpinene	99-85-4	93	784	1062	1062	-	-	-	-	+	-	-	+	+	-	+	+	-	+	+
30	m-Xylene	108-38-3	91	914	872	874	-	-	+	-	-	-	+	+	+	-	+	+	+	-	+
30	2-Furanmethanol	98-00-0	98	796	862	866	-	-	-	-	+	+	+	+	-	-	+	+	-	+	+
30	Methyl furoate	611-13-2	95	888	981	985	-	-	-	+	-	+	+	+	+	+	+	+	+	+	+
30	Acetophenone	98-86-2	105	923	1076	1076	-	-	-	-	-	-	+	+	+	+	+	-	+	+	+
30	o-Ethyltoluene	611-14-3	105	881	983	988	-	-	-	-	-	-	-	+	+	+	+	+	+	-	+
30	Propylbenzene	103-65-1	91	874	956	957	-	+	+	-	-	-	+	+	+	+	+	+	+	+	+
30	Indane	496-11-7	117	787	1041	1048	-	-	-	-	-	-	-	+	+	+	+	+	+	-	+
30	o-Xylene	95-47-6	91	855	896	894	-	-	-	-	-	+	+	+	+	+	+	+	+	-	+
30	p-Cymene	99-87-6	119	841	1029	1026	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+
30	4-Ethylguaiacol	2785-89-9	137	890	1284	1282	-	+	+	-	-	-	-	+	+	-	-	-	+	+	+
30	4-Ethyl phenol	123-07-9	107	681	1183	1178	-	+	+	-	-	-	-	+	+	-	-	-	+	+	+
30	Perilla alcohol	536-59-4	68	727	1303	1295	-	+	-	-	-	-	+	+	+	-	-	+	-	+	+
30	1-Dodecanol	112-53-8	97	707	1479	1483	-	+	-	-	+	-	+	+	+	+	+	+	-	+	+

[¥] Unique ion (*m/z*): used for peak area determination, identified as the unique ion by ChromaTOF data analysis. * Previously confirmed using a wine spiked with IBMP. [₦] RI: retention indices calculated from C8-C20 n-alkanes. [€] RI: retention indices reported in the literature for 5% phenyl polysilphenylene-siloxane capillary GC columns or equivalents (Adams, 2007, Stein, 2009). NOTE: RI (calc) values below 800 are extrapolated using ChromaTOF Software.

The butter aroma was positively correlated with variables from clusters 10, 13, 15, and 16 and negatively correlated with variables in clusters 1, 3, and 4. The oak aroma was positively correlated with variables from clusters 13, 15, and 29 and negatively correlated with variables from cluster 16. PLS1 models for red berry and dried fruit aroma attributes were both positively correlated with variables in cluster 7 including glucose and fructose and negatively correlated with variables in clusters 27, 28, and 29 including anthocyanins (cluster 29). Variables in clusters 2, 4, and 16 were also positively correlated with red berry while variables in clusters 20 and 22 were negatively correlated. The PLS1 model of floral aroma was positively correlated with variables in clusters 2, 3, 4, and 21 and negatively correlated with variables in clusters 11 and 29. It was observed that eucalyptol, titratable acidity, ethanol, glucose and fructose, pH, IBMP, benzenepropyl acetate, hydroxy citronellol, and protein-precipitable tannin were important X-variables while clusters 7, 16, 20, 27, and 29 were important variable clusters for differentiating sensory attributes. PCA of the X-variable correlation coefficient cluster means accounted for 81% of the cumulative variance in the first four principal components (Figure 6.4). The first principal component accounted for 32% of the variance and separated the eucalypt, floral, mint, red berry and sour sensory attributes from the bitter, butter, canned vegetable, earthy, and smoky sensory attributes. The second principal component accounted for 24% of the variance and separated the dried fruit and red berry aroma attributes from the astringent, bitter, eucalypt and mint aroma attributes. The third and fourth principal components accounted for 15% and 11% of the variance, respectively, with the third principal component primarily separating the bell pepper aroma and the fourth principal component differentiates the sour taste attribute from the red berry aroma attribute.

6.4. Discussion

6.4.1. Cabernet sensory attributes

It has been reported that consumers consider taste to be the most important influence on their food choices, followed by cost (Glanz et al., 1998). In turn, this concept can be applied to wine preferences whereby some consumers seek wines that meet particular sensory expectations (Lattey et al., 2010). One objective of this study was to better understand the diversity in sensory characteristics of Cabernet Sauvignon wines from premium wine growing regions in Australia. The study utilised commercially produced wines to better represent the diversity that would be exhibited in commercial products and thus potentially available to the wine consumer. Descriptive sensory analysis demonstrated that, of the 20 sensory attributes assessed, 15 of these were statistically different among the wines. The LC_697, FR_762, MB_457, and MR_175 were different from 28, 27, 26, and 25 of the 30 wines, respectively, due to one or more sensory attributes. This indicated that they were the most different of the wines studied. In contrast, the CV_081 and PA_677 wines were more similar to the other wines in that they were differentiated from only 6 and 8 wines, respectively, due to one or more sensory attributes.

The product set was clearly diverse and suggests that Australian Cabernet Sauvignon wines can have a range of sensory characteristics as there was a significant separation among the wines based on the sensory attributes assessed. Those that were found to have greater scores for floral / dried fruit / red berry sensory attributes were differentiated from those that were more canned vegetable / earthy / smoky in character, and those that were oak-driven from those with more bell pepper / canned vegetable in character (Figure 6.1). For instance the FR_762, FR_855, and MR_871 products were more earthy / vegetal and less fruity than the other wine products, while the WR_462

was found to have higher oak characteristics than the other wines and displayed less bell pepper / canned vegetable character compared to wine CV_553 (Table 6.4). A similar study using descriptive analysis of commercial Cabernet-Sauvignon wines from California found that the wines were distinguished by the contrast between the vegetative and fruity characteristics of the wines (Heymann and Noble, 1987). A number of subsequent studies have observed that the descriptive profile of Cabernet Sauvignon wines shows a dichotomy between the fruity and herbaceous attributes (Chapman et al., 2004a, Chapman et al., 2005, Falcão et al., 2007, Preston et al., 2008). This concept has also been explored in New Zealand Sauvignon Blanc where the results of a sorting task by professionals supported the concept that 'green' and 'ripe' sensory characteristics were mutually exclusive (Parr et al., 2007).

The current study also observed a clear delineation between the wines that were fruity (dried fruit / red berry) from those that were strong in eucalypt and mint sensory attributes, which primarily defined the LC_697 product (Figure 6.1). It was interesting to note that the eucalypt and mint aroma attributes were not distinguished from one another in the first three principal components. This suggests that either these attributes were commonly associated with the LC_697 product or that the terms were essentially interchangeable for the panellists. However, the panellists were perfectly capable of correctly distinguishing the eucalypt and mint sensory standards under randomised and blind conditions prior to each sensory session implying that, in this study, the eucalypt and mint attributes were common to the LC_697 product. A recent study found that eucalyptus sensory characteristics were positively correlated with bell pepper and pepper (black and white pepper) attributes (Preston et al., 2008), whilst another study found that a mint / menthol sensory attribute was positively correlated with a fresh green (green bean, lantana leaf, green capsicum) sensory attribute (Lattey et al., 2010).

This was not the case in the current study where the eucalypt and mint aroma attributes were not correlated with the bell pepper and canned vegetable sensory attributes, which are the most appropriate proxies for the terms used in the previous studies (Preston et al., 2008, Lattey et al., 2010).

The butter and oak sensory attributes, which were statistically higher in the WR_462 product, were also positively correlated in all four principal components indicating that the WR_462 product may have experienced different oak storage compared to the other wine products. The oak attribute was higher in the WR_462 product compared to four other products (Table 6.4) indicating that, although significantly higher, it was not a major attribute differentiating the wine products. This may be because producers were requested to draw wines from one or two year old barrels to reduce overt oak influence on the aroma characteristics of the wines.

With respect to the taste attributes, astringency was the most varied sensory attribute among all of the products assessed. In turn alcohol varied more than sour, which varied more than bitterness which only varied slightly among the products. It was interesting to note that the astringent sensory taste attribute was important in describing both the second and third principal components (Figure 6.1). Astringency is generally viewed as a negative attribute by consumers in food and beverage products (Lesschaeve and Noble, 2005, Lee and Lee, 2008, Lee and Chambers, 2010). However, some studies have identified consumer segments, including winemakers, which prefer wines that are higher in astringency (Varela and Gámbaro, 2006, Lattey et al., 2010). Astringency was negatively correlated with the bell pepper and canned vegetable sensory attributes and was positively correlated with the dried fruit and eucalypt / mint sensory attributes in the second and third principal components, respectively (Figure 6.1). The BV_802, LC_468, and LC_697 wines were all characterised by higher astringency values while

CV_553 and MB_457 were characterised by lower astringency values in the second and third principal components, respectively.

The smoky aroma attribute was highest in the FR_762 wine product which was also high in alcohol, bitterness, and low in red berry and floral sensory attributes (Table 6.4). It could be inferred from the results of a consumer study (Lattey et al., 2010) that FR_762 would be less preferred by consumers when compared to the other wines within the current study. However, this assumes that the intensity of the attributes within the current study were comparable to the products within the previous consumer sensory study.

6.4.2. Relationship between sensory attributes and composition

The results of the current study identified that the sensory attributes of products was varied but that the products were not easily differentiated into clear groups using traditional multivariate techniques such as PCA. Consequently the sensory attributes were modelled individually with PLS regression using the compositional analysis to explain each sensory attribute. The results of the analysis indicated that there were a number of components that were important to the modelling of each attribute. However, some compositional attributes were unique in explaining one sensory attribute as opposed to multiple sensory attributes (Figure 6.2).

Some obvious relationships were clearly observed particularly for the modelling of the alcohol, astringent, and sour taste attributes where ethanol, protein-precipitable tannins, and pH were important predictors, respectively (Table 6.5). The bitterness PLS model was well described by protein precipitable tannin and glucose / fructose which were positively and negatively correlated respectively. It is well understood that bitterness is a taste perception stimulated by flavanoid phenols in red wines (Noble, 1994) which can be enhanced by increasing ethanol concentrations (Fischer and Noble, 1994).

Literature suggests that sweetness generally suppresses bitterness in wine (Noble, 1994). However, a review of taste interactions suggests that sugars at low concentration, which was the case in the current study, have a varied influence on bitterness depending on the compounds involved (Keast and Breslin, 2003). It has also recently been published that phenolic acid ethyl esters might also contribute to the bitterness of red wines (Hufnagel and Hofmann, 2008). Taste interactions in wine warrants further study given the presence of numerous taste stimuli which may have additive and masking effects when part of a complex mixture (Stevens, 1997). It is possible that the bitter PLS model was harder to interpret as a number of these components were not measured or because there were fewer differences among the products with respect to bitterness when compared to the alcohol, astringent and sour taste attributes.

The eucalypt and mint aroma attributes are explained by very similar PLS models due to the LC_697 product being highest in these attributes (Figure 6.3). Both models indicated that eucalyptol and hydroxy citronellol were important X-variables that were positively correlated with the eucalypt and mint aroma attributes. The origin of eucalyptol in wine is still a topic of discussion, with research indicating that it is transmitted from closely located *Eucalyptus* trees (Van Leeuwen et al., 2007). It is reported that it may be a product of acid catalysed transformation of limonene or α-terpineol under elevated temperatures in wine (Farina et al., 2005), or that it may be produced *de novo* in the grape during development (Kalua and Boss, 2009). More recently these different influences have been assessed and it has been concluded that the presence of eucalyptol in young Australian red wine can be primarily attributed to airborne transmission of eucalyptol to grapevines (Capone et al., 2010a). Eucalyptol has an odour described as fresh, camphoraceous and cool (Farina et al., 2005) and in a

survey of 146 commercially available Australian red wines, 40% contained eucalyptol above the reported aroma detection threshold (Van Leeuwen et al., 2007). The highest concentration of eucalyptol in this survey was 19.6 ppb in a Shiraz wine (Van Leeuwen et al., 2007) being lower than a recently reported consumer rejection threshold of 27.5 ppb (Saliba et al., 2009).

The authors are unaware of any previous research that has attempted to investigate which volatile components are related to mint aroma in red wines. Hydroxy citronellol was first identified in grapes and wines by Rapp and co-workers (Rapp et al., 1983) and was identified as a marker for mint aroma in the current study. The compound has been previously described as mild, clean, floral, lily, green and peony (Luebke, 2010). Additional research is required to clarify the contribution of monoterpenes to mint aroma in red wines especially given the lack of previous research in this area. It could be suggested that the presence of eucalypt and mint contributes to the unique aroma of some Cabernet Sauvignon wines. A recent study assessing the consumer rejection threshold of eucalyptol noted that a moderate intensity of eucalyptus character in red wines should not be considered a taint and that moderate intensities may be preferable to no eucalyptus character (Saliba et al., 2009). Further research with larger consumer groups is necessary to confirm consumer preference for wines that exhibit mint and eucalypt characteristics.

The bell pepper sensory attribute was positively correlated with a number of volatile components including the commonly studied IBMP (Table 6.5) (Roujou de Boubée et al., 2000) which is produced in the fruit of Cabernet Sauvignon (Dunlevy et al., 2010, Koch et al., 2010) and is known to have a ‘green-pepper’ sensory characteristic (El-Sayed, 2010). However, it is of equal interest that volatile compounds including δ-octalactone, vitispirane, γ-decalactone, and γ-octalactone, which have ‘sweet’, ‘floral /

fruity', 'dried fruit / peach', and 'coconut / caramel' sensory characteristics, respectively (Eggers et al., 2006, El-Sayed, 2010), were negatively correlated with bell pepper aroma. The gamma-lactones were also negatively correlated with the canned vegetable, earthy, and smoky sensory attributes suggesting that they were deficient in the wine products that are high in these sensory attributes.

Recent research has suggested that the dichotomy between fruity and herbaceous attributes might be attributed to masking and enhancing effects of volatiles such as IBMP and β-damascenone (Escudero et al., 2007, Pineau et al., 2007, Hein et al., 2009). The current study suggests that there are a number of other compounds, including norisoprenoids and delta- and gamma-lactones, which exhibit sweet / fruity notes, and are negatively correlated with vegetal and herbaceous sensory attributes.

It was noted that compounds in clusters 13 and 15, which were positively correlated with the oak and butter sensory attributes (Figure 6.4), included a number of benzene and furan derivatives (Table 6.5). Interestingly, neither the cis- nor the trans-oak lactones were positively or negatively correlated with the oak PLS models, suggesting they were not important in distinguishing this sensory attribute. However, a number of compounds that were important to the butter and oak PLS models have been discussed in previous studies of oak treated wines (Spillman et al., 2004a, 2004b, Prida and Chatonnet, 2010) including anisole (sweet / fragrant), guaiacol (smoky / phenolic), furfural (woody / almond / sweet), and 5-methylfurfural (caramel / burnt sugar) (El-Sayed, 2010). The significance of benzene and furan derivatives compared to the oak lactones may be a function of two characteristics of oak. Firstly, products used in the current study were specifically requested not to be drawn from new oak barrels and were sampled within six months of fermentation. Therefore, as the successive use of oak barrels progressively depletes the volatile compounds in the oak (Gómez-Plaza et

al., 2004) and lactones are found at higher concentrations away from the toasted surface of staves (Hale et al., 1999) it can be suggested that the oak lactones were not extracted at appreciable levels to influence the oak or butter sensory attributes. Secondly, the initial oak toasting level can significantly increase the concentration of furans and benzene derivatives, reducing or having little impact on the concentration of oak lactones which are influenced more so by the oak species and seasoning (Cadahía et al., 2003, Fernández de Simón et al., 2010a). Therefore the positive correlation of these compounds to the oak and butter sensory attributes may be a function of the initial toasting level applied to the oak. A recent study noted that furanic compounds, including furfural and 5-methylfurfural, were positively correlated with the overall oak intensity irrespective of their low odour activity values (Prida and Chatonnet, 2010) suggesting that their sensory contribution may be synergistic or additive. These observations further support the notion that volatile compounds interact to either enhance or mask particular aroma characteristics in wines (Atanasova et al., 2005b, Escudero et al., 2007, Pineau et al., 2007, Pineau et al., 2009).

The current study found that the red berry and dried fruit aromas were defined by similar variables being positively correlated with some ethyl and acetate esters along with glucose and fructose and negatively correlated with anthocyanins (Table 6.5). This was an unexpected observation as it is generally thought that red wine descriptors and colour are positively correlated in red wines (Morrot et al., 2001). This observation could also be attributed to other compositional components that were not measured because they were below the limits of detection of the methodology employed. Black berry aroma was not significantly different among the wines assessed indicating that the variables that were correlated with red berry and dried fruit aroma may not contribute to the perception of black berry aroma. The origin of ‘red’ and ‘black-berry’ aromas in

Bordeaux wines has been discussed in a recent study that categorised the role of ethyl propanoate, ethyl 2-methylpropanoate, and ethyl 2-methylbutanoate in the perception of black-berry aromas and ethyl butanoate, ethyl hexanoate, ethyl octanoate, and ethyl 3-hydroxybutanoate in the perception of red-berry aromas (Pineau et al., 2009). It was observed in the current study that ethyl isobutyrate (ethyl 2-methylpropanoate) and ethyl 2-hydroxyisovalerate were positively correlated, along with benzene propylacetate (sweet / balsam / storax / spicy / cinnamon) (Luebke, 2010), with red berry aroma while hexyl acetate and ethyl octanoate were negatively correlated (Table 6.5). These results neither refute nor support the results of Pineau and co-workers (2009) although it does suggest that esters are important to the fruity characteristics of wines. It is of interest that most of these ethyl esters and alkyl acetates can also be found at similar or higher concentrations in white wines when compared to red wines (Guth, 1997b, Ferreira et al., 2000, Francis and Newton, 2005) suggesting that other intrinsic factors, such as the non-volatile wine matrix, play a role in releasing volatiles and defining the perception of these aromas (Pineau et al., 2007, Robinson et al., 2009). This phenomenon has recently been investigated by Sáenz-Navajas and co-workers (2010) who assessed the perception of various reconstituted red and white wine samples suggesting that the non-volatile matrix exerts a powerful influence on the aroma perception of wine of such a magnitude comparable to that of the volatile composition (Sáenz-Navajas et al., 2010). This may partially explain why the red berry and dried fruit PLS models did not positively correlate many volatile variables with fruity aromas.

The floral sensory term was used to describe the violet / rose like character that is observed in red wines as opposed to the linalool / orange blossom character that is characterised in white wines (Noble et al., 1987). There has been some work conducted on the bergamot (*Citrus bergamia*) aroma of Touriga Nacional wines which suggests

that monoterpene content, particularly linalool and linalyl acetate, was positively correlated to the floral character of the wines (De Pinho et al., 2007). Previous research into Bordeaux wines has identified that β-ionone is found at concentrations above its odour threshold and thus may be important to the violet aroma of red wines (Kotseridis et al., 1999a). The current study observed that neither linalool nor β-ionone were important in the floral PLS model suggesting that the floral characteristic of Cabernet Sauvignon is not defined by either of these compounds (Table 6.5). However, dihydro-α-ionone, which has a warm / woody / earthy / herbal / orris / violet / raspberry odour description (Luebke, 2010), was positively correlated along with a number of sesquiterpenes including α and β-calcorene which the authors could not find aroma descriptions for. This work has not definitively shown that the floral character of Cabernet Sauvignon is caused by any one compound. However, it is highly likely that monoterpenes, norisoprenoids, and sesquiterpenes play an important role in defining this aroma characteristic in red wines and further investigation is warranted.

6.5. Conclusions

This work has characterised the sensory and compositional variation associated with a number of commercially produced Cabernet Sauvignon wines from premium wine producing regions within Australia using chemometric techniques. While these observations are initially limited to the number of samples used from a single vintage, they provide a list of compounds that should be further investigated to confirm their influence on wine sensory attributes and utility as markers for wine characteristics. However, for some attributes, future research is warranted as it is clear that there are nuances that would be better defined with the analysis of additional wines that share these characteristics. There was a clear differentiation between wines that showed fruity characteristics and herbaceous / vegetal characteristics which is an important

differentiation between styles of Cabernet Sauvignon. It is obvious from previous research that these differences appeal to different market segments and an awareness linking wine composition, sensory characteristics, and consumer preferences is important in producing products for specific markets. Compositional results support previous studies that relate eucalyptol to eucalypt and mint aroma attributes while furan and benzene derivatives are positively correlated with the aroma perception of oak. Current literature and the results of this study further demonstrate that the sensory perception of wine is a complex process and involves the interaction of both volatile and non-volatile components. Future research should consider employment of more holistic techniques to develop a greater understanding of the complex interactions between mixtures of volatiles and non-volatiles.

7. Summary

Wine is a complex, multi-faceted, and ever changing system composed predominantly of non-volatile components including ethanol, organic acids, amino acids, sugars, phenolic compounds, proteins, and inorganic ions in water. Although these components contribute directly to the taste of wine it is the human sensory perception of the volatile and semi-volatile compounds in combination with these non-volatile components that continue to captivate consumers resulting in a myriad of products produced globally. Understanding the source of wine volatile compounds and the mechanisms that influence their formation through grape growing, winemaking and storage is essential for wine businesses when developing strategies to produce wines with specific sensory attributes that appeal to target markets. The objective of this research was to develop a greater understanding of the environmental influences that drive flavour formation in grapes and translate this information into awareness of the limitations of site and region in producing wines to specification.

It is important initially to clarify the complex relationship of aroma release from the wine matrix. Therefore a study was undertaken to determine the influence that major grape and wine matrix components have on the partitioning of volatile compounds into the headspace of model solutions and begin to relate the major influences to real wine systems. In summary, the results of this work showed that the presence of glucose increased the concentration of volatiles in the headspace, whereas increasing ethanol concentration was negatively correlated with headspace partitioning of volatiles. The magnitude of each matrix-volatile interaction was ethanol > glucose > glycerol > catechin, whereas proline showed no apparent interaction.

An important observation from these experiments was that increasing ethanol concentrations significantly reduced the headspace concentration of volatile aroma

compounds, which may explain recent sensory research observations that indicate ethanol can suppress the fruit aroma attributes in wine. These findings also have ramifications for the chemical analysis and sensory assessment of wine samples with varied ethanol levels. The results described in this thesis should serve as a primer for further studies into how the solution matrix changes the aroma perception of complex mixtures.

In many instances, it is impractical to concurrently conduct the descriptive sensory analysis of a set of wine samples and undertake detailed chemical analyses. It is thus important to ascertain how storage conditions might change the composition and consequently the sensory characteristics of wine over the course of a study. This is further complicated when wine may need to be shipped between sites for analysis as was the case in the current study. Consequently, a study was designed to assess the sensory repercussions of adverse temperature conditions during the storage / transport of white and red wines. In these experiments, the wines were exposed to simulated shipping conditions and then evaluated using sensory descriptive analysis and HS-SPME GC-MS. Four white wines and four red wines were exposed to four different storage conditions to create 32 treatments. Storage conditions included 20°C, 40°C, 20/40°C (reflecting diurnal cycle in temperatures), and a sample that travelled in the trunk of a car for three weeks.

The higher temperature treatment produced the most significant differences among the white wines by increasing diesel, oxidized, and rubber aromas and decreasing citrus, floral, and tropical fruit aromas. The magnitude of the effect was significant, although less pronounced, in the red wines, with increased dried fruit and canned vegetable aromas. Differences were noted for a number of compounds in the headspace of the treated wines compared to controls including higher levels of vitispirane 1&2, TDN, p-

Cymene and reductions in several esters and acetates. These experiments confirmed that the conditions of transport and storage of the experimental wines produced for other parts of this project were unlikely to compromise the integrity of the samples. Furthermore, this work has identified potential chemical markers of wines that have experienced high temperatures during transport and storage, and has determined the sensory consequences of these adverse conditions on red and white wines.

A major goal of wine flavour and aroma research is to be able to predict sensory attributes from the chemical composition of a wine. An understanding of how differences in the chemical composition of a wine will influence its sensory attributes requires improved analytical methods which allow the monitoring of a large number of volatiles, including those present at low concentrations, in a single analysis. To these ends, a novel analytical method was developed using headspace solid-phase microextraction (HS-SPME) for analysis of wine volatiles by comprehensive two-dimensional gas chromatography (GC×GC) time-of-flight mass spectrometry (TOFMS). This represents an important advancement in wine volatile analysis as the method allows for the simultaneous analysis of a significantly larger number of compounds found in the wine headspace compared to other current single dimensional GC-MS methodologies.

This is the first documented application of GC×GC-TOFMS for non-targeted wine volatile profiling and the first to clearly show that the use of elevated temperatures during the incubation step of HS-SPME analysis of wine does generate artefacts. A considerable list of compounds that have been observed in Cabernet Sauvignon wines from Australia has been included in the publication for future reference. This list is unambiguous and will aid research groups in identifying new and novel compounds which may play a role in wine aroma. It is not intended that this HS-SPME GC×GC-

TOFMS method be used for high throughput or routine analysis of wine volatiles due to the high costs currently associated with the instrumentation. However, as research in this field becomes progressively more multidisciplinary, metabolomic studies will require analytical methodologies that provide accurate and comprehensive detail about the volatile composition of wine in an effort to better understand flavour and aroma formation in the grape and during fermentation. This non-targeted GC×GC-TOFMS method represents a step change in the volatile analysis of wines and this is due not only to the two-dimensional nature of the method but also to the extended run time which maximises sensitivity and chromatographic separation to yield quality data.

The HS-SPME GC×GC-TOFMS methodology was applied in conjunction with descriptive sensory analysis to Cabernet Sauvignon wines from Western Australia produced from field trials exploring the influence of yeast strain, canopy management, and vineyard site on wine volatile composition and sensory characteristics. The compounds quantified included potent aroma compound classes such as monoterpenes, norisoprenoids, sesquiterpenes, and alkyl-methoxypyrazines, which have been documented to contribute to wine aroma. The wines produced in this study had significantly different sensory attributes and chemical compositions. An important observation was that, in many cases, multiple factors influenced the abundance of certain wine volatile compounds.

Site had the most significant influence on sensory scores and wine composition, followed by canopy management. Unexpectedly, yeast strain had no significant sensory effect despite the fact that a number of volatile compounds were significantly different in the wines made from different strains. It should be noted that the conclusions made from these observations are limited to the scope of the current study. However, it does suggest that the composition of grapes has an important role to play in determining the

sensory characteristics and chemical composition of wines, and in this experiment, more influence than the yeast strains. This is important as it implies there is merit in pursuing further studies where wine composition and sensory characteristics are compared across viticultural influences (e.g. region, management and environment) to help develop strategies to grow grapes for specific sensory outcomes.

The field trial discussed above showed that site was a major influence on Cabernet Sauvignon wine composition and sensory characteristics. Therefore, an extensive study exploring the chemical composition and sensory attributes of a number of commercially produced Cabernet Sauvignon wines from ten wine growing regions of Australia was undertaken to characterise the effect of site across a broader range of samples. There was a clear differentiation between wines that showed fruity characteristics and herbaceous / vegetal characteristics which are commonly described in different styles of Cabernet Sauvignon wines and appear to have an antagonistic sensorial relationship. Compositional results showed that eucalyptol was associated with eucalypt and mint aroma attributes while furan and benzene derivatives were positively correlated with the aroma perception of oak. Many other associations between volatile components of the wines and sensory scores were noted and these results will form the basis for more targeted studies aimed at determining the chemical basis of wine sensory attributes.

The experiments described in this thesis demonstrate that the sensory perception of wine is a complex process and involves the interaction of both volatile and non-volatile components. The study increases our understanding of commercial wine composition and its relationship with sensory attributes seen in Cabernet Sauvignon wines from Australia. Future research will build on this knowledge and should consider employment of more holistic techniques to develop a greater understanding of the complex interactions between mixtures of volatiles and non-volatiles. Further, there is a

disconnect in the current literature with regards to linking wine composition, sensory characteristics, and consumer preferences, which is important to producing products for specific markets. Thus, the results of the current study should be extended to develop a better understanding of how these attributes relate to consumer segments.

Understanding the various influences including grape growing, winemaking and storage that influence the formation of wine volatile compounds is essential to wine businesses when developing strategies to produce wines with specific sensory attributes that appeal to target markets. This project moves us closer to this goal.

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