Oxidation treatments affecting Sauvignon blanc wine sensory and chemical composition

by

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Summary

This study focussed on the effect of oxygen on the chemical and sensory evolution of Sauvignon blanc wine under controlled oxidation conditions. The sensorial interactive effects between Sauvignon blanc varietal aroma compounds and compounds that typically arise during oxidation of white wines were also investigated.

In the first research chapter the sensorial interactive effects of Sauvignon blanc impact compounds with aldehydes typically originating from oxidation of white wines were investigated. Four compounds, 3-mercaptohexan-1-ol (3MH), 3-isobutyl-2-methoxypyrazine (IBMP), 3-(methylthio)-propionaldehyde (methional) and phenylacetaldehyde were added together in a model wine medium at varying concentrations. The concentrations chosen were according to those reported in literature to occur in Sauvignon blanc wines. The sensory effects of these compounds were profiled using a trained sensory panel. Compounds were first profiled individually and results showed that the change in comcpound concentration not only led to a change in intensity ratings but also in some cases a change in the descriptor.

All four compounds in the same sample showed complicated interactive effects. Data were statistically analysed using relatively novel techniques such as statistical networks that allowed deeper insights into the interactions involved. Various observations were made such as the contribution of 3MH to the 'green' character of the wine, the potent suppressing abilities of methional on 3MH and IBMP and the additive effect of methional and IBMP contributing to the 'cooked' character of the sample.

In the second research chapter the effect of repetitive oxidation on a fresh and fruity style Sauvignon blanc wine was investigated. Results showed the progress and evolution of aromatic and non-aromatic compounds during an oxidative aging period. A large range of chemical analyses were conducted together with extensive sensory profiling. Results showed a decrease in volatile thiols responsible for the fruity nuances and an increase in oxidation-related compounds, such as acetaldehyde, during the course of the oxidation. Sensory profiling showed the evolution of the wine aroma during oxidation. The wine evolved from a fresh and fruity wine to slight oxidation and then developed extreme oxidative characteristics. The Control samples (no oxygen added) developed a 'cooked' character which could indicate the formation of reductive compounds in these wines. Conversely, the wines that received a single dose of oxygen did not develop this flavour and were perceived to be more fresh and fruity than the Control samples. The evolution of the wine colour was also monitored using a spectrophotometer as well as a sensory panel and results suggest that the colour of the wine evolved before the disappearance of the pleasant aroma. The advantages and disadvantages of oxygen exposure to this type of wine style are discussed as well as the complexity of the wine matrix and sensory interactions occurring in the specific wine.

The aim of the third research chapter was again to investigate further sensory interactions between Sauvignon blanc varietal aroma compounds and an oxidation-related compound. After the observations reported in the second research chapter, the inclusion of acetaldehyde in an interaction study seemed to be of critical importance. Acetaldehyde can reach significant concentrations during oxidation and can have a detrimental effect on wine aroma. This interaction study included three compounds, 3MH, IBMP and acetaldehyde. Acetaldehyde was able to effectively mask the 'green' character of the sample, while it also enhanced certain fruity nuances when present at specific concentrations. 3MH was able to suppress the oxidising character of acetaldehyde when present at sufficient concentrations.

The results from this study clearly showed the complexity of the wine medium and the interactions involved. It also highlighted the importance of performing these types of sensory studies in a simple medium opposed to a complex matrix such as wine. The effect of oxygen on various aspects of the wine was investigated and the combination of chemical and sensory data delivered some interesting conclusions also involving interactions that occurred. This study paved the way for future investigations on the sensory relationships of Sauvignon blanc aroma compounds and the role of proper oxygen management in the production of quality wines.

Opsomming

Die doelwit van die studie was om die effek van gekontroleerde oksidasie op die ontwikkeling en verloop van verskeie chemiese komponente asook die ontwikkeling van die sensoriese profiel van ‘n Sauvignon blanc wyn te ondersoek. Bykomende studies ondersoek die interaksie/wisselwerking tussen aromakomponente in ‘n eenvoudige matriks. Interaksies tussen spesifieke Sauvignon blanc kultivar-geassosieerde aromakomponente en komponente wat normaalweg tydens die oksidasie van witwyne ontwikkel, word ondersoek.

Die eerste navorsingshoofstuk het ten doel om die interaksie tussen tipiese Sauvignon blanc aromakomponente (of impakkomponente) met aldehiede wat normaalweg tydens oksidasie van witwyne ontwikkel, na te vors. Vier komponente is in ‘n modelwyn gevoeg teen verskeie konsentrasies, wat oor die algemeen in die literatuur gerapporteer is om voor the kom in Sauvignon blanc wyne. Die komponente wat ondersoek is, is: 3-merkaptoheksanol (3MH), 3-isobutiel-2-metoksiepirasien (IBMP), 3-(metieltio)-propionaldehied (methional) en fenielasetaldehied. Die sensoriese effekte van die komponente is deur ‘n opgeleide sensoriese paneel geëvalueer. Komponente is eers individueel geanaliseer en die resultate het getoon dat dat die intensiteit van die spesifieke aroma verander namate die konsentrasie verander. In sommige gevalle het die beskrywende woord vir die aroma ook verander. Deur al vier komponente in dieselfde monster te voeg word die ondersoek gekompliseer. Die data is statisties geanaliseer deur gebruik te maak van relatiewe nuwe tegnieke soos statistiese netwerke wat dieper insig in die betrokke interaksies bewerkstellig. Verskeie waarnemings word gerapporteer onder andere die bydrae van 3MH tot die ‘groen’ karakter van die wyn, die kragtige onderdrukkingsvermoë van methional op 3MH en IBMP asook die opbouende effek van methional en IBMP wat bydra tot die ‘gekookte’ karakter van die monster.

Die tweede navorsingshoofstuk is daarop gemik om die effek van herhalende oksidasie op 'n vars en vrugtige styl Sauvignon blanc wyn te ondersoek en om die vordering en ontwikkeling van aromatiese en nie-aromatiese komponente gedurende hierdie tydperk te analiseer. ‘n Wye reeks chemiese komponente is geanaliseer tesame met omvangryke sensoriese analise. ‘n Afname in die vlugtige tiole, wat verantwoordelik is vir die vrugtige geure, is gevind tesame met ‘n toename in oksidasie-verwante komponente (soos asetaldehied). Sensoriese ondersoeke toon ook die evolusie van die wynaroma tydens oksidasie. Die wyn het ontwikkel van ‘n vars en vrugtige styl na effense oksidasiegeure waarna ekstreme oksidasiekarakters waargeneem is. Die Kontrole monsters het ‘n ‘gekookte’ karakter ontwikkel wat ‘n aanduiding van die ontwikkeling van ‘reduktiewe’ komponente in hierdie wyn kan wees. Aan die ander kant het wyne wat een suurstofdosering ontvang het, geen van hierdie geure ontwikkel nie en die wyn is as varser en vrugtiger beskryf in vergelyking met die Kontrole monsters. Die ontwikkeling van die wynkleur is ook gemonitor deur gebruik te maak van ‘n spektrofotometer asook 'n sensoriese paneel. Resultate stel voor dat die kleur van die wyn ontwikkel voor die aangename geure begin verdwyn. Die

voor- en nadele van suurstofblootstelling aan hierdie tipe wynstyl word bespreek asook die kompleksiteit van die wynmatriks en sensoriese interaksies wat in hierdie spesifieke wyn voorkom.

Die derde navorsingshoofstuk is weereens daarop gemik om die sensoriese interaksie tussen tipiese Sauvignon blanc kultivar-geassosieerde aromakomponente en nog ‘n oksidasie-geassosierde aromakomponent te ondersoek. Die resultate vanuit die tweede navorsingshoofstuk het die insluiting van asetaldehied in die interaksiestudie genoodsaak. Asetaldehied kan betekenisvolle konsentrasies tydens oksidasie behaal en kan ook nadelige effekte op wynaroma hê. Hierdie interaksiestudie het die volgende drie komponente ingesluit: 3MH, IBMP en asetaldehied. Asetaldehied het die ‘groen’ karakter van IBMP effektief gemaskeer terwyl dit die waarneming van die vrugtige aroma ondersteun en selfs verhoog het wanneer dit teen sekere konsentrasies teenwoordig was. 3-Merkaptoheksanol het die oksidasiekarakter van asetaldehied onderdruk wanneer dit teen genoegsame konsentrasies teenwoordig was.

Die kompleksiteit van wyn as ‘n navorsingsmedium is duidelik vanuit die studie veral in die ondersoeking van interaksie-effekte tussen komponente. Die belangrikheid van die gebruik van 'n eenvoudige medium teenoor ‘n komplekse medium vir soortgelyke studies is dus duidelik. Die effek van suurstof op verskeie aspekte van witwyn is ondersoek en die kombinasie van chemiese en sensoriese data het interessante gevolgtrekkings gelewer. Die studie het die pad vir toekomstige studies gebaan in terme van sensoriese interaksies met betrekking tot Sauvignon blanc aroma. Die belangrikheid van oordeelkundige suurstofbestuur tydens die produksie van kwaliteit wyne is ook uitgelig.

Hierdie proefskrif is aan my ouers opgedra.

This dissertation is dedicated to my parents.

Biographical sketch

Carien Coetzee was born on 31 January 1986 in Bellville, South Africa. She went to DF Malan High School and after matriculating in 2004 enrolled for a BScAgric degree, majoring in Viticulture and Oenology at Stellenbosch University. In 2009, she enrolled for a MScAgric degree in Oenology at the same University. She completed both degrees *cum laude* in 2008 and 2011 respectively. She enrolled for a PhD in Oenology in 2011. As part of her PhD studies, she visited other Universities such as the University of Auckland in New Zealand as well as Universidade Católica Portuguesa in Porto, Portugal to enhance her research experience and obtain further results regarding her studies. In 2013 she was awarded the TATA Africa Scholarship for Women in Science as part of the Department of Science and Technology's National Women in Science Awards for outstanding ability and potential in research.

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Preface

This dissertation is presented as a compilation of six chapters. Each chapter is introduced separately and is written according to the style of the *South African Journal of Enology and Viticulture*. Each chapter should be regarded as an individual entity and therefore some repetition between chapters may occur.

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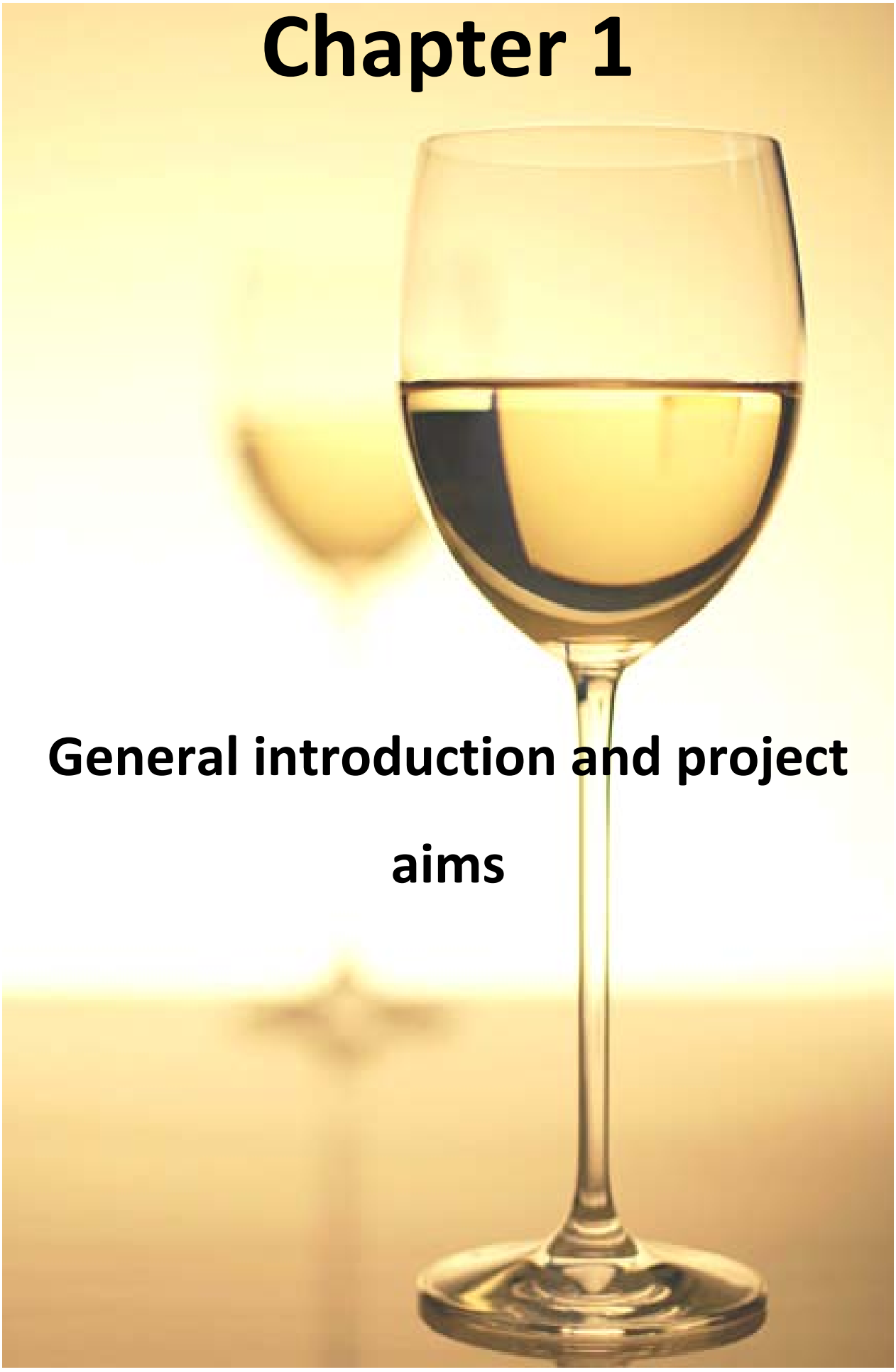
CHAPTER 6: General discussion and conclusions

Chapter 1



General introduction and project aims

Chapter 1:

General introduction and project aims

1.1 INTRODUCTION

South African Sauvignon blanc wine has distinctive sensory characteristics. Both fruity ("passion fruit, guava, grapefruit, tropical") and green ("green pepper, grassy, asparagus") descriptors have been used to describe the unique aroma of this cultivar. Virtually nothing was known about the volatile compounds responsible for these attributes until the mid-1990s, except for the role of 3-isobutyl-2-methoxypyrazine in the "green pepper" character thanks to the work of Augustyn and Allen in South Africa and Australia (Augustyn *et al.*, 1982; Allen *et al.*, 1991). Today, the compounds responsible for Sauvignon blanc flavours have been, for the most part, identified. Various grape and fermentation derived compounds contribute to the aroma of Sauvignon blanc wine. However, many of the mechanisms responsible for the formation of these compounds are still not fully understood. Compounds such as the volatile thiols and methoxypyrazines are key aroma compounds responsible for the typical Sauvignon blanc aroma contributing to 'fruity' and 'herbaceous' nuances respectively, while other aroma compounds such as esters, alcohols and acids can also contribute to the wine aroma (Swiegers *et al.*, 2006; Lund *et al.*, 2009).

Oxidation of white wines is a constant problem for winemakers worldwide. Oxidation may drastically affect wine quality and is identified by a loss in pleasant aroma together with an increase in unwanted aroma nuances. In general, it is accepted that certain grape varieties are especially sensitive to oxygen, suggesting that some of the chemical components key to their sensory attributes are strongly modulated by oxygen. Sauvignon blanc is a well documented example of an oxygen-sensitive wine (Coetzee & Du Toit, 2012). Understanding the stability of various compounds is crucial in order to preserve the fresh and fruity characters of Sauvignon blanc wines over a long period of time.

The term "oxygen management" refers to one or multiple operations in which a well controlled amount of oxygen is delivered to the wine in order to achieve optimal expression of desirable sensory attributes, however it is also important to maintain the optimal chemical composition, especially antioxidant content. Obtaining a better understanding on this topic will give wine producers the upper hand to compete on a commercial scale and place on the market a product with a quality that is better and more consistent than their competitors. Any defect in wine quality should thus be identified rapidly and rectified if possible. In the case of oxidation, the prevention of defects by proper oxygen management is important.

This study on oxygen management is not only linked to what happens to the wine during aging, but takes a holistic approach to oxygen in wine, as we know today that the winemaking process and oxygen "history" of the wine is extremely important (Ugliano, 2013). It is essential to understand the chemistry behind oxygen exposure to control and manage the development of various characteristics in wine. Given the intrinsic diversity existing across individual wines, the great challenge in this regard remains

the ability to predict the tendency of a wine to develop certain characters and, in general, to define how much oxygen a wine might need to express improved sensory characteristics.

There seems to be a lack of information especially on South African Sauvignon blanc wine and the reaction of various chemical compounds during oxidative processes. To date, no scientific literature has focussed on a relatively large range of aroma compounds during oxidation of South African wines and the combination of extensive chemical data with sensory profiling can deliver valuable information regarding the evolution of these important compounds. A number of studies have investigated the effect of enhanced oxidation on the chemical composition of white wines (Ferreira *et al.*, 1997; Escudero *et al.*, 2002; Silva Ferreira *et al.*, 2003; Loscos *et al.*, 2010; Cejudo-Bastante *et al.*, 2013), but little is known about the effect of controlled repetitive oxygen additions on Sauvignon blanc wine. A need also exists to elucidate the interactive effect between Sauvignon blanc aroma impact compounds and oxidative derived volatiles at concentration ranges found in white wines.

1.2 PROJECT AIMS

The objectives of this study were to improve the understanding of:

- The evolution of a large range of compounds (aromatic and non-aromatic) during controlled Sauvignon blanc wine oxidation
- The sensory impact of controlled Sauvignon blanc wine oxidation and how the sensory relates to chemical data
- Sensory interactions between Sauvignon blanc impact aroma compounds and aroma compounds typically linked to oxidation

Other objectives to which the results from this study could contribute are

- to formulate possible mechanisms to improve Sauvignon blanc wine quality and longevity
- to provide credible information and integral solutions on how to manage the impact of oxygen on the wine, thereby improving the winemaker's control over the final wine profile through the use of oxygen management
- to reduce wine faults

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Chapter 2

Literature review

Sauvignon blanc wine: Contribution of aging and oxygen on aromatic and non-aromatic compounds and sensory composition

Chapter 2: Literature Review

Sauvignon blanc wine: Contribution of aging and oxygen on aromatic and non-aromatic compounds and sensory composition

2.1 INTRODUCTION

Aroma is an important factor in the quality of all foods, but in wines the aroma is probably one of the most important aspects contributing to the overall quality. A large number of chemical compounds with different volatilities and polarities are responsible for the aroma of wine (Arrehnius *et al.*, 1996) and during winemaking and aging various reactions and interactions occur that can influence the perception of the wine bouquet.

One of the most important oenological problems in winemaking is premature wine oxidation, particularly the oxidative spoilage of young white wines. With the exception of sherry and sherry-like wines, white wine quality, in general, will decrease with oxidation (Singleton *et al.*, 1979). This oxidation can take place in a short amount of time during which a significant loss of fresh and fruity aroma takes place, followed by a colour change as well as the development of unwanted oxidation odours.

Aroma attributes that develop due to the formation of new chemical compounds have been described as “honey-like”, “farmfeed”, “hay”, “woody-like”, “toasted”, “dry fruits”, “caramel”, “overripe fruit”, “apple”, “oxidised apple”, “acetaldehyde”, “cooked”, “aldehyde” and “liquor” (Toukis, 1974; Noble *et al.*, 1987; Renouil, 1988; Halliday & Johnson, 1992; Chrisholm *et al.*, 1995; Escudero *et al.*, 2002; Silva Ferreira *et al.*, 2002b). These types of unpleasant descriptors are unwanted in wines and winemakers go to great lengths to avoid the formation of the chemical compounds responsible for these attributes.

Young wines contain high concentrations of oxygen reactive species, triggering a chain of chemical reactions consequently altering the wine content. Various closure permeability studies have been performed to assess the effect of oxygen and aging on the quality of young white wines (Godden *et al.*, 2001; Brajkovich *et al.*, 2005; Lopes *et al.*, 2005; Skouroumounis *et al.*, 2005). Generally speaking, the conclusion of these studies are that less permeable closures allow better preservation of the fresh and fruity character of the wine, presumably by preventing the oxidative loss of pleasant aroma compounds (Lopes *et al.*, 2009).

Too little oxygen exposure has been associated with ‘reductive’ off-odours. Closures not allowing sufficient oxygen ingress could lead to the formation of the compounds contributing to the ‘reductive’ aroma (Godden *et al.*, 2001; Skouroumounis *et al.*, 2005; Kwiatkowski *et al.*, 2007; Lopes *et al.*, 2009). Conversely, oxidative loss of aromatic compounds and the evolution of undesirable aroma compounds will occur with excessive oxygen exposure. Figure 2.1 shows the evolution of aroma compounds during aging of wines exposed to low and high oxygen concentrations.

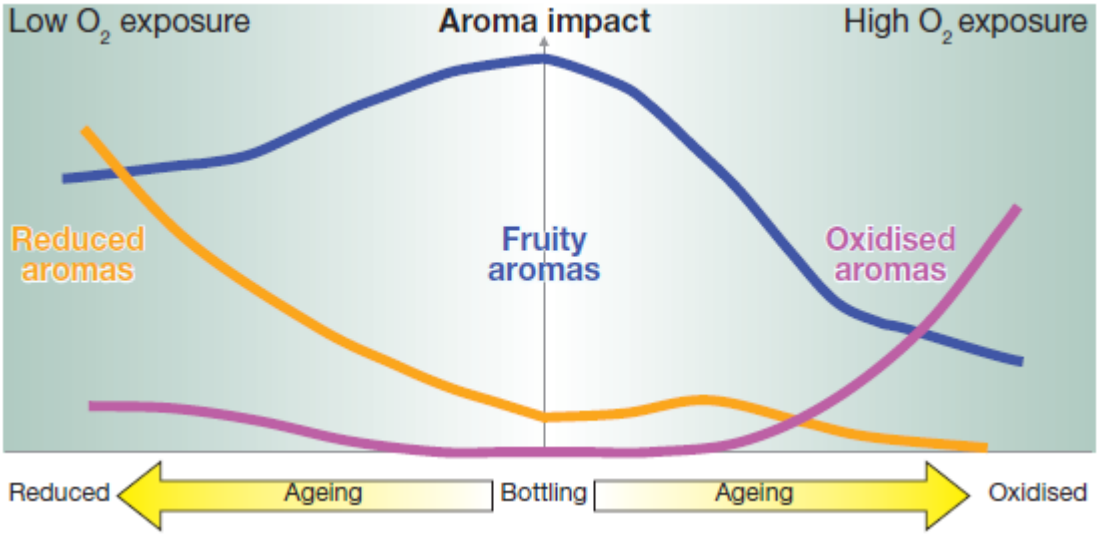


Figure 2.1 The effect of oxygen exposure on the aroma of bottled wine (Ugliano *et al.*, 2010). Reproduced with permission from The Australian Wine Industry Technical Conference Inc.

It can be concluded that a moderate degree of oxygen exposure allows expression of optimal aroma attributes (Brajkovich *et al.*, 2005; Lopes *et al.*, 2009; Ugliano *et al.*, 2009; Nygaard *et al.*, 2010), however the actual degree of oxygen exposure that is necessary to achieve this has not been determined. Very few such studies have actually included precise oxygen measurements (Brajkovich *et al.*, 2005; Lopes *et al.*, 2009; Ugliano *et al.*, 2009; Nygaard *et al.*, 2010), thus still making it difficult to define how much oxygen would be beneficial for wine during bottle aging. The type (variety and composition) and style (winemaking practices such as wood contact) of the wine would also play a crucial role in the amount of oxygen required to achieve the ideal wine.

While the chromatic changes during wine aging are well documented, little is known about the aromatic deterioration in relation to white wine oxidation, even though it seems to take place prior to discoloration (Li *et al.*, 2008). Other than that, studies investigating oxidative spoilage of wines were often carried out under conditions accelerating oxidation such as high oxygen exposure or high temperatures (Ferreira *et al.*, 1997; Escudero *et al.*, 2000a; Escudero *et al.*, 2002). Conversely, oxidative processes taking place during aging are usually rather mild and the significance of such levels of oxidation on a wide range of chemical compounds and sensory profile of the wines remain to be established. The interactions between aroma compounds present at any one time in the wine during bottle aging could also significantly influence the wine's character by changing the perception of certain attributes and should be investigated. This review will focus on the various wine constituents of a typical Sauvignon blanc wine and the reactions thereof towards oxidation. Sensory aspects and interactions between aromatic compounds will also be discussed.

2.2 WHITE WINE OXIDATION

2.2.1 Oxygen during wine processing

During winemaking, the grape must and wine are exposed to different levels of oxygen. This has been described as macro, micro and nano concentrations. Figure 2.2 provides a theoretical illustration of the oxygen concentration during wine processing (Ugliano *et al.,* 2010), however studies have shown that these circumstances are not always applicable and large spikes of oxygen can be introduced late in the process.

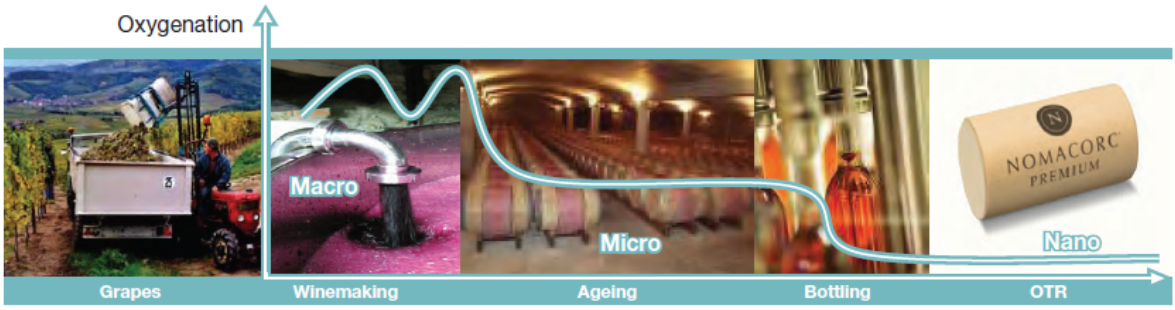


Figure 2.2 Representation of the ideal oxygen management (Ugliano *et al*., 2010). Reproduced with permission from The Australian Wine Industry Technical Conference Inc.

Any oenological practices involving air will undoubtedly cause oxygen dissolution and wines saturated with oxygen will contain about 8 mg/L oxygen at cellar temperatures and atmospheric pressure (Singleton *et al.*, 1985). Wine is capable of consuming a considerable amount of dissolved oxygen which has been ascribed to the total phenol content, which explains why red wine can consume more oxygen than white wine (Rossi & Singleton, 1966).

The effect of single oenological processes on the level of dissolved oxygen in wines can be classified as 'high enrichment' and 'low enrichment' treatments (Castellari *et al.*, 2004). A study done on over 500 red and white wines has identified 'high enrichment' practices as racking, centrifugation, refrigeration, continuous tartaric stabilization and bottling (Castellari *et al.*, 2004). Dissolved oxygen concentration after these treatments ranged from 1 mg/L to about 8.5 mg/L with refrigeration and cold stabilization ranked as the two treatments causing the highest oxygen pickup. 'Low enrichment' treatments are practices like pumping, filtration, heat exchange and electrodialysis and caused an uptake of up to 0.6 mg/L with filtration causing the most oxygen pickup (Castellari *et al.*, 2004). During and after bottling, both wine and the gaseous headspace in the bottle will contain a substantial amount of oxygen. The total packaged oxygen as the sum of these two components can typically reach 1-9 mg/L (Ugliano *et al.*, 2013) depending on the specific handling of the wine (care taken to exclude oxygen). In the case of

some closures such as corks or similar products, additional oxygen will be released into the bottle after bottling.

The solubility of oxygen in wine is influenced by the wine composition (e.g. ethanol content), but depends primarily on the temperature and the partial pressure of the gas, with greater solubility at lower temperatures and when pure oxygen is used instead of air (Waterhouse & Laurie, 2006). The rate of the oxidation reactions, however, increases with increased temperature (Margalit, 1997; Vivas de Gaulejac *et al.*, 2001; Ribéreau-Gayon, 2006). The contact of wine with oxygen can be minimised by the use of inert gasses such as nitrogen, carbon dioxide and even argon gas, which can displace the air in a tank or barrel.

2.2.2 Oxygen and phenolics

Phenolic compounds are characterised by an aromatic ring containing one or more hydroxyl groups (-OH), including their functional derivatives. The concentration of phenolics in white wine will depend on the variety, cultivation conditions, climate, grape maturity, winemaking techniques as well as aging conditions. The polyphenol content of white wines is substantially lower than in red wines due to different winemaking techniques favouring phenolic extraction during red wine production. Phenolics are strong hydrogen donating species which makes them ideal oxidation substrates (Wilderandt & Singleton, 1974).

Phenolic molecules originating from grapes can be divided into non-flavonoids and flavonoids. The non-flavonoids are grape-derived and consist among others of hydroxybenzoic acids, hydroxycinnamic acids and stilbenes and are normally the principal phenolic molecules in white wines. Compounds such as the caffeic acid, *p*-coumaric acid and ferulic acid and their tartaric acid esters are examples of non-flavonoids and are the main phenolic molecules in white wine that did not receive prolonged periods of skin contact. Of these, *trans*-caftaric acid is the predominant hydroxycinnamate ester in grape juice and wine, together with smaller quantities of coutaric and fertaric acid (Singleton *et al.*, 1978; Singleton *et al.*, 1984; Vrhovšek, 1998). The naturally occurring tartaric esters are susceptible to hydrolysis, liberating the corresponding free hydroxycinnamic acids. The hydroxybenzoic acids represent a minor class of white wine polyphenols of which gallic acid, vanillic acid and syringic acid are a few examples.

The major classes of flavonoids in grapes and wine are flavan-3-ols, flavonols and anthocyanins. Anthocyanins are pigments significant only in red grape varieties and are in general absent from white wines. The main flavan-3-ols found in grapes are (+)-catechin and (-)-epicatechin as well as the galate ester (-)-epicatechin-3-*O*-gallate (Tsai Su & Singleton, 1969), while the flavonols mainly consist of quercetin, kaempferol and myricetin (Monagas *et al.*, 2005; Ribéreau-Gayon, 2006).

In grape must, enzymatic oxidation of phenolic compounds takes place due to the presence of oxidation enzymes (e.g. polyphenol oxidase) which catalyse the oxidation process, while in wine, non-enzymatic

chemical oxidation is the predominant oxidation reaction due to the absence or inhibition of oxidation enzymes. Although polyphenol oxidation is extremely slow (Oszmianski *et al.*, 1985), it affects a wide range of polyphenols, depending on their individual redox potentials (Kilmartin *et al.*, 2002). The oxidation process will depend on a number of factors such as oxygen concentration, temperature, presence of catalysts, the nature and composition of polyphenols, pH, ethanol content and presence of antioxidants (Berg & Akiyoshi, 1956; Cilliers & Singleton, 1989; Kilmartin *et al.*, 2001; Waterhouse & Laurie, 2006).

Oxygen in wine is in the unreactive triplet state, and its ability to react directly with most wine components is low (Waterhouse & Laurie, 2006). The presence of a catalyst (particularly iron and copper) increases the reaction speed by donating an electron to oxygen resulting in a superoxide ion which exists as a hydroperoxyl radical at wine pH (Figure 2.3). The radical has relatively low reactivity in the wine environment and will react with strong hydrogen donating species such as phenolic molecules (Wilderandt & Singleton, 1974). The reaction of the superoxide ion with *o*-diphenols forms H_2O_2 and *o*-quinones at wine pH (Figure 2.3).

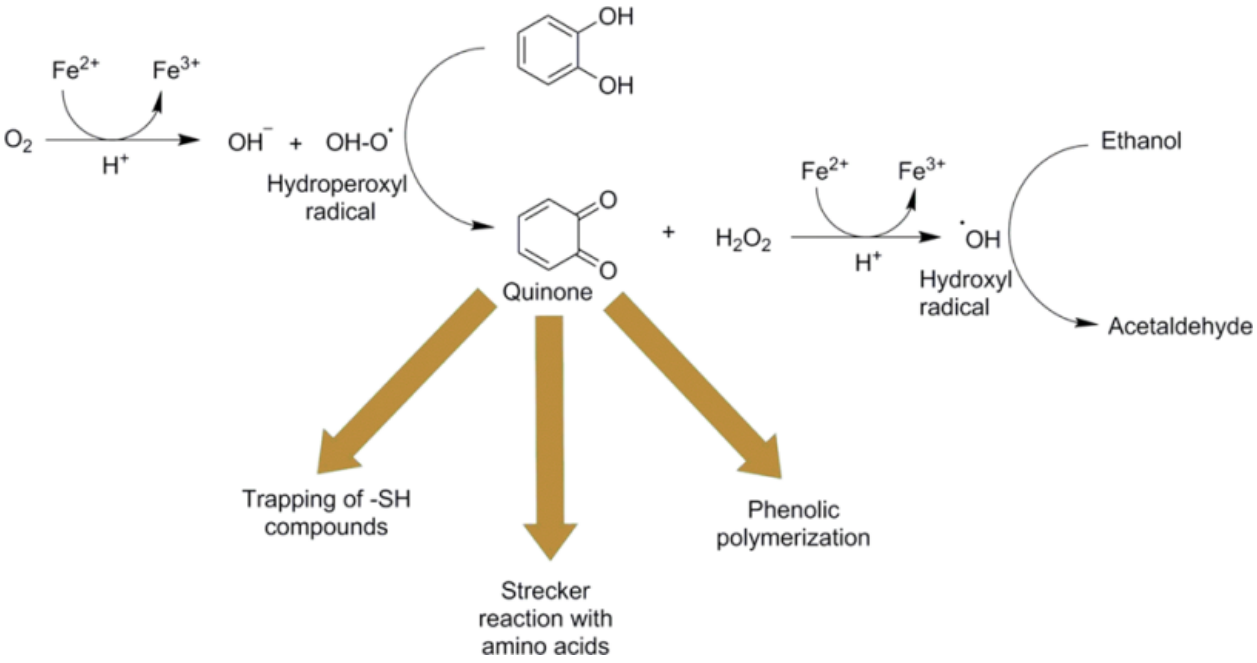


Figure 2.3 Formation of *o*-quinones and hydrogen peroxide and the consequent oxidation of ethanol to form acetaldehyde and other reactions in the wine matrix. Reprinted with permission from Ugliano, M., 2013. Copyright 2013 American Chemical Society.

The H_2O_2 can react with ferrous ions via the Fenton reaction to produce a hydroxyl radical which is extremely reactive and can react with various wine constituents (e.g. alcohols, organic acids and sugars) in proportion to their concentration, causing the formation of aldehydes and ketones. In this case, ethanol will be primarily oxidised to produce acetaldehyde (Waterhouse & Laurie, 2006). The *o*-quinone

is unstable and very reactive and can react further with other molecules with lower redox potentials such as other phenolic molecules, SO_2 and thiol containing compounds including glutathione and amino acids. This array of reactions can cause a substantial change in the composition of wine.

2.2.3 The role of antioxidants

The most common antioxidants (other than phenols) present in wine are sulphur dioxide (SO_2), ascorbic acid and glutathione (GSH). These compounds interfere in the phenol oxidation process, either by removing oxygen from the wine or by reversing or altering the oxidation process.

Sulphur dioxide is an effective and low cost additive for the preservation of wines and other food products (Doyle & Beuchat, 2007). In wine, it serves as the main preservative to prevent oxidation and decrease microbial activity. Even though SO_2 occurs naturally in all wines as a by-product of yeast metabolism during fermentation (Rankine & Pocock, 1969), it is usually added at several stages in the process of conventional winemaking such as during crushing, settling or after primary and secondary fermentation (Paul, 1975). Unfortunately, excessive use of SO_2 can be not only detrimental to organoleptic quality of a wine, but poses a health risk for sensitive consumers (especially asthmatics) and its presence requires mandatory label warning statements in most jurisdictions (Kleinhans, 1982). The equilibrium established upon dissociation of SO_2 in wine is shown in Figure 2.4.

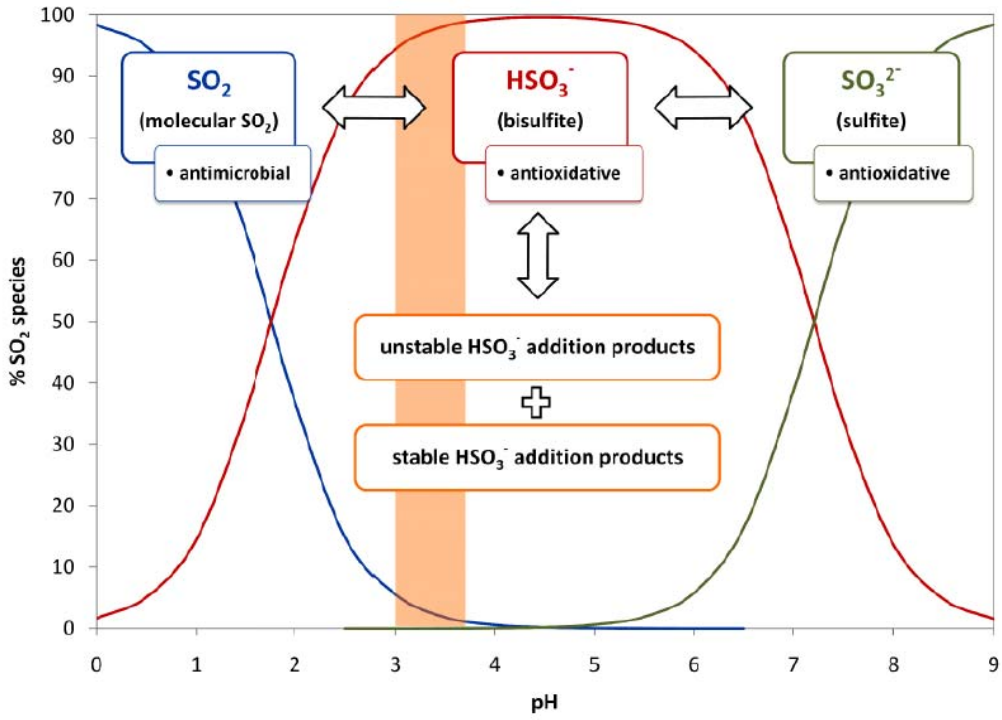


Figure 2.4 Distribution of SO_2 species at different pH values (Sneyd *et al.*, 1993; Herbst, 2010). Adapted with permission from The Australian Wine Industry Technical Conference Inc.

Sulphur dioxide exists in wine in both free and bound form (the sum equalling total SO_2). At wine pH (pH 3 to 4), free SO_2 can exist in three forms: molecular SO_2, bisulphite (HSO_3^-) and sulphite (SO_3^{2-}). The equilibrium is pH-dependant and affected by the presence of wine constituents that bind the bisulphite as well as wine temperature (Usseglio-Tomasset, 1992). The molecular form is mainly responsible for antimicrobial properties due to its ability to penetrate the cellular membranes of microorganisms (Beech *et al.*, 1979). However, at wine pH only a small proportion of the free SO_2 is in molecular form, the predominant form being bisulphite (94-99%) which can bind a large range of wine components, consequently producing bound SO_2 (Zoecklein *et al.*, 1995; Oliveira *et al.*, 2002). The sulphite can react directly with oxygen, but is present at extremely low concentrations at wine pH. Direct reaction of bisulphite with oxygen is slow and the antioxidant activity lies in the ability to reduce the H_2O_2 to water, convert *o*-quinones back to *o*-diphenols and react directly with *o*-quinones to form sulphonic acids (Figure 2.5) (Danilewicz, 2007).

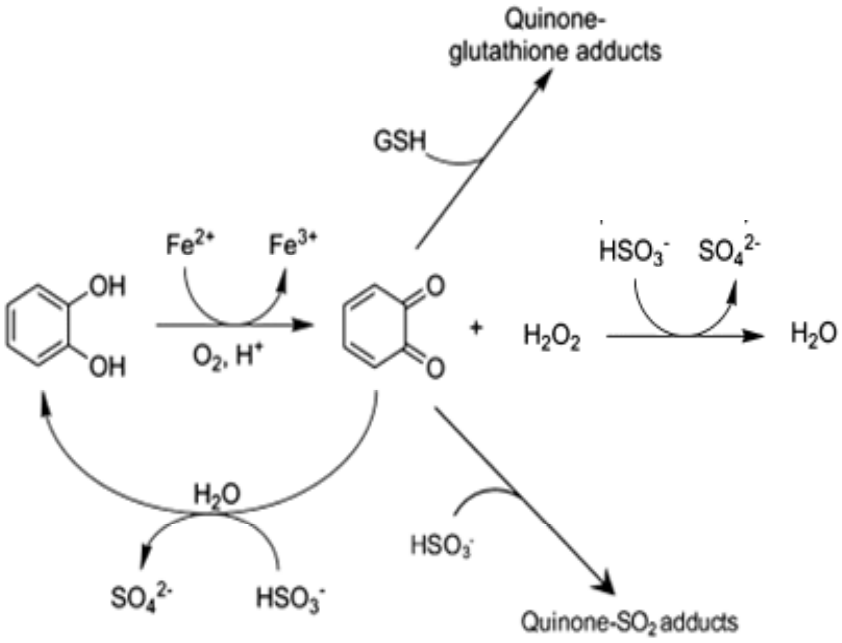


Figure 2.5 Proposed mechanism illustrating SO_2 and GSH antioxidant protection in wine. Reprinted with permission from Ugliano, M., 2013. Copyright 2013 American Chemical Society.

A number of carbonyl compounds (mainly acetaldehyde, pyruvic acid and α-keto-glutaric acid) can bind with free SO_2 individually to form complex compounds (Jackowetz & De Orduña, 2013). The bisulphite-acetaldehyde addition product accounts normally for the majority of the bound SO_2 in wine. Reactions with carbonyl compounds such as acetaldehyde leads to the formation of bisulphite addition products in reversible reactions, however other reactions such as those between bisulphite and *o*-quinones form

stable adducts and are irreversible (Laurie *et al.*, 2012). Nevertheless, there is a general trend in the wine industry towards minimising SO_2 content and appropriate substitutes and supplements are a popular topic in research. To date, no single replacement for SO_2 has been found that combines antimicrobial and antioxidant characteristics.

Glutathione is a sulphur-containing tripeptide (L-γ-glutamyl-L-cysteinyl-glycine) and a naturally occurring antioxidant from the grapes and yeast metabolism (Figure 2.6). The concentration in the must is influenced by the nitrogen uptake of the vine (Choné *et al.*, 2006) and it is accumulated in the berry at the onset of véraison (Adams & Liyanage, 1993). Concentrations of GSH in 28 young Sauvignon blanc wines averaged at 12.5 mg/L (Janes *et al.*, 2010) and winemaking conditions promoting oxygen exposure led to a decrease in GSH concentrations while higher concentrations have been observed in juices treated reductively (Du Toit *et al.*, 2007; Maggu *et al.*, 2007).

GSH has an electron-rich nucleophilic mercapto group which can be spontaneously substituted by 1,4-Michael addition into the electrophilic centre of the *o*-quinone formed during oxidation. The product is a thioether, 2-S-glutathionyl-caftaric acid or Grape Reaction Product (GRP) (Figure 2.6). The formation of GRP traps the *o*-quinone thus preventing any further reactions taking place (Kritzinger *et al.*, 2013a). Glutathione can also react with oxygen species such as H_2O_2 (Anderson, 1998) to be oxidised to glutathione disulphide (Figure 2.6). It has been argued that the disulphide can also be formed by reducing the *o*-quinone back to the *o*-diphenol (Cilliers & Singleton, 1990). This may explain increasing *trans*-caftaric and coutaric acid concentrations in must after GSH additions as reported by Penna *et al.* (2001).

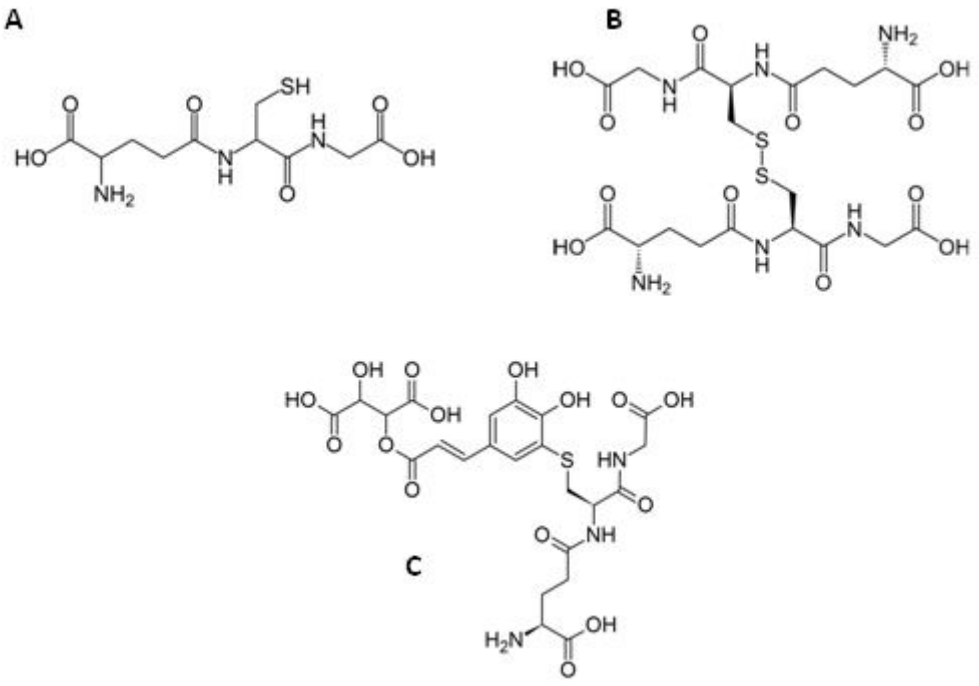


Figure 2.6 Molecular structures of glutathione (A), glutathione disulphide (B) and grape reaction product (C).

Glutathione is also capable of performing nucleophilic reactions with other compounds such as aldehydes (Cheynier *et al.*, 1986; Cheynier & Van Hulst, 1988; Sonni *et al.*, 2011) and has protective abilities toward important aroma compounds such as esters, monoterpenes and volatile thiols (Papadopoulou & Roussis, 2001; Lavigne-Cruège & Dubourdieu, 2003; Roussis *et al.*, 2007; Papadopoulou & Roussis, 2008). However, GSH has also been shown to induce production of H_2S during wine aging in low oxygen conditions (Ugliano *et al.*, 2011).

Glutathione has been linked to the aroma potential of Sauvignon blanc grapes due to the identification of glutathionylated precursors for 3MH and 4MMP (Peyrot des Ganchos *et al.*, 2002). The addition of GSH to must or wine is not legally permitted. However, yeast extracts (which contains GSH) claim to preserve the freshness and increase the mouthfeel as well as the aromatic complexity of white wines (Kritzinger *et al.*, 2013b). These extracts are commercially available.

Ascorbic acid is naturally present in grapes, but disappears rapidly following crushing and is therefore mostly added just prior to bottling by wine producers. It is able to react rapidly with the molecular oxygen present in wine, thereby preventing oxidative browning and prolonging shelf life of white wines (Kielhöfer & Würdig, 1958). This reaction produces dehydroascorbic acid as well as H_2O_2. It is thus important to have sufficient SO_2 present to react with the formed H_2O_2 in order to prevent further oxidation reactions from taking place (Peng *et al.*, 1998). Consequently, the complete replacement of SO_2 with ascorbic acid as an antioxidant in white wine is not recommended and ascorbic acid should only be used as a supplement to scavenge oxygen.

The potential of ascorbic acid to recycle *o*-quinones back to *o*-diphenols has also been suggested (Cilliers & Singleton, 1990; Isaacs & Van Eldik, 1997; Danilewicz, 2003). However, recent studies showed no indication of rapid interaction between ascorbic acid and *o*-quinones on the cyclic voltammograms (Makhotkina & Kilmartin, 2009). The function of ascorbic acid as an antioxidant has been questioned as ascorbic acid can lead to increased browning during storage together with an accelerated consumption of free SO_2 (Kielhöfer & Würdig, 1960). The pro-oxidative activity of ascorbic acid in white wines under certain conditions has been researched since (Peng *et al.*, 1998). Ascorbic acid initially functions as an antioxidant, however over time the presence of ascorbic acid can lead to enhanced SO_2 consumption and oxidation of phenolic compounds resulting in browning (Peng *et al.*, 1998; Bradshaw *et al.*, 2001; Bradshaw *et al.*, 2003; Bradshaw *et al.*, 2004; Clark *et al.*, 2008).

2.2.4 Effect of oxygen on white wine colour

The colour of white wine is one of the important quality parameters. Dark yellow or brown colour usually indicates oxidation or spoilage of white wine. A positive correlation between total phenolic content and browning has been reported (Simpson, 1982). However the content of hydroxycinnamic

acids in the wines correlated poorly with browning (Fernandez-Zurbano *et al.*, 1995). The hydroxycinnamic acids may, however, contribute to the browning through coupled oxidation reactions (Simpson, 1982; Fernandez-Zurbano *et al.*, 1995). The monomeric flavan-3-ols and dimeric procyanidins plays an important role in white wine colouration with (-)-epicatechin more positively correlated to the brown colour compared to (+)-catechin (Simpson, 1982). Browning in white wine can be due to different mechanisms. Firstly, the oxidation of phenolic molecules to their corresponding *o*-quinones leads to further reactions with phenolic compounds to produce dimers which appear to be more susceptible to oxidation, and thus accelerate phenol polymerisation and autocatalytic oxidation in wine (Singleton, 1987). The formation these polymers can result in the formation of more intensely coloured yellow-brown compounds (Es-Safi *et al.*, 1999).

The second mechanism is the oxidative degradation of tartaric acid leading to the formation glyoxylic acid which can mediate condensation reactions of flavan-3-ols, potentially contributing to browning in white wines. The formation of yellow pigments in an oxygenated model wine medium containing the flavan-3-ol (+)-catechin, tartaric acid and ferrous ions (Fe^{2+}) has been observed (Oszmianski *et al.*, 1996). The glyoxylic acid reacts with flavan-3-ol units (via a carboxymethine bridge) generating colourless and yellow compounds in model wine. The major colourless product has been identified as a (+)-catechin dimer (linked by a carboxymethine bridge) (Fulcrand *et al.*, 1997). The yellow pigments have been identified as xanthylium salts deriving from dehydration and oxidation of the colourless carboxymethine linked dimer (Es-Safi *et al.*, 1999). The absorbance maxima of xanthylium cations are close to 420 nm, the standard wavelength to measure browning of white wine. Other carbonyl substances such as acetaldehyde could also potentially mediate the condensation reaction (Es-Safi *et al.*, 1999; Lopez-Toledano *et al.*, 2004; Drinkine *et al.*, 2005; Monagas *et al.*, 2005).

2.3 SAUVIGNON BLANC AROMA AND EVOLUTION DURING AGING AND OXIDATION

Vitis vinifera L. cv. Sauvignon blanc is a grape variety native to Bordeaux (Graves) and the Loire Valley (Sancerre and Pouilly Fumé) (Adams & Liyanage, 1993). This variety is now widely cultivated in many other wine-growing regions across the globe including South Africa, Australia, New Zealand, Chile and the United States (Jackson, 2008). The grapes ripen to moderate to high levels of acidity, producing fresh, crisp and dry white wines with pungent aroma and flavour (Cooper, 2008). Generally, South African Sauvignon blanc wines are cold-fermented in stainless steel tanks and not exposed to oak. In this way, the terroir (often delivering fresh and fruity aromas) is reflected in the wine.

Depending on the climate, Sauvignon blanc wines can offer a range of wine styles. On the one hand, the wine can deliver fresh and fruity characters reminiscent of “guava”, “grapefruit”, “gooseberries” and “passion fruit” which usually originate from grapes grown in a warmer climate. On the other hand the

more “green” style Sauvignon blanc can be produced from cooler grape growing regions delivering aroma nuances such as “green pepper”, “grassy” and “asparagus” (Lund *et al*., 2009b). The aroma of Sauvignon blanc is known to change dramatically over a period of just a year in the bottle, meaning that the wine should be drunk early to experience the intended aromatic bouquet of the wine (Herbst, 2010).

Various chemical compounds contribute to the aromatic composition of a wine. Sauvignon blanc impact compounds such as the volatile thiols and the methoxypyrazines can contribute significantly to the typical character of the wine. Other aroma groups such as ester, alcohols, acids and monoterpenes can also contribute to the pleasant wine aroma. Wines developing a ‘reductive’ aroma will typically have negative aroma descriptors due to low oxygen exposure, while various oxidation-related compounds such as aldehydes, lactones and acetals will contribute to unpleasant aroma. In the following sections details regarding the most important aroma contributing groups will be discussed.

2.3.1 Volatile thiols

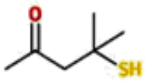
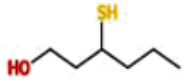
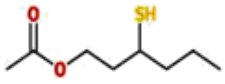
Thiols (more traditionally referred to as mercaptans) are sulphur-containing compounds possessing a sulfhydryl group (-SH). Various sulphur-containing compounds occur in wine and can contribute to a range of aromatic nuances, depending on the type of compound. Certain thiols typically contribute to the fruity characters of a wine and attributes such as “grapefruit”, “passion fruit”, “box tree” and “gooseberry” have been used to describe the odour (Darriet *et al.*, 1995; Tominaga *et al.*, 1998a). These compounds will be referred to as the volatile thiols.

Other sulphur-containing compounds can contribute to a ‘reductive’ aroma due to low oxygen content in certain wines and the aroma reminiscent of “rotten egg”, “garlic” and “cabbage” occurs (Rauhut, 1993; Brajkovich *et al.*, 2005). Compounds such as furfural and 5-hydroxymethylfurfural (5-HMF) (also sulphur-containing compounds) can also impart significant aroma to a wine. The contribution of the sulphur-containing compounds other than the volatile thiols, will be discussed in Chapter 2, sections 2.4 and 2.5.

Although first identified in Sauvignon blanc wine during the 1990s, the presence of volatile thiols has also been reported in wines made from other varieties (both red and white) such as Riesling, Colombard, Semillon, Cabernet Sauvignon and Merlot (Bouchilloux *et al.*, 1998; Tominaga *et al.*, 2000; Murat *et al.*, 2001b). Along with methoxypyrazines, volatile thiols are considered to be impact odourants for Sauvignon blanc wines due to the fact that they form part of the typicality of the variety and are highly sought after by consumers (Goniak & Noble, 1987; Rauhut, 1993; Tominaga *et al.*, 2004; Lund *et al.*, 2009b; King *et al.*, 2011). The volatile thiols playing an important role in Sauvignon blanc wine aroma are mainly 4-mercapto-4-methylpentan-2-one (4MMP), 3-mercaptohexan-1-ol (3MH) and 3-mercaptohexan-1-ol acetate (3MHA) (Table 2.1) (Darriet *et al.*, 1995; Tominaga *et al.*, 1996; Tominaga *et*

al., 2000; Tominaga *et al.*, 2004; Coetzee & Du Toit, 2012). Other volatile thiols have been identified, however the concentration of these compounds in wines are usually below the perception threshold (Tominaga *et al.*, 1998a).

Table 2.1 Volatile thiols involved in Sauvignon blanc aroma (Coetzee & Du Toit, 2012).

Compound	Abbreviation	Structure	Olfactory description	Range in wines [a] ng/L	Perception threshold [b] ng/L
4-Mercapto-4-methylpentan-2-one	4MMP		box tree, passion fruit, broom, black current[c]	4-40	0.8
3-Mercaptohexan-1-ol	3MH		passion fruit, grapefruit, gooseberry, guava[d]	26-18000	60
3-Mercaptohexan-1-ol acetate	3MHA		passion fruit, grapefruit, box tree, gooseberry, guava[e]	0-2500	4.2

a) Range in wines from France and New Zealand (Tominaga *et al.*, 1998a; Ribéreau-Gayon *et al.*, 2006; Lund *et al.*, 2009b; Mateo-Vivaracho *et al.*, 2010)

b) Perception threshold is the minimum detectable concentration for 50% of the tasters; these values have been tested in model wine solution (Tominaga *et al.*, 1996; Tominaga *et al.*, 1998b; Dubourdieu *et al.*, 2006)

c) Darriet *et al.* (1995)

d) Tominaga *et al.* (1998a); Dubourdieu *et al.* (2006)

e) Tominaga *et al.* (1996); Dubourdieu *et al.* (2006)

3MHA has been described as “sweet-sweaty passion fruit”, “grapefruit”, “box tree”, “gooseberry” and “guava” (Tominaga *et al.*, 1996; Nicolau *et al.*, 2006) and has a very low perception threshold of 4.2 ng/L in a model wine solution (Tominaga *et al.*, 1996). 3MH has been described as “passion fruit”, “grapefruit”, “gooseberry” and “guava” (Swiegers *et al.*, 2005; Nicolau *et al.*, 2006; Van Wyngaard, 2013) with a perception threshold of 60 ng/L (Tominaga *et al.*, 1998a), while 4MMP has been described as “box tree”, “passion fruit”, “broom” and “black current bud” (Darriet *et al.*, 1995) with a perception threshold of 0.8 ng/L in a model wine solution (Tominaga *et al.*, 1998a). The low perception thresholds reported makes these compounds potent aromatic contributors in wine.

In a survey done on 24 South African Sauvignon blanc wines (2011 vintage) the average thiol concentrations were 10.1 ng/L, 157.96 ng/L and 969 ng/L for 4MMP, 3MHA and 3MH respectively (Van Wyngaard, 2013). These concentrations were in line with those found in other studies investigating Sauvignon blanc wines from all over the world including New Zealand, Australia, France and Chile (Benkwitz *et al.*, 2012b), with the exception of 3MH for which the concentrations were lower (Lund *et al.*, 2009b; Benkwitz *et al.*, 2012b).

Unlike the methoxypyrazines which are present as such in the grapes, the volatile thiols (4MMP and 3MH) are thought to be in part released by the yeast from odourless, non-volatile precursors during fermentation (Darriet *et al.*, 1995; Tominaga *et al.*, 1996). Some precursors in the juice have been identified as being cysteinylated or glutathionylated precursors. However, studies have shown that these precursors only account for a fraction of the total amount of thiols present in the wine (Darriet *et al.*, 1995; Tominaga *et al.*, 1998c; Peyrot des Ganchos *et al.*, 2002; Subileau *et al.*, 2008a; Fedrizzi *et al.*, 2009), moreover no direct correlation between precursor concentration and the amount of free volatile thiols in the resulting wines have been observed (Capone *et al.*, 2010; Roland *et al.*, 2011). Other than the release from precursors, the biogenesis of volatile thiols has been proposed. The direct addition of H_2S or another sulphur donor to conjugated carbonyl compounds such as *(E)*-2-hexenal and mesityl oxide followed by a reduction step could be another source of volatile thiol formation (Schneider *et al.*, 2006). The formation of the volatile thiols is not fully understood, since the main precursor has yet to be elucidated and the production of these volatile thiols needs further investigation.

3MHA is formed by the esterification of 3MH with acetic acid during fermentation. The final concentration of 3MHA (and other fermentative esters) depends on the balance of activities of alcohol acetyltransferase (promoting estrification of the corresponding alcohol) and esterase (promoting hydrolysis). Yeast strains differ in their ability to release the volatile thiols from their precursors and also in their ability to convert 3MH to 3MHA (Murat *et al.*, 2001a; Swiegers *et al.*, 2005; Swiegers *et al.*, 2006; Anfang *et al.*, 2009). 3MHA typically amounts to up to 10% of the concentration of 3MH (Tominaga *et al.*, 2000; Dubourdieu *et al.*, 2006).

The formation of volatile thiols can be manipulated through both viticultural and oenological operations. Research on the impact of viticultural practices on the production of volatile thiol precursors is limited and under question due to the main mechanisms of volatile thiol formation from precursors not yet being identified. The effect of factors such as nitrogen fertilization, water availability and *Botrytis cinerea* infection on certain precursors has been reported (Choné, 2001; Peyrot des Ganchos *et al.*, 2005; Choné *et al.*, 2006; Sarrazin *et al.*, 2007; Thibon *et al.*, 2009). During winemaking, various processes can also be adapted to maximise the precursor extraction and/or formation as well the liberation of the thiol from the precursor. Practices such as mechanical harvesting, skin contact, pressure during pressing and oxygen exposure could significantly increase the precursor content in the juice as well as the volatile thiol concentration in the corresponding wines (Maggu *et al.*, 2007; Patel *et al.*, 2010; Roland *et al.*, 2010; Capone & Jeffery, 2011). The liberation of the volatile thiols from the respective precursors depends on conditions such as yeast strain, nitrogen availability and fermentation temperature (Murat *et al.*, 2001a; Masneuf-Pomarède *et al.*, 2006; Swiegers *et al.*, 2006; Subileau *et al.*, 2008b; Anfang *et al.*, 2009; Harsch *et al.*, 2013). More detail regarding the influence of these processes on the volatile thiol concentrations can be obtained in a review article published in 2012 (Coetzee & Du Toit, 2012).

The volatile thiols are particularly susceptible to oxidation during aging (Murat *et al.*, 2003; Blanchard *et al.*, 2004) and various packaging and aging studies have reported the decrease of the volatile thiols during storage (Murat, 2005; Lopes *et al.*, 2009; Herbst-Johnstone *et al.*, 2011; Ghidossi *et al.*, 2012). Three mechanisms have been identified via which the thiol content can decrease (Figure 2.7). Volatile thiols can oxidise easily in the presence of oxygen and iron to form the corresponding disulphides (reaction 10) (Jocelyn, 1972; Kotserides *et al.*, 2000). Furthermore, these thiols are nucleophilic and capable of addition reactions with electrophiles such as polymeric phenolic compounds (Ribéreau-Gayon, 1998, 2004b) and participate in chemical reactions (1,4-Michael-type addition) with products of phenolic oxidation such as *o*-quinones (reaction 8) (Herbst *et al.*, 2008; Nikolantonaki *et al.*, 2010). The formed adducts are non-volatile and would cause a loss in varietal character. Recent observations reported the *o*-quinone trapping as the main mechanism accounting for 3MH loss in wine under oxidative conditions, while other reactions seems to contribute marginally (Kreitman *et al.*, 2013).

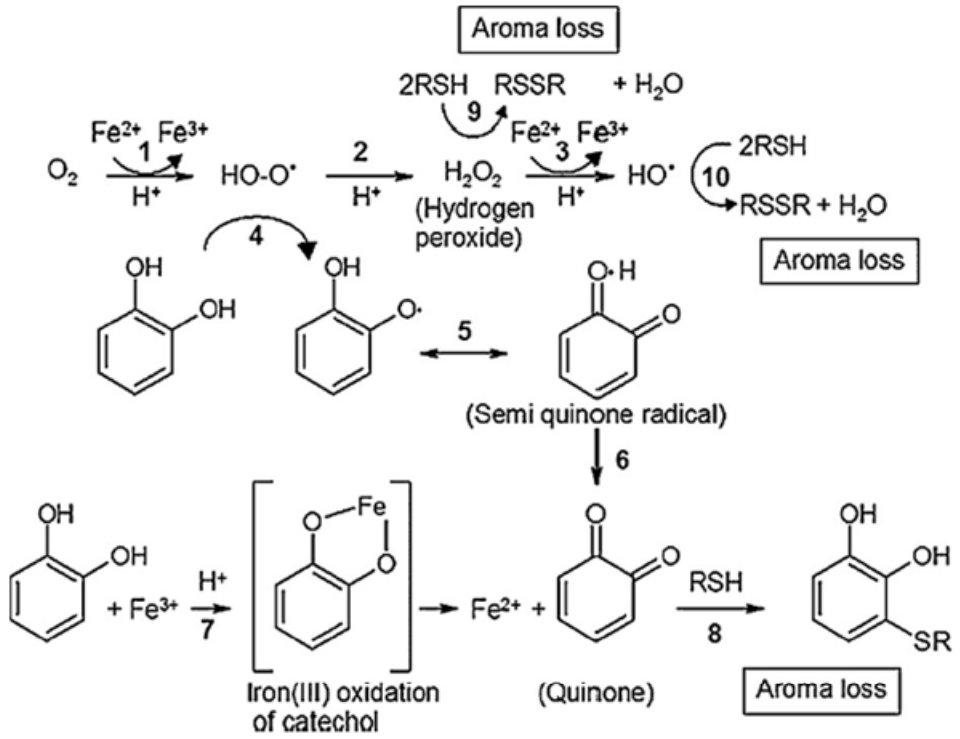


Figure 2.7 Possible mechanistic pathways for the the degradation of the volatile thiols in wine under oxidative conditions. Reprinted with permission from Nikolantonaki *et al.*, 2010. Copyright 2010 American Chemical Society.

The rate of these reactions are pH dependant, since at wine pH the concentration of the thiolate ion (RS^-), which is more reactive than its protonated form (RSH), are low with pK_a values for thiols being typically between 9 and 12. The greater proportion of thiolate anions at higher pH may explain the consistently lower levels of 3MH in these conditions (Blanchard *et al.*, 2004).

The decrease in especially 3MHA can also be attributed to acid-catalysed hydrolysis at wine pH (Figure 2.8) (Tominaga *et al.*, 2004). The ester structure of 3MHA makes it susceptable to this type of reaction. Volatile acetate esters are introduced enzymatically to the wine during fermentation by the action of yeast via a combination of acetyl-CoA with an alcohol which is catalysed by alcohol acetyltransferase. The products of this reaction are 3MH and acetic acid. The hydrolysis reaction is expected to be accelerated by higher temperature, but not directly affected by oxidative conditions (Makhotkina *et al*., 2012).

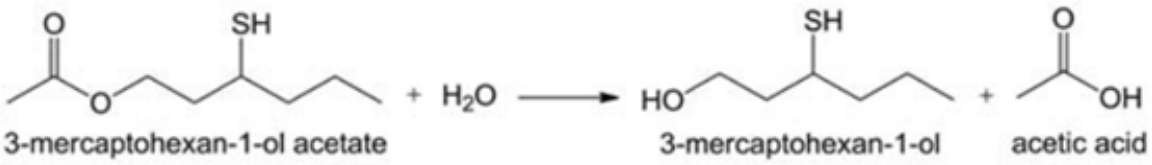


Figure 2.8 Hydrolysis of 3-mercaptohexan-1-ol acetate to 3-mercaptohexan-1-ol and acetic acid (Herbst-Johnstone *et al.*, 2011).

Acid hydrolysis was found to have a stronger influence than *o*-quinone trapping on the decrease of 3MHA concentrations during bottle aging of Sauvignon blanc wine under highly hermetic conditions such as screw caps (Herbst *et al.*, 2008; Herbst-Johnstone *et al.*, 2011). As 3MH has a higher perception threshold than 3MHA, the hydrolysis is expected to result in lower aromatic intensity and possibly different aroma.

The presence of SO_2 was shown to prevent the loss of volatile thiols due to its ability to recycle the *o*-quinones to the original phenol, bind them directly, or reduce H_2O_2 to water (Danilewicz *et al.*, 2008; Nikolantonaki *et al.*, 2010; Laurie *et al.*, 2012). However, the formation of the *o*-quinone adducts was shown to occur even in the presence of SO_2, although to a lower extent (Nikolantonaki *et al.*, 2012).

2.3.2 Methoxypyrazines

Methoxypyrazines are nitrogen-containing ring structures and secondary plant metabolites which are responsible for aroma descriptors such as “green pepper”, “asparagus”, “grassy”, “herbaceous” and “vegetative”. The aromatically potent alkyl methoxypyrazines are the main compounds thought to be responsible for these flavours in especially Sauvignon blanc wines. Three main methoxypyrazines exist in wines, namely 3-isobutyl-2-methoxypyrazine (IBMP), 3-isopropyl-2-methoxypyrazine (IPMP) and 3-*sec*-butyl-2-methoxypyrazine (SBMP) (Figure 2.9) (Allen *et al.*, 1991; Lacey *et al.*, 1991; Marais, 1994).

These compounds have a very low odour threshold value of 1-2 ng/L in water (Buttery *et al.*, 1969; Allen *et al.*, 1991; Lacey *et al.*, 1991; Marais, 1994) and surveys done on South African Sauvignon blanc wines

reported the IBMP (the most important methoxypyrazine) concentrations to be in the range of 1.2 to 40 ng/L (Alberts *et al.*, 2009; Van Wyngaard, 2013). SBMP is rarely detected in Sauvignon blanc wines, whereas IPMP can occur at concentrations above its perception threshold (up to 13.7 ng/L) (Lubbers *et al.*, 1994; Guyot *et al.*, 1995; Kwiatkowski *et al.*, 2007).

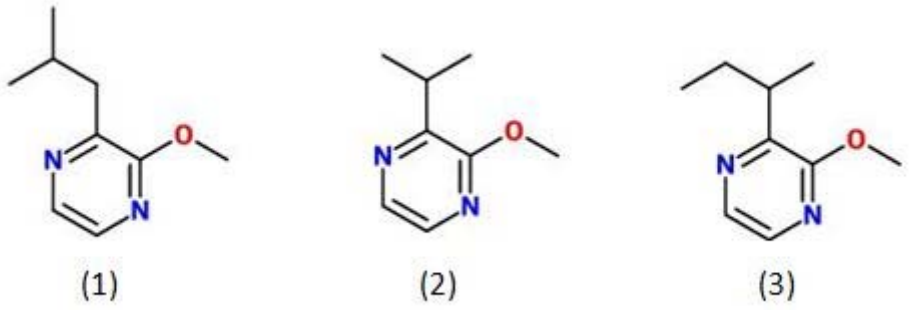


Figure 2.9 3-Isobutyl-2-methoxypyrazine (IBMP) (1), 3-isopropyl-2-methoxypyrazine (IPMP) (2) and 3-*sec*-butyl-2-methoxypyrazine (SBMP) (3).

The "herbaceous" notes of methoxypyrazines are usually associated with under-ripe grapes and are undesirable for most varieties, however Sauvignon blanc seems to be the exception. The formation of these compounds in the vine has not been fully elucidated (Ebeler & Thorngate, 2009). The structure would suggest their origin as a secondary product during the metabolism of amino acids (Marais, 1994; Swiegers *et al.*, 2006; Ebeler & Thorngate, 2009). The methoxypyrazine concentration in the berry evolves during the maturation with the maximum concentration found at vériason. After this, the concentrations decrease with ripening (Lacey *et al.*, 1991). Elevated levels of these compounds are normally found in cooler climates (Lacey *et al.*, 1988; Green *et al.*, 2011). Warmer climates usually deliver lower concentrations which is thought to be due to increased light exposure during ripening (methoxypyrazines are light sensitive) (Hashizume & Samuta, 1999; Ryona *et al.*, 2008) in these areas as well as the elevated average temperatures (Falcao *et al.*, 2007). In general, the methoxypyrazine content is more affected by the viticultural practices than by the vinification processes (Marais, 1994; De Boubee *et al.*, 2002; Hunter *et al.*, 2004; Maggu *et al.*, 2007).

The methoxypyrazines are highly extractable from Sauvignon blanc grapes. Skin contact has a greater impact on the IBMP concentration compared to the pressure applied during pressing (Maga, 1989). However, settling of the juice before fermentation can lead to a decrease in methoxypyrazine concentrations (Kotserides *et al.*, 2008), while concentrations have been reported to remain stable during fermentation (Sala *et al.*, 2004). The compounds are generally resilient to standard wine fining practices (Pickering *et al.*, 2006) and during aging concentrations did not differ when exposed to various aging conditions such as light exposure and temperature variations (Blake *et al.*, 2010). Even

hyperoxidation of musts and wine did not alter the methoxypyrazine content (Marais, 1998; Coetzee *et al.*, 2013). Surprisingly, a poor correlation was obtained when comparing methoxypyrazine concentration with the aromatic intensity of the "green pepper" attribute. The contribution of other aromatic compounds such as the varietal thiols and dimethyl sulphide to these types of attributes can be significant in Sauvignon blanc wines and help explain the weak correlation observed previously (Park *et al.*, 1994; Marais *et al.*, 1998; Lund *et al.*, 2009b; King *et al.*, 2011; Van Wyngaard, 2013).

2.3.3 Esters, higher alcohols, fatty acids

Esters constitute one of the most important classes of aroma compounds and are largely responsible for the fruity aromas associated with wine (Lilly *et al.*, 2000; Swiegers *et al.*, 2006). Esters are mainly produced during fermentation by the condensation of an alcohol and a coenzyme-A-activated acid, by the action of alcohol acetyl transferase (Lambrechts & Pretorius, 2000). A large variety of esters can be formed as all of the alcohols (especially ethanol) and fatty acids may react to form esters. Acetate esters of the higher alcohols and the ethyl esters of straight chain, saturated fatty acids are the most significant esters produced in wine (Lambrechts & Pretorius, 2000). Some of the most quantitatively significant esters in wine have been identified to be isoamyl acetate, ethyl hexanoate and 2-phenylethyl acetate (Thurston *et al.*, 1981).

Ester concentration can decrease during aging due to chemical hydrolysis (Marais, 1978; Ferreira *et al.*, 1997; Lambropoulos & Roussis, 2007) or oxidation by hydroxyl-radical oxidation-related processes (Litchev, 1989; Escudero *et al.*, 2000a) which can both lead to a loss in the fruity character of a wine. The hydrolysis is favoured at elevated temperatures and low pH values (Ramey & Ough, 1980). The decline in especially the acetate esters contributes to the loss of freshness and fruitiness in white wines during bottle aging. Acetate esters of higher alcohols tend to diminish more rapidly during aging compared to ethyl esters of fatty acids. Ethyl esters are thought to be close to their chemical equilibrium in young wines (Simpson, 1978) and the hydrolysis that occurs during storage happens relatively slowly (Ramey & Ough, 1980).

Higher alcohols are also a product of alcoholic fermentation and can be important precursors for ester formation (Soles *et al.*, 1982). At concentrations below 300 mg/L they generally contribute to the complexity of the wine aroma (Rapp & Mandery, 1986). However, at higher concentrations the aroma can become too intense and can contribute to a strong pungent smell and taste (Nykänen, 1986), thus possibly masking other aroma contributors. Higher alcohols can be anabolically synthesized from intermediates of the sugar metabolism or catabolically synthesized from branch-chain amino acids, through the Ehrlich pathway (Nykänen, 1986; Boulton *et al.*, 1996; Dickinson *et al.*, 1997; Dickinson *et al.*, 2003). During aging, alcohols can be oxidised to form aldehydes (Marais & Pool, 1980), causing the concentration to decrease, however many studies report stable alcohol concentrations during the aging

of wines (Marais, 1978; Roussis *et al.*, 2005; Roussis *et al.*, 2007; Blake *et al.*, 2009). Unlike other higher alcohols, hexanol concentration can increase during storage, which is probably due to the oxidation of linoleic and linolenic acids (Oliveira *et al.*, 2006).

Fatty acids, of which the most abundant are acetic, hexanoic, octanoic and decanoic acid contribute to the fresh flavour of wine. However, at too high concentrations fatty acids it can lead to unwanted flavours associated with "rancid", "cheesy" and "vinegar" aroma (Schreier, 1979; Lambrechts & Pretorius, 2000). Medium chain fatty acids, such as hexanoic, octanoic and decanoic acid are produced by the yeast as intermediates in the biosynthesis of long chain fatty acids. The hydrolysis of ethyl esters during aging can lead to an increase in the corresponding acid. However, an increase in these acids has not always been observed as studies have shown that the volatile fatty acid stability is not uniform as some compounds increase while others decrease or remain stable during aging (Roussis *et al.*, 2005; Câmara *et al.*, 2006; Blake *et al.*, 2009; Lee *et al.*, 2011).

Many factors can influence the formation and degradation of esters, higher alcohols and fatty acids during fermentation, aging or oxidation. Fermentation conditions such as temperature, juice clarification, yeast strain as well as other parameters such as oxygen exposure and SO_2 additions are some of the most important factors (Bertrand, 1968; Daudt & Ough, 1973; Marais, 2001; Garde-Cerdán & Ancín-Azpilicueta, 2007; Coetzee *et al.*, 2013).

2.3.4 Monoterpenes

Monoterpenes are known for their "floral", "fruity", "citrus" and "perfume" odours usually expressed by geraniol, linalool, nerol and α-terpineol (Marais, 1983). Sauvignon blanc can be classified as a member of intermediate class between monoterpene-dependant floral grapes and monoterpene-deficient non-floral grapes (Benkwitz *et al.*, 2012a). Most terpenes increase during ripening (Bayonove & Cordonnier, 1971) with some studies reporting a decrease at the overripe stage (Versini *et al.*, 1981). A considerable proportion of these compounds is in the bound form in the juice and is released during fermentation by the yeast, however some can become aromatic by chemical rearrangement (Williams *et al.*, 1980; Loscos *et al.*, 2007). During aging, wines are known to lose some of the floral aromas associated with monoterpenes (Rapp & Mandery, 1986; Rapp, 1988).

Linalool specifically is known to decrease during storage (Ferreira *et al.*, 1997; Lambropoulos & Roussis, 2007) while α-terpineol initially increased (probably due to the oxidation of other terpenols) and then decreased at a later stage (Ferreira *et al.*, 1997). Terpenes are sensitive to acidic conditions, storage time and temperature and can be transformed into other compounds which could contribute to a different aroma and have different perception thresholds (Papadopoulou & Roussis, 2001; Roussis *et al.*, 2005; Roussis *et al.*, 2007). The importance of monoterpenes in wines is accentuated by the fact that

they act synergistically in a wine medium and can thus influence the aromatic composition of a wine (Ribéreau-Gayon *et al.*, 1975).

2.4 REDUCTIVE AROMA

'Reductive' aroma properties have been described as "rotten egg", "cabbage" and "garlic". The compounds responsible for these aroma attributes usually form after a period in the bottle. The occurrence of these odours has been attributed to the presence of low molecular weight sulphur compounds. Hydrogen sulphide (H_2S) and methyl mercaptan (MeSH) have been identified to be primarily responsible for the post-bottling reduction (Lopes *et al.*, 2009; O'Brien *et al.*, 2009; Ugliano *et al.*, 2011; Ugliano *et al.*, 2012). H_2S can accumulate during bottle aging and concentrations 3-4 times higher than its perception threshold has been reported after 6 months in the bottle (Ugliano *et al.*, 2011).

The mechanisms for the formation of the reductive compounds are not fully understood, however the formation of H_2S from a cysteine precursor in the presence of a dicarbonyl compound as well as the direct reduction of sulphate or sulphite have been suggested (Pripis-Nicolau *et al.*, 2000; Lopes *et al.*, 2009). Interestingly, studies have shown an increase in the formation of 'reductive' odours in wines that were treated with copper sulphite at bottling, which is a common practice amongst winemakers to prevent the formation of these odours (Ugliano *et al.*, 2011). H_2S can also participate in other reactions such as the reaction with benzaldehyde to form methyl mercaptan which can contribute to the 'reductive' aroma of the wine with "smokey/empyreumatic", "cabbage" and "sewage" nuances, however this has not been conclusively demonstrated (Tominaga, 2003; Tominaga *et al.*, 2003; Ugliano, 2013).

Low levels of oxygen exposure during bottle aging correspond to a larger accumulation of H_2S and MeSH (Lopes *et al.*, 2009; Ugliano *et al.*, 2011; Ugliano *et al.*, 2012) with the accumulation of MeSH mainly occurring between the first 6 to 12 months of bottle storage (Ugliano *et al.*, 2012). Dimethyl sulphide (DMS) also increases during bottle aging and can have an important contribution to wine aroma (Segurel *et al.*, 2005; Escudero *et al.*, 2007). The formation of DMS does not seem to be affected by oxygen exposure (Ugliano *et al.*, 2012), although a decrease in DMS has been reported in the presence of excess oxygen (Silva Ferreira *et al.*, 2003d; Fedrizzi *et al.*, 2011). Oxygen exposure will also lead to a decrease in H_2S and MeSH concentrations. Oxidation of mercaptans to the corresponding disulphides has been proposed, but not proven (Limmer, 2005) and recent data did not find a direct relationship between the mercaptan and the disulphides (Nguyen *et al.*, 2010; Ugliano *et al.*, 2012). The reaction of reductive compounds with *o*-quinones resulting from phenolic oxidation could explain the decrease of especially H_2S and MeSH in an oxygenated wine due to the high reactivity of H_2S toward *o*-quinones in wine-like

solutions (Nikolantonaki & Waterhouse, 2012). The presence of oxygen at the during the early stages of bottle aging will thus prevent the formation of these compounds, possibly at a precursor level, however this needs further investigation (Ugliano, 2013).

2.5 OXIDATION AROMA

Early work on oxidative spoilage of white wines indicated that oxidation brought about sensory characters described as “honey”, “farmfeed”, “woody” and “cooked vegetables” (Escudero *et al.*, 2002; Silva Ferreira *et al.*, 2003c). A number of trace aroma compounds have been identified as major contributors to these aroma attributes, including various aldehydes, lactones and acetals (Escudero *et al.*, 2000a; Escudero *et al.*, 2000b; Escudero *et al.*, 2002; Silva Ferreira *et al.*, 2002b; Silva Ferreira *et al.*, 2003c). The aldehydes are especially important due to their possible impact on wine aromatic composition (Escudero *et al.*, 2000b; Silva Ferreira *et al.*, 2003c; Culleré *et al.*, 2007). The various chemical compounds potentially contributing to wine oxidation aromas will be discussed in the following sections.

2.5.1 Aldehydes

Acetaldehyde is one of the most important carbonyl compounds in wine and constitutes up to 90 % of the total amount of aldehydes found in wine (Nykänen, 1986). Its organoleptic influence and its ability to combine rapidly with SO_2 even at low temperatures makes this compound one of the critical markers during winemaking (Burroughs & Sparks, 1973). Acetaldehyde is formed by the yeast during alcoholic fermentation (Margalit, 1997) and can also originate from microbial activity of other microbes such as lactic acid bacteria and acetic acid bacteria (Drysdale & Fleet, 1988; Liu & Pilone, 2000). However, winemaking practices that enhance acetaldehyde formation are usually post-fermentation and can lead to moderate to important acetaldehyde increases (Jackowetz & De Orduña, 2013).

The most important production of acetaldehyde is during the oxidation of ethanol (Wilderandt & Singleton, 1974; Ribéreau-Gayon, 1998, 2004b). This reaction is not direct, but rather via coupled auto-oxidation of certain phenolic compounds (Figure 2.3) (Wilderandt & Singleton, 1974) and the formation will depend on the amount of oxygen present (Kielhöfer & Würdig, 1960; Schneider, 2003, 2005). Free SO_2 present in the wine will prevent this oxidation by reacting with intermediate oxidation products as well as the formed acetaldehyde resulting in an odourless sulphite combination, known as hydroxysulphonate, which is stable in the acid medium (Figure 2.10) (Waterhouse & Laurie, 2006).

$$CH_3\text{-}CHO + HSO_3^- \rightleftharpoons CH_3\text{-}CHOH\text{-}SO_3^-$$

Figure 2.10 Reaction of acetaldehyde with bisulphite

The reaction between acetaldehyde and bisulphite is rapid and at a pH of 3.3, 98% of the acetaldehyde will be combined with the sulphite within 90 minutes. It has been estimated that only 0.04% of acetaldehyde is in the free form in the presence of 30 mg/L free SO_2 (Blouin, 1965). Thus only when all the free acetaldehyde has been consumed can SO_2 exist in the free form in wine. Acetaldehyde has been identified as the most important SO_2 binder in a study screening 237 table wines and accounted for 55% to over 70% of the estimated bound SO_2 for red and white wines respectively (Jackowetz & De Orduña, 2013). The production of acetaldehyde will thus effectively cause a decrease in free SO_2 concentration with the subsequent increase in bound SO_2. Due to the decreased preservative activity of the bound form (Rankine, 1968), the addition of further SO_2 will be required to efficiently maintain antimicrobial and antioxidant properties. Acetaldehyde also readily binds to proteins and especially to GSH or individual amino acids (Liu & Pilone, 2000). Cysteine additions reduced acetaldehyde concentrations in beer (Suovaniemi *et al.*, 2006) and the use of other sulphur-containing compounds as additives to bind acetaldehyde in beverages should be investigated. Acetaldehyde concentration (free and bound) in dry white wines has been reported to range between 7 and 240 mg/L averaging at 40 mg/L (Lopes *et al.*, 2009; Jackowetz & De Orduña, 2013), while the highest concentrations were reported in fortified wines ranging from 12-800 mg/L (Lachenmeier & Sohnuis, 2008).

Acetaldehyde in the free form is a key impact aroma compound for sherry wines and can reach high concentrations in these types of wines (Martínez *et al.*, 1998) due to the absence of free SO_2. Odours associated with the presence of free acetaldehyde have been described as “green apple”, “overripe bruised apple”, “grassy”, “pungent”, “nutty” and “sherry” (Margalith, 1981; Henschke & Jiranek, 1993; Miyake & Shibamoto, 1993; Frivik & Ebeler, 2003). At low concentrations, the presence of free acetaldehyde could contribute to the pleasant fruity aroma of a wine, however at higher concentrations the typical oxidation-related nuances will develop (Miyake & Shibamoto, 1993; Zea *et al.*, 2010). The bound form reduces the sensory effect of acetaldehyde (Jackowetz *et al.*, 2011) and it is thus recommended to maintain a positive level of free SO_2 to ensure fixation of acetaldehyde (Somers & Wescombe, 1987).

Other aldehydes can contribute to “honey”, “boiled vegetable” and “rotten potato” nuances in oxidised wines (Escudero *et al.*, 2000b; Culleré *et al.*, 2007). Of these aldehydes, the Strecker aldehydes, 3-(methylthio)-propionaldehyde (methional) and phenylacetaldehyde, have been identified as major contributors (Figure 2.11) (Escudero *et al.*, 2000a; Escudero *et al.*, 2000b; Escudero *et al.*, 2002; Silva Ferreira *et al.*, 2002b; Silva Ferreira *et al.*, 2003c). The formation of these aldehydes has been proposed

to occur via the Strecker reaction of dicarbonyl compounds with amino acids, methionine and phenylalanine to form methional and phenylacetaldehyde respectively (Figure 2.12) (Escudero *et al.*, 2000b; Pripis-Nicolau *et al.*, 2000; Silva Ferreira *et al.*, 2002b; Rizzi, 2006).

Wine contains several types of dicarbonyl compounds. Dicarbonyls (such as *o*-quinones) can be formed via oxidation of a phenolic molecule, while other dicarbonyls such as diacetyl, glyoxal and methylglyoxal originate from microbial origin (Pripis-Nicolau *et al.*, 2000). The formation of aldehydes via the Strecker reaction in wine has been brought under question as the reaction rate between 4-methyl-1,2-benzoquinone and the α-amino acids in a synthetic wine medium was essentially zero (Nikolantonaki & Waterhouse, 2012), compared to a neutral aqueous solution without added ethanol at 22˚C, conditions that are different from the acidic wine matrix (Rizzi, 2006). The pH of the medium could have an important role in controlling the rate of α-amino addition (Youngblood, 1986; Modica *et al.*, 2001).

A B

Figure 2.11 Strecker aldehydes. A) Methional; B) Phenylacetaldehyde

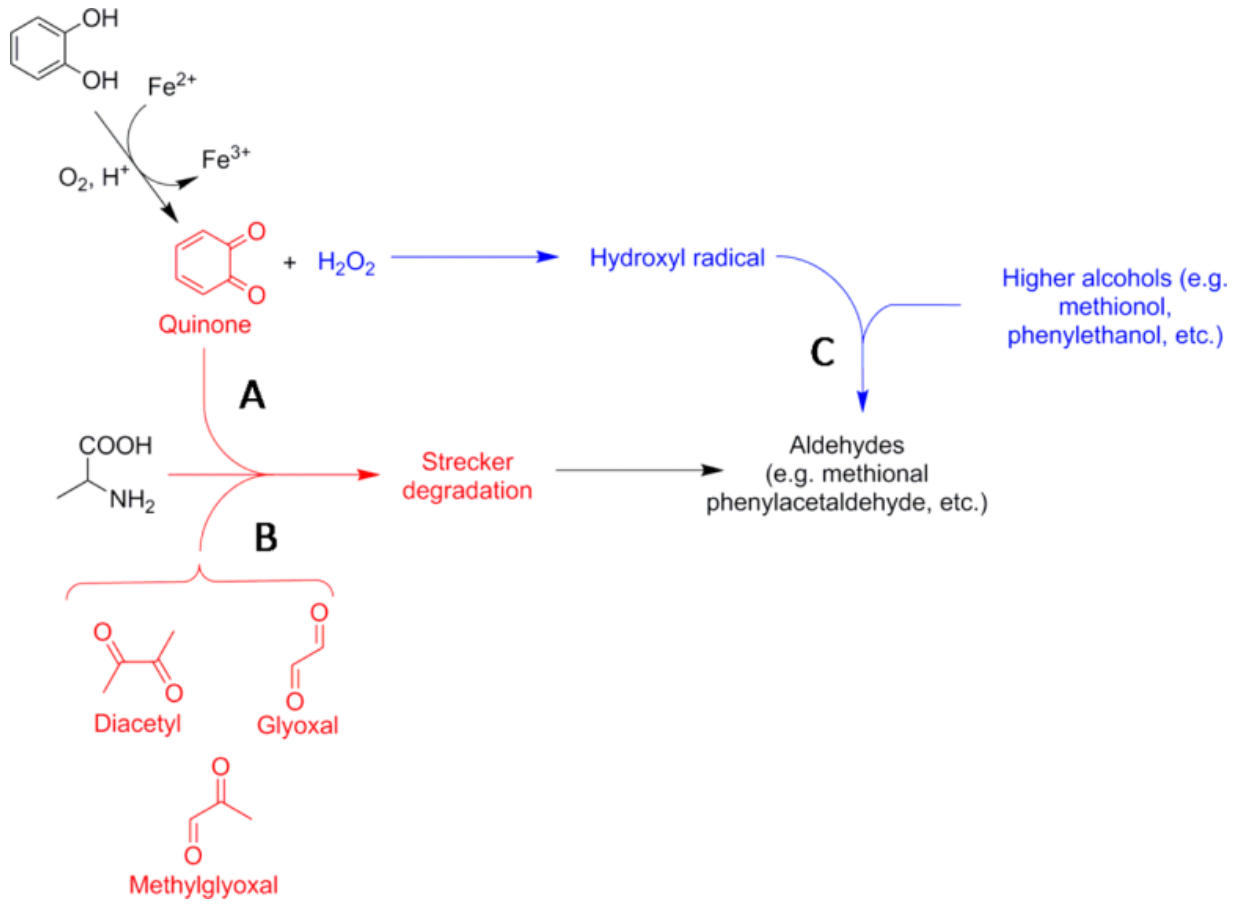


Figure 2.12 Mechanism of formation of branched-chain aldehydes in wines via (A) Strecker degradation of amino acids involving *o*-quinones derived from phenolic oxidation, (B) Strecker degradation involving dicarbonyl compounds of microbial origin, or (C) oxidation of higher alcohols by hydroxyl radical.
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The formation of aldehydes from the oxidation of related fermentation derived alcohols (phenylethanol and methionol) has also been suggested (Escudero *et al.*, 2000a; Escudero *et al.*, 2000b; Pripis-Nicolau *et al.*, 2000; Silva Ferreira *et al.*, 2002b; Rizzi, 2006; Loscos *et al.*, 2010; Fedrizzi *et al.*, 2011; Nikolantonaki & Waterhouse, 2012) and a good correlation between the alcohol and corresponding aldehyde has been observed in aged wines (San Juan *et al.*, 2012). Such a mechanism would be analogous to the one generating acetaldehyde from ethanol.

Methional and phenylacetaldehyde have perception thresholds of 0.5 µg/L and 1 µg/L in a synthetic wine medium respectively (Escudero *et al.*, 2000b; Culleré *et al.*, 2007) and concentrations in a young Sauvignon blanc wine just after bottling were found to be below 0.5 and 5 µg/L respectively. After 18 months aging at 20˚C, the concentrations increased to about 5.5 and 34 µg/L respectively (Ghidossi *et al.*, 2012). Higher concentrations of these compounds have been reported in more extreme storage conditions such as higher dissolved oxygen concentrations and higher storage temperatures (Silva Ferreira *et al.*, 2003c).

The formation of methional and phenylacetaldehyde are greatly affected by temperature and the dissolved oxygen concentration (Silva Ferreira *et al.*, 2002b). However, both factors are not required for the formation of the aldehydes as it was found in beer that the aldehydes formed in the absence of oxygen (<0.2 mg/L) (Soares da Costa *et al.*, 2004). Oxidation of the alcohols as well as the Strecker degradation reaction will be prevented by the presence of SO_2 (Silva Ferreira *et al.*, 2003c), probably due to its ability to reduce H_2O_2 to water, convert the *o*-quinone back to the *o*-diphenol and also reversibly reacting with the microbial-derived dicarbonyls. Thus SO_2 should effectively be able to prevent the formation of these aldehydes.

Furfural and other furanic aldehydes such as 5-methylfurfural (5-MF) and 5-hydroxymethylfurfural (5-HMF) usually originate from the heating process of oak during barrel toasting (Moutounet *et al.*, 1989). The thermal degradation of polysaccharides produces these furanic aldehydes from carbohydrate polymers. The formation of furfural due to the non-oxidative decay of ascorbic acid has also been reported (Bauernfeind & Pinkert, 1970; Yuan & Chen, 1998; Wallington *et al.*, 2013), however in the case of aged wines (especially those not containing ascorbic acid), the occurrence of these compounds is probably due to the degradation of carbohydrates (Câmara *et al.*, 2004). A good correlation between aging time and the concentration of these furanic compounds were found in Port and Madeira wines (Silva Ferreira *et al.*, 2003a; Câmara *et al.*, 2004). Ferreira *et al.* (1997) also reported a correlation between furfural concentration and wine browning during aging. The concentration of furfural and 5-MF in a range of white wines oxidised for 1 week was found to be between 16-342 µg/L and 10-60 µg/L respectively (Escudero *et al.*, 2002). Perception thresholds of 150 mg/L, 20 mg/L and 100 mg/L have been reported for furfural, 5-MF and 5-HMF respectively (Meilgaard, 1975; Câmara *et al.*, 2004) which would suggest minor organoleptic contribution due to the concentrations being below the perception threshold (Ribéreau-Gayon, 2006). However, the contribution of these compounds to “woody” or “maderized” nuances during the oxidation of white wines has been reported and could explain the perception of these odours in wines that did not receive any wood contact (Escudero *et al.*, 2002; Campo *et al.*, 2008).

Benzaldehyde is always present in wine and is produced by alcoholic fermentation in concentrations up to 0.5 mg/L. During wine aging, benzaldehyde formation has been attributed to phenylalanine oxidation (Loyaux *et al.*, 1981), while other authors reported its origin from amygdalin (Nykänen & Suomalainen, 1982). The concentration of benzaldehyde in a range of white wines oxidised for 1 week was found to be between 20-313 µg/L (Escudero *et al.*, 2002) and the compound correlated well with the term “liquor”(Escudero *et al.*, 2002). The odour detection threshold for benzaldehyde is 0.35 mg/L in water (Belitz *et al.,* 2009).

2.5.2 Other carbonyl compounds and acetals

Sotolon is a chiral furanone (lactone) (Figure 2.13) and a very powerful odourant that has been described to have the odour of "curry" and "myrrh" at high concentrations with "roasting", "maple syrup", "burnt sugar" and "caramel" at lower concentrations (Escudero *et al.*, 2000a; Ghidossi *et al.*, 2012). Sotolon has been demonstrated to be a key odourant in sherry, port, "vin jaune", botrytized and "Tokai wines" (Masuda *et al.*, 1984; Martin & Etiévant, 1991; Guichard *et al.*, 1992; Martin *et al.*, 1992; Silva Ferreira *et al.*, 2003a; Collin *et al.*, 2012), but can have a detrimental effect when present in dry white wines (Escudero *et al.*, 2002; Silva Ferreira *et al.*, 2003c; Lavigne *et al.*, 2008).

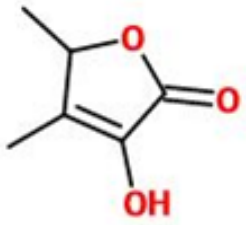


Figure 2.13 Chemical structure of sotolon.

The mechanism for the formation of this compound is still not fully understood, however a few pathways have been suggested and the connection between oxidation and sotolon formation seems to be evident (Pham *et al.*, 1995; Cutzach *et al.*, 1999; Silva Ferreira *et al.*, 2003a; Silva Ferreira *et al.*, 2003c; Escudero *et al.*, 2011). The formation of sotolon can occur via the enzymatic or chemical deamination of threonine followed by aldol condensation with acetaldehyde in various wine media (Takahashi *et al.*, 1976; Pham *et al.*, 1995; Cutzach *et al.*, 1998). Threonine is deaminated by threonine deaminase, forming α-ketobutyric acid. The condensation reaction between α-ketobutyric acid and acetaldehyde followed by lactonisation can then form sotolon.

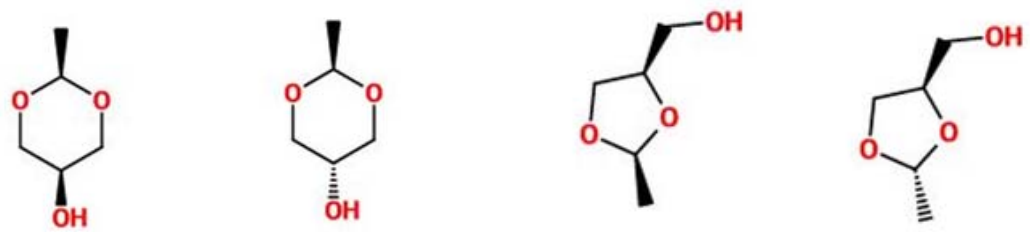
Sotolon can also be formed from the oxidative degradation of ascorbic acid in the presence of ethanol (König *et al.*, 1999; Pons *et al.*, 2010). This mechanism is most likely to occur in dry white wines, especially considering the common practice of adding ascorbic acid to Sauvignon blanc must/wine during winemaking. During the oxidative decomposition of the ascorbic acid, α-ketobutyric acid is formed as degradation intermediate. Maillard reactions in several combinations of binary mixtures of cysteine and three sugars, ribose, glucose and rhamnose also resulted in the formation of sotolon (Hofmann & Schieberle, 1995, 1997). This seems to be a less likely mechanism in the case of white wines, as these reactions occur under much harsher conditions than those found during aging (Pons *et al.*, 2010).

The concentrations of this compound in dry white wines are much less compared to other alcoholic beverages (fortified wine, port, sherry) (Salmon *et al.*, 1999). Concentration in young Sauvignon blanc

wines just after bottling were found to be below 0.5 µg/L while Sauvignon blanc wine stored under various types of closures during a 24 month period had a sotolon content ranging from 0.1-1.1 µg/L (Lopes *et al.*, 2009; Ghidossi *et al.*, 2012). Another study measured concentrations of up to 10 µg/L for Sauvignon blanc wines aged between 2 and 32 years (Lavigne *et al.*, 2008). The formation of this compound in white wines seems to be somewhat dependant on temperature and a concentration of around 8 µg/L were obtained during a forced aged experiment conducted at 60˚C (Silva Ferreira *et al.*, 2003b). The odour threshold for this compound has been determined to be 15 µg/L in flor sherry (Martin *et al.*, 1992), 2 µg/L in model wine (Pons *et al.*, 2010) and 8 µg/L in dry white wines (Lavigne *et al.*, 2008).

Acetals are formed by the condensation reaction between glycerol and acetaldehyde in an acid medium. Four isomers can be formed via this reaction: *cis*- and *trans*-5-hydroxy-2-methyl-1,3-dioxane and *cis*- and *trans*-4-hydroxymethyl-2-methyl-1,3-dioxolane (Figure 2.14). In Port wine, isomers of glycerol and acetaldehyde acetals have been found at total concentrations ranging from 9.4 to 175.3 mg/L (Silva Ferreira *et al.*, 2002a). During oxidation of wine large amounts of the dioxanes and dioxolanes are expected to be produced due to the fact that ample quantity of substrates is available.

The aromatic impact of these compounds could contribute significantly to the oxidised odour perceived in some wines. Odour evaluation by sniffing in gas chromatography rated the *trans*-5-hydroxy-2-methyl-1,3-dioxane as the compound with the highest intensity aroma contributing to a “sweet” and “old port-like” aroma (Silva Ferreira *et al.*, 2002a).



A B C D

Figure 2.14 Isomers of acetaldehyde and glycerol acetals. A) *cis*-5-hydroxy-2-methyl-1,3-dioxane ; B) *trans*-5-hydroxy-2-methyl-1,3-dioxane; C) *cis*-4-hydroxymethyl-2-methyl-1,3-dioxolane; D) *trans*-4-hydroxymethyl-2-methyl-1,3-dioxolane.

2.6 SENSORY SCIENCE

Evaluating the chemical composition of a wine can deliver valuable information such as the type and quantity of specific aromatic compounds present, however the use of the chemical composition only is not adequate to predict the aroma profile and wine quality. This is important in a complex medium such

as wine where interactions between all compounds present could influence the sensory perception of the aroma. Sensory analysis (especially descriptive analysis) of wines is thus of critical importance for the evaluation of wine aroma (Stone & Sidel, 1993; Lawless & Heymann, 1998). The combination of the chemical and sensory data could deliver valuable information concerning the aroma profile of the wine and how various compounds interact.

2.6.1 Descriptive analysis

Descriptive analysis is a standard profiling method that has been used since 1974 to define the sensory attributes for a specific product set (Chollet *et al.*, 2011). According to Lawless & Heymann (2010), descriptive analysis methods such as Quantitative Descriptive Analysis® (QDA) are considered to be the most sophisticated tools in sensory science. These tests identify, describe and quantify the difference in sensory attribute intensities between samples within a sample set by using a small panel of usually 8 to 12 people (Lawless & Heymann, 2010) whom are extensively trained for a specific product category and are expected to then work as an analytical instrument (Nestrud & Lawless, 2008; Næs *et al.*, 2010).

With proper training, the researcher can expect little variance between individual results which justifies the use of a smaller panel. The error in variance can thus be lowered by intensive training, whilst still maintaining statistical power and test sensitivity (Lawless & Heymann, 2010). There are various descriptive analysis tests such as the Flavor Profile® method (Caul, 1957); the Texture Profile® method (Brandt *et al.*, 1963; Szczesniak, 1975); Quantitative Descriptive Analysis® (Stone & Sidel, 2004); the Spectrum Method® (Meilgaard *et al.*, 2006) and other hybrid versions of these techniques that can be used by researchers (Einstein, 1991). These techniques are classified as the most comprehensive and informative sensory methods in the history of sensory science (Lawless & Heymann, 2010).

The procedure of QDA starts with the generation of attributes to describe the products. Thereafter physical or chemical standards are selected which act as references for the selected attribute terms during the training period. Association of an attribute with a single word of phrase is a critical step during QDA. After concept formation and verbalisation training, assessors are trained to rate all the applicable attribute intensities on an intensity line scale (Chollet *et al.*, 2011). Intensity scales can be presented as various numberings with an upper and a lower limit or as a structured or unstructured line scale (Næs *et al.*, 2010). Repetitive training using the line scale is then done to profile the specific sample set according to the attribute list. The final profiling test does not include a discussion and the samples are randomised per panellist in order to prevent any first-order effect to influence the panellist's perception of a specific sample.

Due to the fact that panellists are intensively trained to identify differences in specific sensorial attribute intensities, one can use QDA on product sample sets that are similar to one another. Data collected from QDA tests can be analysed statistically by using parametric statistics such as analysis of variance

(ANOVA), whereas the relationships between the attributes are often described using multivariate methods such as principle component analysis (PCA).

QDA does have a few disadvantages. This technique is time consuming. Panel members must thus commit to the training procedures which can take up to months due to critical language development and calibration (Chollet *et al.*, 2011). The long periods of time needed for training and testing as well as large volumes of wine needed, make this test procedure quite expensive. Another disadvantage is the fact that the attributes generated for a specific product cannot be used as a generic for other products. This would effectively mean that a panel needs to be trained anew for each new product set adding more hours to the analysis which can become financially too strenuous for many companies and researchers (Bitnes *et al.*, 2007). Consequently most industries cannot routinely use this technique (Stone & Sidel, 2004; Meilgaard *et al.*, 2006; Kemp *et al.*, 2009).

More cost-efficient methods such as free choice profiling (Williams & Langron, 1984), flash profiling (Dairou & Sieffermann, 2002) and projective mapping (Napping®) (Risvik *et al.*, 1994; Risvik *et al.*, 1997) have been developed in the past few years in an attempt to replace QDA (Guinard *et al.*, 2001). QDA could also potentially create comprehension and agreement problems within the panel due to the importance of language (Chollet *et al.*, 2011). The difference in the use of the intensity scales by the various panellists is also a disadvantage. However, this can be corrected by continuously checking the panel for consensus and consistency using specific software packages such as PanelCheck® (V1.4.0, NOFIMA, Norway) which is a freeware R-based program (Lawless & Heymann, 2010). Methods to evaluate the panel such as Tucker plots and the use of ANOVA have proven to deliver best results, however the combination of the two methods would be considered ideal. Tucker plots are used to assess each panel member's performance in relation to a specific attribute or the whole panel. It will identify which panellists struggle to differentiate between different samples, while ANOVA is used to elucidate the importance of an attribute and the significance of that attribute in the wines tested (Tomic *et al.*, 2007; Tomic *et al.*, 2010).

QDA describes the wine attributes and the intensities at which these attributes occur in each sample. Intensive training would also allow the identification of small but significant differences between the samples even in cases where the samples are sensorially similar.

Descriptive analysis has successfully been used as a tool to differentiate between Sauvignon blanc wines from different vintages and countries (Lund *et al.*, 2009b; Green *et al.*, 2011). These techniques have also been used to investigate interactions between various compounds in Sauvignon blanc wine (King *et al.*, 2011; Benkwitz *et al.*, 2012a) as well as to identify off-flavours in oxidation spoiled white wines (Silva Ferreira *et al.*, 2003c).

2.6.2 Sensory interaction studies

Over the last two decades aroma reconstitution approaches have added a new dimension to sensory investigations. In this way, sensory interactions of chemical compounds at different concentrations and in various media are investigated (Guth, 1997; Lee, 2003; Ferreira *et al.*, 2006). Studies indicated that wine aroma is influenced by complex interactions between various wine constituents and rarely dominated by a single component (Escudero *et al.*, 2007).

In a basic or neutral wine the mixture of ethanol, esters, alcohols and acids will create an aroma "buffer" (Ferreira *et al.*, 2008). This is considered to form the base of the wine aroma and consists of various aromatic compounds usually present in concentrations above their perception threshold. This buffering property can attenuate the addition or omission effect of aroma compounds. The compounds are not perceived as individual entities but rather as an integrated complex medium (Ferreira *et al.*, 2002; Escudero *et al.*, 2004). The ability of an odourant to break the buffer and change the aromatic composition of the medium will define or classify the odourant (Ferreira *et al.*, 2008).

One or more of the following mechanisms could break the buffer: 1) a single type of molecule present at high enough concentrations 2) a group of compounds closely related in terms of chemical or aromatic properties 3) a large group of aroma compounds with similar descriptors 4) an association between an aroma enhancer and a molecule unable to break the buffer itself (Ferreira *et al.*, 2008). Impact compounds are compounds that can break the buffer when present at 'normal' concentrations. These compounds can transmit the specific aroma without the support of other chemicals in wine. Aroma compounds with the ability to do this include volatile thiols, methoxypyrazines and oxidation compounds such as sotolon, phenylacetaldehyde and methional.

Wine is a complex medium containing various types of aroma compounds that can all have an effect (differing degrees of suppression and masking) on each other (Francis & Newton, 2005). This complicates the investigation of interactive effects between compounds. Over the years, researchers have been trying to understand the interactions of aroma compounds in various media as different compounds can manifest differently in different media (Maga, 1989; Marais & Swart, 1999; Campo *et al.*, 2005; Escudero *et al.*, 2007).

Several studies have indicated difficulties when working in a wine matrix resulting in conflicting results when comparing matrices such as water, model wine and real or dearomatized wines (Aronson & Ebeler, 2004). Even dearomatized wines still had sufficient chemical compounds present to influence interaction studies. In these dearomatized wines most of the aroma compounds would be removed by using resins such as amberlite or charcoal. The remaining matrix would still contain an unknown amount of unidentified chemical compounds that could potentially influence the results of the aromatic interaction study. Lund *et al.* (2009a) proved the significant effect of non-volatile polyphenols on the perception of some aromatic compounds, while other studies also noticed the effect of compounds

present at their perception thresholds influencing the perception of other aromatic compounds (Culleré *et al.*, 2007; Van Wyngaard, 2013). This indicates that aromatic compounds could still have an enhancing or suppressing effect on other compounds even when it is not volatile or not detectable by humans.

Various studies have undertaken the task to elucidate interactive effects between compounds in wines. Ethanol has been found to play a very important role as it not only influences the solubility of odourants but it can have an impact on the actual chemoreceptor system. A higher ethanol concentration will increase the solubility of the odourant causing a decrease in the amount of volatiles reaching the pituitary gland. Even more, ethanol has proven to mask or suppress the fruity notes of esters (Escudero *et al*., 2007). The addition of β-damascenone and β-ionone to the mixture of esters brought a clear increase of the fruity note (Pineau *et al.*, 2007). Dimethyl sulphide also intensified the fruity aroma especially when β-damascenone was also present. The study concluded that the fruity notes of red wines are the result of a complex interaction between esters, ethanol, norisoprenoids, dimethyl sulphide and other volatiles (Escudero *et al.*, 2007).

Other studies investigated interactive effects between aroma impact compounds. Combinations containing high concentrations of 4MMP, 3MH and 3MHA not only delivered high intensity ratings for the "cat urine/sweaty" attribute but also the "cooked green vegetal" descriptor thus demonstrating the contribution of the thiols to the 'green' characteristics of a wine (King *et al.*, 2011). Synergism between IBMP and 4MMP has also been reported. When added individually at low concentrations (2 ng/L for IBMP; 0.2 ng/L for 4MMP), "dusty" was the only attribute generated, however when added together at the same concentration, the amount of attributes generated by the panel increased to "dusty", "grassy" and "herbaceous" (Marais & Swart, 1999). When added at higher concentrations, a mutual suppression between the volatile thiols and the methoxypyrazines was observed (Marais & Swart, 1999; Campo *et al.*, 2005; King *et al.*, 2011; Van Wyngaard, 2013). The addition of esters to the base wine increased the perception of the "confectionary" attribute and also increased the ratings of "overall fruit aroma", "tropical" and "cooked green vegetal" attributes (attributes also associated with thiols). Esters are known to be particularly important in the bouquet of young white wines and interact in an additive manner (Van der Merwe & Van Wyk, 1981; Campo *et al.*, 2005). High thiol concentrations in the presence of an ester combination decreased the ratings for "confectionary", indicating a suppressive effect of the thiols on the esters when present at high levels. To the contrary, in the presence of moderate to high thiol concentrations, the esters actually enhanced some thiol-related sensory attributes (Ferreira *et al.*, 2002; Campo *et al.*, 2005; King *et al.*, 2011; Benkwitz *et al.*, 2012a). Overall, the addition of IBMP had a greater impact on the aroma of a wine when compared to the addition of the thiols. The effect of IBMP has been described as being dominant and in wines containing both thiols and IBMP, the aroma is often driven by IBMP (Hein *et al.*, 2009; King *et al.*, 2011; Van Wyngaard, 2013).

Lund *et al.* (2009a) investigated the interaction of non-volatile wine compounds such as polyphenols and volatile aroma compounds in a diluted wine medium. They found that the perception of IBMP, 3MH and

ethyl decanoate were largely suppressed by the addition of polyphenols ((+)-catechin, quercetin and caffeic acid). On the other hand the addition of specifically caffeic acid enhanced the perception of 3MH. This could be due to the suppression of other aroma compounds that initially masked the 3MH aroma (Lund *et al.*, 2009a). The aroma perception of 3MHA was least affected by the polyphenol additions when compared to the other aroma compounds tested in the study. The presence of the ester functional group is thought to make it less susceptible for interaction with the polyphenol when compared to the alcohol group of 3MH (Lund *et al.*, 2009a).

Very few studies have investigated the interactive effect of unpleasant aroma compounds such as compounds related to oxidation. The addition of branched aliphatic aldehydes and (E)-2-alkenals generated attributes such as “sweet orange”, “dried fruit”, “fusel”, “closed room”, “dirty”, “mouldy” and “rancid” and an additive effect between these aldehydes were observed (Culleré *et al.*, 2007). There seems to be a lack of information regarding these types of studies, especially considering that no research has been done to date to investigate the interactions between character impact compounds and potent compounds originating due to oxidation.

2.7 CONCLUSION

Oxygen management related issues are estimated to compromise the quality of approximately 3% of all wines before the bottles reach the retailer (Nygaard, 2010). Consumers are sensitive to wine faults, such as ‘reductive’ and oxidative aromas and this will decrease the liking scores and purchase intent (Nygaard, 2010). The management of oxygen during winemaking is thus generating considerable interest in the wine world. If oxygen exposure is too high or too low, wines can develop defects that can compromise their sensory quality. Too little oxygen exposure could lead to a wine with ‘reductive’ odours, while too much oxygen can result in an oxidised wine displaying other unpleasant aromas together with the decrease in pleasant fruity aromas. The challenge is thus to identify the right amount of oxygen needed for a specific wine to maintain the perfect balance between chemical composition and sensory perception. Other than that, research focussing on the evolution of the compounds during oxidative aging of wines is needed to better understand the relationships between compounds and mechanisms occurring. Such research should also be conducted under conditions which the wine might encounter in a commercial cellar situation and not only be performed under conditions of enhanced oxidation.

The complexity of a wine as a medium greatly complicates research attempts. Sauvignon blanc wine especially is known for the large diversity of flavours which will add to the complexity. The volatile thiols and the methoxypyrazines are the main compounds involved in the unique character of Sauvignon blanc wines, however by themselves these key molecules do not account for the aromatic complexity of high

quality wines. Various interactions occur between both volatile and non-volatile wine constituents and these interactions can change as the concentrations change. Studies attempted to identify some of these interactive effects, however much more research is needed to successfully address this issue.

2.8 ABBREVIATIONS USED

3-mercaptohexan-1-ol (3MH); 3-mercaptohexan-1-ol acetate (3MHA); 4-mercapto-4-methylpentan-2-one (4MMP); 3-isobutyl-2-methoxypyrazine (IBMP); 3-isopropyl-2-methoxypyrazine (IPMP); 3-*sec*-butyl-2-methoxypyrazine (SBMP); glutathione (GSH); oxidised glutathione (GSSG); grape reaction product (GRP); methyl mercaptan (MeSH); dimethyl sulphide (DMS); 5-hydroxymethylfurfural (5-HMF); 5-methylfurfural (5-MF); analysis of variance (ANOVA); principle component analysis (PCA); quantitative descriptive analysis® (QDA).

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Chapter 3

Research results

Sensory interaction studies combining varietal and oxidation-related compounds that occur in Sauvignon blanc wines

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3.1 INTRODUCTION

Wine aroma is not the result of single aroma active compounds, but rather due to the various complex interactions between specific compounds (Escudero *et al.*, 2007; Fischer, 2007). These interactions give rise to a wine's taste and aroma (Dall'Asta *et al.*, 2011). Over the last two decades aroma reconstitution approaches have added a new dimension to sensory investigations. Sensory interactions of chemical compounds at different concentrations and in various media has been investigated (Ribéreau-Gayon *et al.*, 1975; Van der Merwe & Van Wyk, 1981; Maga, 1989; Guth, 1997; Marais & Swart, 1999; Ferreira *et al.*, 2002; Lee, 2003; Aronson & Ebeler, 2004; Campo *et al.*, 2005; Francis & Newton, 2005; Ferreira *et al.*, 2006; Culleré *et al.*, 2007; Escudero *et al.*, 2007; Pineau *et al.*, 2007; Lund *et al.*, 2009a; King *et al.*, 2011; Benkwitz *et al.*, 2012; Van Wyngaard, 2013). Studies performed by adding a range of aroma compounds to various media show complex interactions between groups of aroma compounds such as esters, thiols, alcohols, pyrazines, ketones and terpenes (Ribéreau-Gayon *et al.*, 1975; Ferreira *et al.*, 2002; Campo *et al.*, 2005; Escudero *et al.*, 2007; Pineau *et al.*, 2007; King *et al.*, 2011).

Interactive studies between thiols, esters and 3-isobutyl-2-methoxypyrazine (IBMP) demonstrated the complex sensory interactions (synergism, suppression and enhancement) of the compounds and unexpected contributions of certain thiols and a combination of esters and thiols to the 'green' characteristics (Marais & Swart, 1999; King *et al.*, 2011). Furthermore, it has previously been shown that non-volatile polyphenols can affect the perception of various aroma compounds including thiols, methoxypyrazines and esters (Lund *et al.*, 2009a). The above mentioned studies almost exclusively investigate the effect of positively-related compounds on each other. Limited research has been performed on the intensity of sensory attributes generated or the modification of these attributes by varying concentrations of individual aroma compounds or combinations of compounds. The suppression/masking/enhancing effect of off-odours or faulty odours should be investigated as it can develop in a wine during aging.

Sauvignon blanc is a complex cultivar and can yield very different wine styles. The volatile thiols (4-mercapto-4-methylpentan-2-one (4MMP), 3-mercaptohexan-1-ol (3MH) and 3-mercaptohexan-1-ol acetate (3MHA)) can lead to tropical and fruity style wines, while the methoxypyrazines (3-isobutyl-2-methoxypyrazine (IBMP), 3-isopropyl-2-methoxypyrazine (IPMP) and 3-*sec*-butyl-2-methoxypyrazine (SBMP)) can contribute "green pepper", "grassy" and "asparagus" aroma of the wine (Lacey *et al.*, 1991; Tominaga *et al.*, 1998; Swiegers *et al.*, 2006; Coetzee & Du Toit, 2012). Both of these odour groups are often well sought after for the production of quality wines (Lund *et al.*, 2009b; King *et al.*, 2011).

The volatile thiols, 3MH and 4MMP, are released from their precursors by yeast during alcoholic fermentation (Subileau *et al.*, 2008). 3MHA results from the esterification of 3MH by yeast (Swiegers *et al.*, 2009). The volatile thiols are known to be sensitive to oxidation and acid hydrolysis and their concentrations decrease during aging (Tominaga *et al.*, 2004; Herbst *et al.*, 2008). The methoxypyrazines are, however, not sensitive to oxidation and studies have shown methoxypyrazine concentrations to remain constant during oxidative handling of juice and wine (Marais, 1998; Coetzee *et al.*, 2013). Methoxypyrazines are present in grapes and their concentration decreases during grape maturation (Lacey *et al.*, 1991). A comprehensive review on Sauvignon blanc aroma (with a focus on volatile thiols) and the reaction of various aroma groups to oxidation has been published previously (Coetzee & Du Toit, 2012).

During aging, new aroma compounds form which can change the aromatic composition of a wine. In white wine, compounds such as acetaldehyde, 3-(methylthio)-propionaldehyde (methional), phenylacetaldehyde and sotolon have been shown to play a key role in the developed aging character of white wines. These compounds contribute to the typical aging character by lending aroma attributes such as “green apple skin”, “cooked potato”, “flower”, “honey” and “curry”. (Silva Ferreira *et al.*, 2002; Frivik & Ebeler, 2003; Silva Ferreira *et al.*, 2003a; Jackowetz & De Orduña, 2013). A study done on oxidation-related aldehydes and how they affect each other showed the additive/enhancing effect of the aldehydes on each other as well as the importance of the compounds even when present at concentrations below their perception thresholds (Culleré *et al.*, 2007). The study also demonstrated the effect of groups of compounds (branched aliphatic aldehydes vs *(E)*-2-alkenals) on each other. However, to date very little research has focussed on a mixture of fresh and oxidative or aged aromas and the question still remains how these aroma compounds will interact when combined in a wine-like medium. The sensory impact of the compounds at a wide range of concentrations (including below and at the perception threshold) would also be important to investigate.

In this study, the sensory interactions between the varietal characters of Sauvignon blanc and typical white wine oxidation compounds were investigated. Various compounds were added singularly and in combination to a model wine medium and evaluated by a trained sensory panel using descriptive analysis. Testing the interactions should clarify the evolution of white wine aroma during bottle aging and possibly serve as a model to evaluate at what concentrations interactions become detrimental to the wine aromatic composition.

3.2 MATERIALS AND METHODS

3.2.1 Medium

Model wine was the preferred choice of spiking medium as the complexity of wine could interfere with the interaction study. Model wine consisted of distilled water, 5 g/L tartaric acid and 12% ethanol and pH adjusted to 3.5 using sodium hydroxide. After preparing the model wine, the composition was confirmed using WineScan FT 120 instrument (FOSS Analytical, Denmark).

3.2.2 Chemicals and spiking

The compounds used in this study were 3MH, IBMP, methional and phenylacetaldehyde. Solutions of 714.86 mg/L 3MH (Interchim), 250 mg/L IBMP (Sigma), 1585 mg/L methional (Sigma) and 1561 mg/L phenylacetaldehyde (Sigma) were prepared in 99.5% ethanol (Merck Chemicals, South Africa). The exact concentration of 3MH was also confirmed by Ellman's reagent (Ellman, 1959). 3MH and IBMP solutions were stored in the dark at -80˚C, while methional and phenylacetaldehyde solutions were stored at 4˚C. These solutions were used to spike the model wine to the desired concentrations one hour prior to tasting.

3.2.3 Experimental design

Initially each compound was spiked individually in order to assess the effect of concentration changes on the aromatic perception of each compound. The concentrations used throughout this study were kept constant and can be seen in Table 3.1. This was done to compare profiles of both trials (singular additions and multiple additions). Samples are numbered from 1 to 5 according to the level of each of the compounds. Using the level as the indicator of the concentration simplifies the interpretation of the various figures in this study. Refer to Table 3.1 for the concentration of each compound level.

A central composite design was used for the sensory analysis of the samples containing multiple compounds (to assess interactions). The suitability of the central composite design lies in the fact that it can be used to test interactions between different factors. Ideally a factorial design could be used to test all combinations, however this design would result in a very large number of combinations to be assessed. The central composite design only selects a certain amount of combinations specifically chosen by the design. The use of this method led to a design with eight star

points, sixteen cube points and one centre point which resulted in 25 samples in total to be tested (Table 3.2).

Table 3.1 Concentrations tested.

	Level and concentration				
Compound	1	2	3	4	5
3-Mercaptohexan-1-ol (ng/L)	40.0	60.0	500.0	2000.0	6000.0
3-Isobutyl-2-methoxypyrazine (ng/L)	1.0	2.0	10.0	20.0	40.0
Methional (µg/L)	0.3	0.5	3.0	6.0	15.0
Phenylacetaldehyde (µg/L)	0.5	1.0	30.0	80.0	130.0

Table 3.2 Composition (concentration levels) of various samples in the central composite design.

	Compound			
Sample	**3-Mercapto-hexan-1-ol**	**3-Isobutyl-2-methoxypyrazine**	**Methional**	**Phenylacetaldehyde**
Centre	3	3	3	3
Cube 1	2	2	2	2
Cube 2	2	2	2	4
Cube 3	2	2	4	2
Cube 4	2	2	4	4
Cube 5	2	4	2	2
Cube 6	2	4	2	4
Cube 7	2	4	4	2
Cube 8	2	4	4	4
Cube 9	4	2	2	2
Cube 10	4	2	2	4
Cube 11	4	2	4	2
Cube 12	4	2	4	4
Cube 13	4	4	2	2
Cube 14	4	4	2	4
Cube 15	4	4	4	2
Cube 16	4	4	4	4
Star 1	1	3	3	3
Star 2	5	3	3	3
Star 3	3	1	3	3
Star 4	3	5	3	3
Star 5	3	3	1	3
Star 6	3	3	5	3
Star 7	3	3	3	1
Star 8	3	3	3	5

3.2.4 Sensory analysis

The sensory panel consisted of 11 judges (all female between the age of 25 and 54). Intensity rating was done using a 100 mm unstructured line scale, ranking intensity from “none” to “intense”. Testing was done in booths using standard ISO wine tasting glasses. The booths have standard artificial daylight lighting and the temperature was controlled at 20±2˚C. Sample glasses were marked with random three-digit codes unique for each judge and glasses were covered with a plastic lid prior to sensory assessment to prevent the aroma contaminating the laboratory environment. The order of the samples was randomised and balanced across the assessors. Along with the set of samples containing the spiked compound, a blank glass containing the unspiked model wine only, was also provided for comparison. Panellists evaluated the samples orthonasally only and the data was collected on a paper ballot. For reasons of human ethics, a brief explanation of the addition of flavour to the samples was given prior to testing, although care was taken to exclude any information that could have caused bias.

Compounds were first analysed individually. Eight training sessions (one hour each) were conducted in an attempt to reach consensus regarding different samples and terminology used. In this way, the panellists were exposed to each compound twice. At each training session, the panel received all five levels of a compound as well as a blank sample consisting only of model wine. A range of reference standards was also given. During the first discussion session, the panel generated descriptors and a brief line scaling exercise was done. During the second training session the descriptors and the sample position on the line scale was finalised. Each individual compound was analysed during a formal test session in triplicate.

The profiling of the samples containing multiple compounds was done after the evaluation of the singular compounds. At this stage the judges were already familiar with the compounds. During profiling, the samples were divided into three subsets as all samples could not be analysed in one session. Subsets were trained and tested on individual days to avoid fatigue. The first subset was cube points 1-8, the second subset was cube points 9-16 and the third subset was the star points 1-8 as suggested by Esbensen, (2002). The experimental layout and the combinations of compounds for each sample and subset can be seen in Table 3.2. Two training sessions consisting of two hours each were used for each subset (4 hours per subset). The centre sample (all compounds present at level 3) accompanied each set during training and testing. During training, the panellists were not informed of the composition of each sample and reference standards were available for the panellists during the trial (section 3.3.2).

Samples were scaled on a 100 mm unstructured line scale using the attributes generated during the profiling of the singular compounds. Analysis were performed as 3 different tests (each subset separately) with 3 replications each (three two hour sessions). With the set of eight spiked samples (along with the centre sample), a blank glass containing model wine only, was also provided for comparison. Cube points 1-8 were tested first, followed by cube points 9-16 and star points 1-8. The judges took regular breaks between samples to prevent fatigue.

3.2.5 Data Analysis

Assessor performance was evaluated using PanelCheck (Version V1.4.0, Nofima, Tromsø, Norway) according to the workflow as described by Tomic *et al.* (2010). Sensory descriptive data for each attribute was analysed using mixed model analysis of variance (ANOVA). Principle component analysis (PCA) biplots were created using the correlation matrix of the mean data. Post-hoc Fisher's LSD tests were used to test for significance of sensorial differences between compound combinations and the significant statistical relationships were modelled as a network and visualised with Cytoscape 3.0.1. (Shannon *et al.*, 2003). In a custom-built python program using networkx and scipy libraries, t-tests were used to test for the significance of the sensorial differences between compound combinations, and the significant statistical relationships were modelled as a network in which coloured nodes indicated the intensity of a sensory attribute compared to the control levels (that were indicated by an uncoloured node located at the centre of the network). Response surface plots were constructed (Statistica 10, Statsoft Inc., Tulsa, USA) to investigate interactions. A p value threshold of 0.05 ($p<0.05$) was used for the determination of statistical significance.

3.3 RESULTS AND DISCUSSION

3.3.1 Panel evaluation

Panel performance was monitored as suggested by Tomic *et al.* (2010). 3-Way ANOVA was used to determine significant attributes and Tucker plots were used to assess the consensus amongst judges (Figure 3.1). For all the significant attributes generated there was a definite grouping of the panellists. For most of the attributes this grouping was situated between the inner and outer ellipses. This shows good panel consensus with 50-100% of the variance explained by the judges.

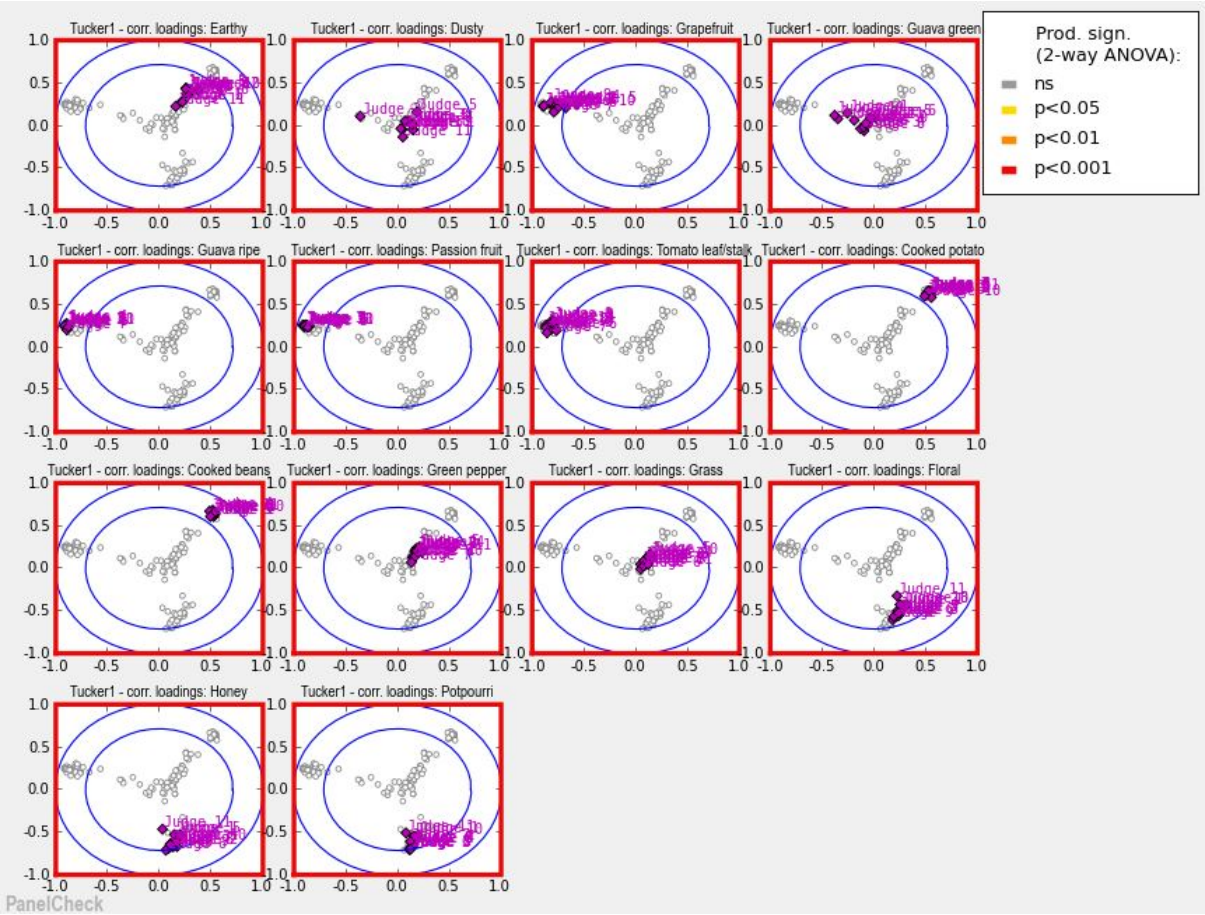

Figure 3.1 Tucker plot showing significance of all the attributes generated by the panel.

3.3.2 Individual compound evaluation

Model wine was used as the testing medium as even a neutral or dearomatized wine has sensory properties due to certain aromatic compounds that remain in the wine. For the purpose of this study, the absence of any other aromatic and non-aromatic compounds (that could influence the perception of the aroma) was critical (Lund *et al.*, 2009a).

The concentrations were chosen based on concentrations of the aroma compounds found in white wines as reported in literature, as well as a preliminary sensory assessment of the individual aroma compounds and mixtures of the compounds. Pre-screenings by experienced wine tasters (all who completed a certificate course in wine evaluation) were done before finalizing the concentrations to ensure that the levels chosen fulfil the requirements for the aim of this study.

The concentrations (levels) for this study was chosen as follows: the first level was chosen to be below the perception threshold, as this would give an indication of enhancing or suppressive effects of these compounds even when they are not present in concentrations high enough to be

individually perceived. The second level was at the perception threshold as reported in literature. A complete detection threshold test was not done for each compound as concentrations reported were deemed sufficient (especially when reported in model wine) (Buttery *et al.*, 1969; Escudero *et al.*, 2000; Dubourdieu *et al.*, 2006; Culleré *et al.*, 2007). The third level was at a low to medium concentration while the fourth level was at medium to high concentration found in wines according to literature. The fifth level was considered to be very high according to levels found in white wines in general (Silva Ferreira *et al.*, 2003a; Silva Ferreira *et al.*, 2003b; Dubourdieu *et al.*, 2006; Culleré *et al.*, 2007; Alberts *et al.*, 2009; Swiegers *et al.*, 2009). This way the effect of these compounds below, at and above their perception threshold, could be investigated.

The attributes generated by the panel for each compound are shown in Table 3.3 and Figure 3.2. In Table 3.3 the definition of each descriptor is shown as defined by the panel. The reference standards used for each attribute are also defined (Table 3.3). The list of descriptors did not change between the two trials (individual or a combination of compounds), however not all the attributes were used by the judges. For example, the descriptor *earthy* was not used by the panel when evaluating the samples according to the central composite design as this attribute did not describe the odour of the samples when the compounds were present in combination. A correlation network was used in order to visualise the (Pearson) correlations between compounds and the sensory attributes associated with them. Figure 3.2 shows the correlation network constructed from data obtained from analysing the compounds individually.

Table 3.3 Attributes and reference standards used for descriptive analysis of spiked model wines at various concentrations

Descriptor	Definition	Reference standard
Earthy	Smell associated with soil	none
Dusty	Smell associated with a closed basement or cupboard	none
Grapefruit	Typical grapefuit	Grapefuit pieces in model wine
Guava green	Unripe guava (green skin)	Unripe green guava pieces in model wine
Guava ripe	Very ripe guava (pink skin)	Ripe guava pieces in model wine
Passion fruit	Typical passion fruit	Passion fruit pieces in model wine
Tomato leaf/stalk	Fresh tomato plant stalk	Fresh cherry tomato on the vine with long stalk
Cooked potato	Thoroughly cooked potato	Steamed potato pieces in model wine
Cooked beans	Cooked green beans	Canned cooked beans in model wine
Green pepper	Typical green pepper	Fresh green pepper
Grass	Freshly cut grass	Freshly cut grass
Floral	Bouquet of fresh flowers	none
Honey	Typical honey	2 teaspoons honey dissolved in model wine
Potpourri	Dried flower leaves (mostly rose petals)	Dried rose petals (various colours)

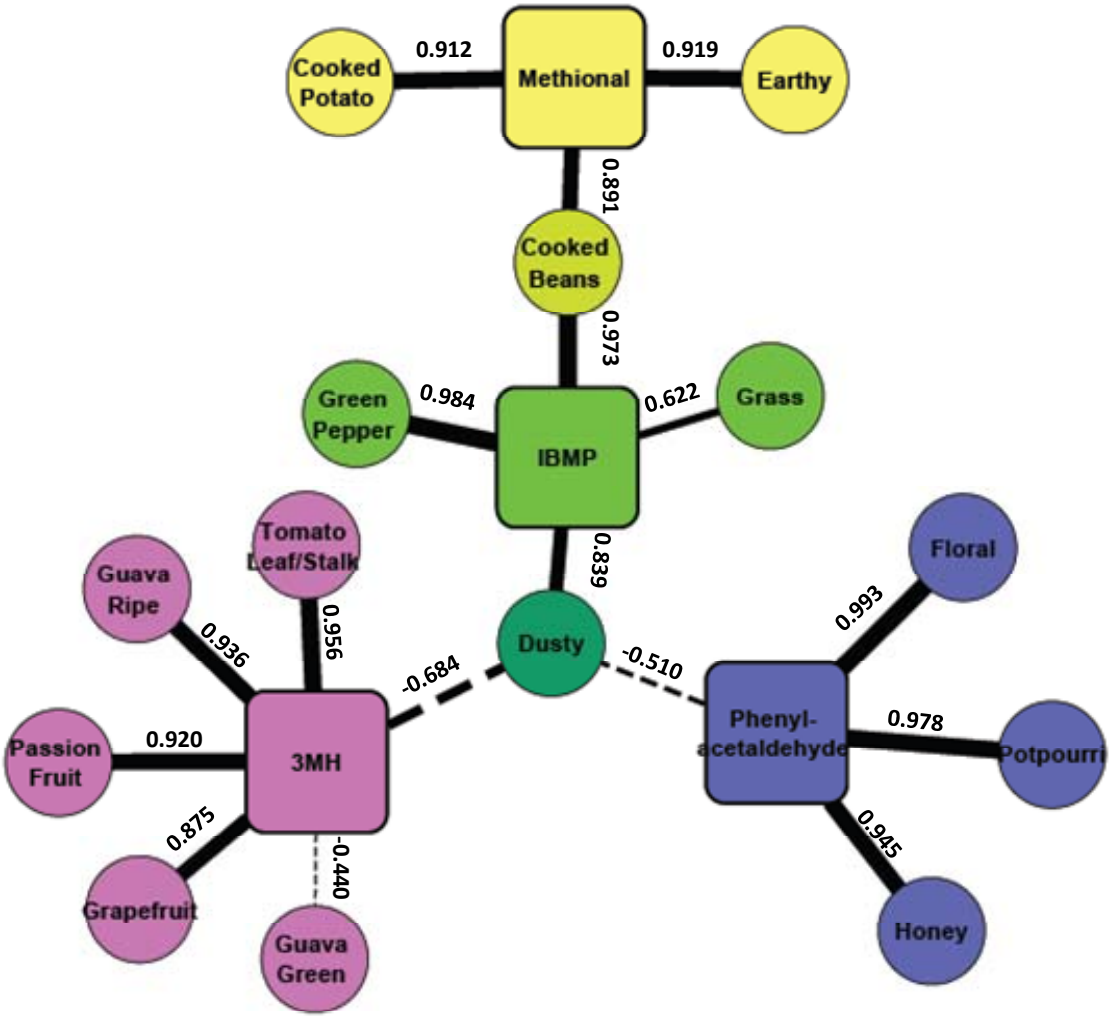

Figure 3.2 A correlation network showing the attributes generated by adding individual compounds to a model wine medium at the various levels. Values displayed on the edge are the correlation between the compound concentration and intensity of the attribute. Thickness of the edges indicates the strength of the correlation. Broken line indicates a negative correlation.

Correlations between the compound concentration and attribute intensity can be seen on the edges between the compound and the attribute. *Dusty* was used as a descriptor for IBMP, 3MH and phenylacetaldehyde. The correlation between *dusty* and IBMP was high (0.839) unlike the correlation with the other two compounds which were weakly negative. The other descriptor used for more than one compound was *cooked beans* and was used as an attribute for both methional and IBMP. Both of these compounds contributed to this descriptor with strong correlations, with methional (0.891) having a lower correlation than IBMP (0.973). This could indicate a possible enhancement effect when these two compounds are present in the same solution. *Guava* was divided into two classes. *Guava green* was described as the odour from the unripe fruit while *guava*

ripe refers to the fully ripened or even overripe fruit. *Guava ripe* had a very strong correlation (0.936) with 3MH, while *guava green* showed a weak negative correlation with 3MH (-0.440). The use of the *guava* descriptor has rarely been reported in literature (Swiegers *et al.*, 2006). However, Van Wyngaard (2013) showed *guava* to also be one of the dominant attributes for 3MH. This could be due to the South African panel being more familiar with the odour of this fruit when compared to a French panel (Valentin, 1997).

With the exception of *grass* (0.622), all other attributes (*cooked potato, earthy, green pepper, tomato leaf/stalk, passion fruit, grapefruit, floral, potpourri* and *honey*) had high positive correlations (>0.875) with the corresponding compound. The use of most of these attributes to describe the various compounds have previously been reported in literature (Van Straten *et al.*, 1981; Augustyn *et al.*, 1982; Tominaga *et al.*, 1996; Tominaga, 1998; Tominaga *et al.*, 1998; Escudero *et al.*, 2002; Silva Ferreira *et al.*, 2003a; Dubourdieu *et al.*, 2006; Van Wyngaard, 2013). The use of *tomato leaf* as an attribute for Sauvignon blanc is not uncommon and the presence of 3MH has been identified in tomato leaf as well as rhubarb petiole (Tominaga, 1998).

The effect of varying 3MH levels on the attribute intensities can be seen in Figure 3.3. No specific descriptor was used to describe the faint odour observed at 40 and 60 ng/L, however *dusty* did occur at low intensities (<10 intensity units). Mateo-Vivaracho *et al.* (2010) also found 3MH concentrations close to the perception threshold (21 or 81 ng/L) not to contribute significantly to the flavour.

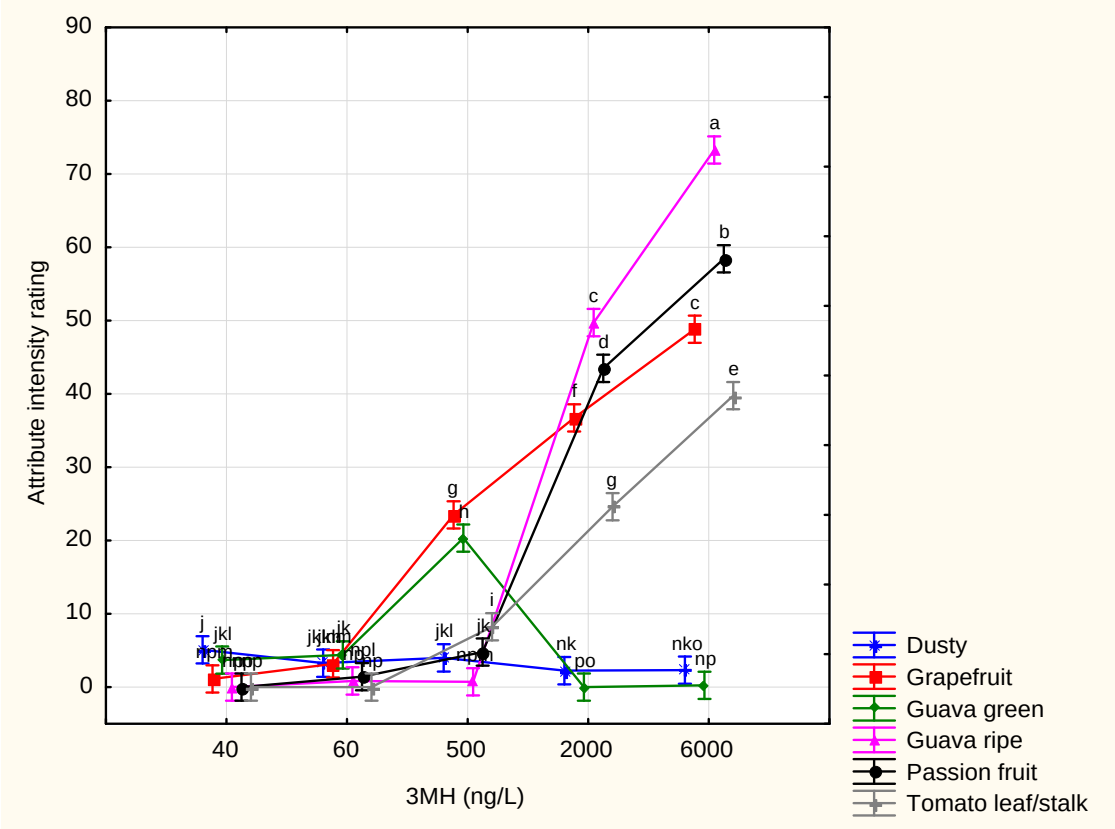

Figure 3.3 Effect of 3MH concentration on the various attribute intensities. Different letters indicate significant differences at $p<0.05$.

Dusty remained low and decreased slightly during the increase of 3MH concentrations. At 500 ng/L, a significant increase in *grapefruit* and *guava green* intensities was observed. *Tomato leaf/stalk* also increased, but the intensity was still relatively low. At 2000 ng/L *guava green* decreased significantly to below 10 units and remained low even at 6000 ng/L of 3MH. This is a good indication of a non-linear relationship between the 3MH concentration and the attribute perception and could apply to other descriptors and compounds as well. This phenomenon should be kept in mind when generating attributes for a compound without testing various concentrations as often a linear relationship between concentration and an increase in sensory intensity is assumed. *Passion fruit* and *guava ripe* increased to an intensity of above 40 units and surpassed the other descriptor intensities at 2000 ng/L. The two descriptors remained dominant at 6000 ng/L with *guava ripe* reaching an intensity of above 70. *Grapefruit* and *tomato leaf/stalk* also significantly increased from 500 to 6000 ng/L. Mateo-Vivaracho *et al.* (2010) reported the contribution of 3MH to the fruitiness of a wine when present in concentrations above 1497 ng/L, while King *et al*. (2011) also reported

increases in the *cooked green vegetal* attribute with an increase in thiol concentrations which shows the contribution of 3MH to "green" attributes.

Dusty reached higher intensities when IBMP was added to the medium compared to 3MH (Figure 3.4). The intensity increased significantly from 2 to 10 ng/L after which no further intensity increases were observed. *Green pepper* was identified as the main attribute used to describe the odour of IBMP when added at 10 to 40 ng/L.

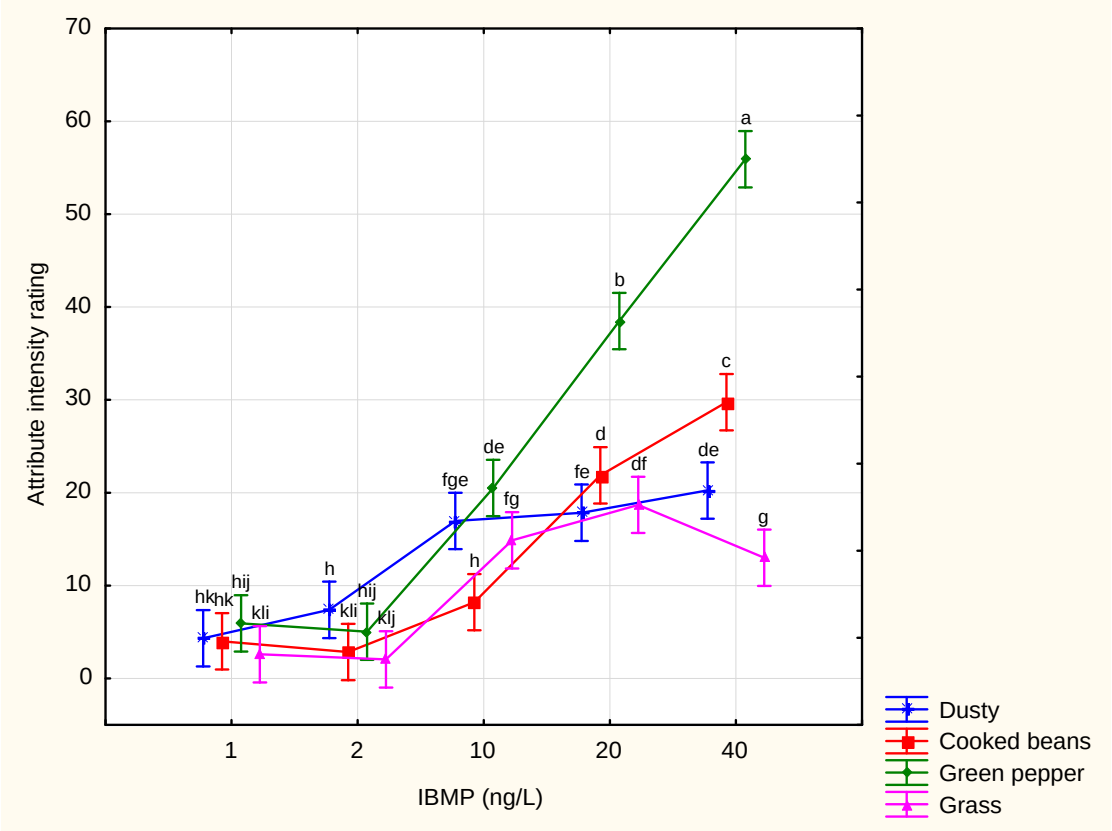

Figure 3.4 Effect of IBMP concentration on the various attribute intensities. Different letters indicate significant differences at $p<0.05$.

The intensity of *green pepper* also increased with large intervals reaching an intensity of above 50 units at 40 ng/L. *Cooked beans* also significantly increased with intensity from 2 ng/L as IBMP concentrations increased. *Grass* did not always increase with increased IBMP concentrations and the intensity was significantly lower at 40 ng/L compared to 20 ng/L. This is contradictory to results reported by Van Wyngaard, (2013) where a further increase in the intensity of the grass attribute was reported from 15 (about 20 intensity units) to 40 ng/L (about 30 intensity units). The use of a different panel and marix could explain the differences observed.

The three descriptors used to describe the odour of methional all increased linearly from 0.5 µg/L with increased methional concentrations (Figure 3.5). *Cooked potato* was used as the main attribute at all concentrations and reached the intensity level of about 60 units at 15 µg/L methional. After *cooked potato*; *cooked beans* and *earthy* followed in descending order with intensities averaging at 38 and 24 units at 15 µg/L methional respectively. Even though the use of these attributes have been used to describe the compound (Escudero *et al.*, 2000), the change in intensity as the concentration is altered has not been reported.

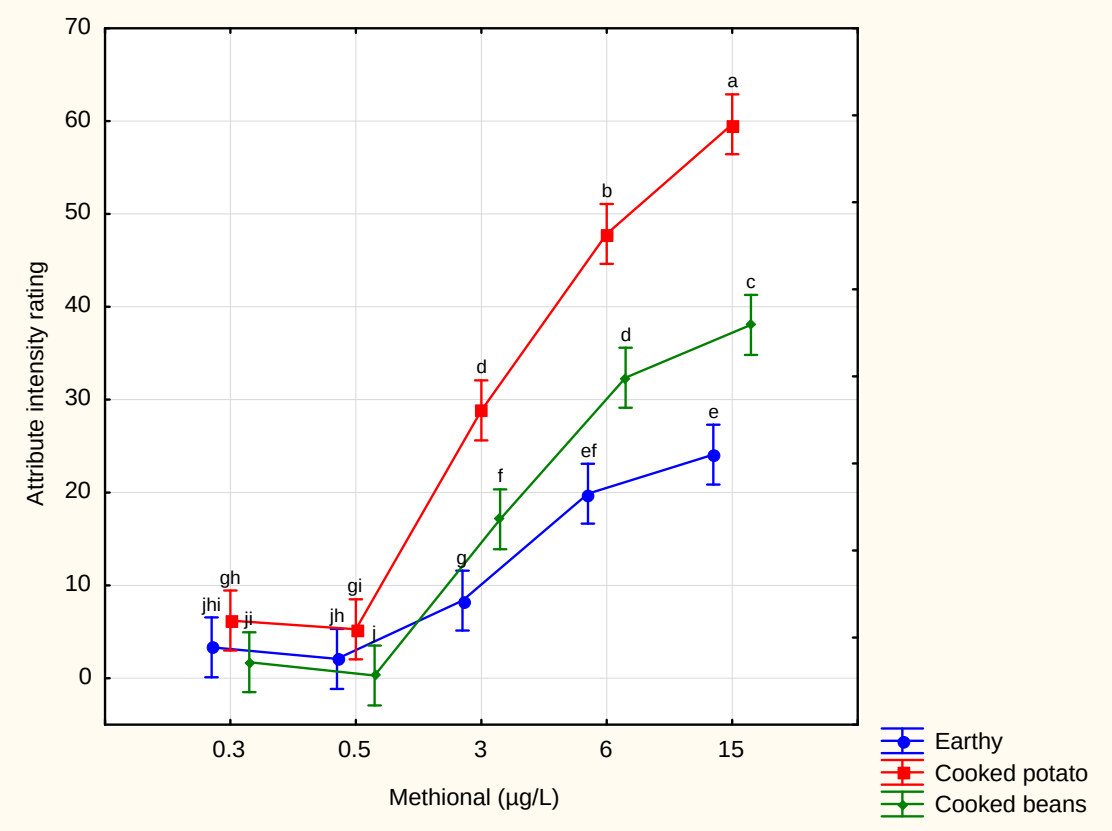

Figure 3.5 Effect of methional concentration on the various attribute intensities. Different letters indicate significant differences at $p<0.05$.

Floral was mainly used to describe the odour caused by phenylacetaldehyde (Figure 3.6). The intensity significantly increased from 0.5 to 130 µg/L phenylacetaldehyde. Only at 130 µg/L did the *potpourri* attribute surpass the intensity of floral. *Potpourri* did not increase initially and only occurred at 80 and 130 µg/L where the intensities were rated relatively high (30.5 and 55.9 intensity units respectively). *Honey* increased with increased phenylacetaldehyde concentrations, but reached a plateau at 80 µg/L. The use of the *honey* attribute has been reported (Silva Ferreira *et al.*, 2003a),

however the attributes *floral* and especially *potpourri* in relation to wine have very little to no references in literature.

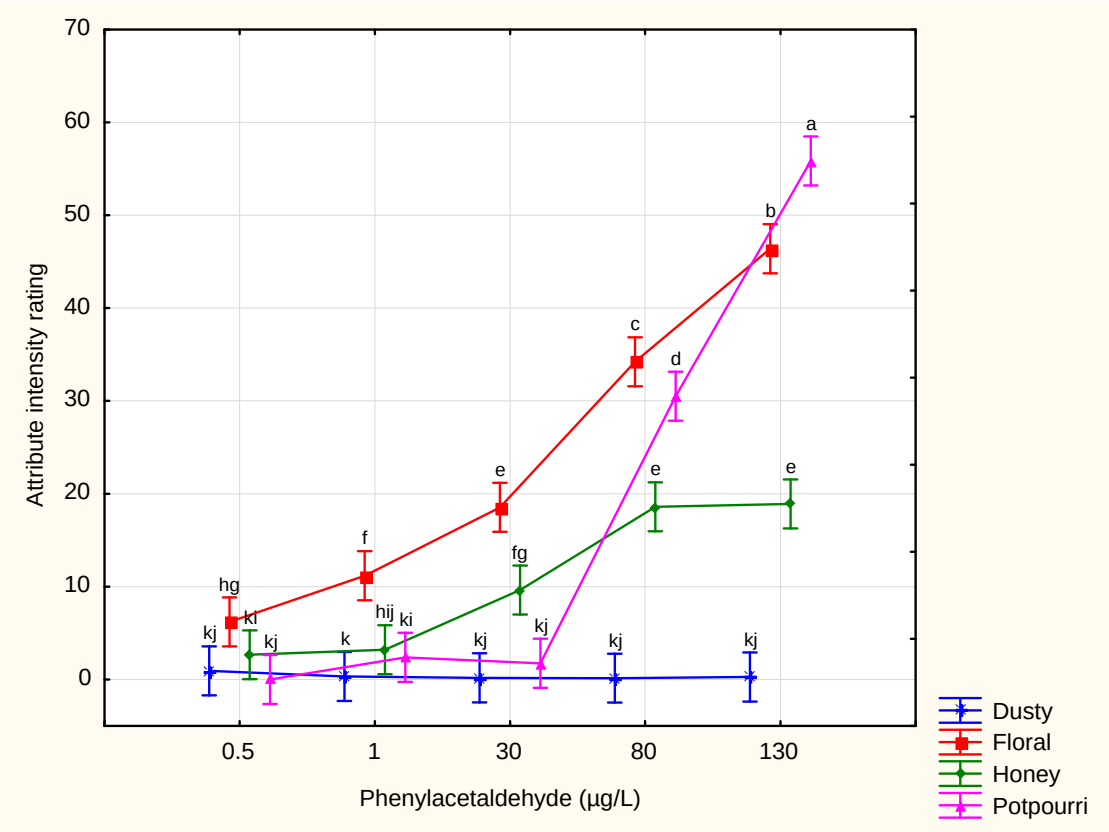

Figure 3.6 Effect of phenylacetaldehyde concentration on the various attribute intensities. Different letters indicate significant differences at $p<0.05$.

3.3.3 Multiple compound evaluation

Samples containing multiple compounds will be referred to as a four digit code in the text. The codes are an indication of the level (concentration) of the specific compound as reported in Table 3.1. For example, code 1-3-5-2 would represent the sample containing level 1 of 3MH (40 ng/L), level 3 of IBMP (10 ng/L), level 5 of methional (15 µg/L) and level 2 of phenylacetaldehyde (1.0 µg/L).

PCA biplots were constructed to investigate the interactions that occurred when multiple compounds were added to the same sample. In a PCA biplot, the correlation of variables with objects (and between variables themselves) depends on many factors. The plot may be interpreted using different approaches, including trends in the magnitude of the variables, angles and distances between variables as well as the distance between the data points.

Sixty percent of the variation can be explained by the first two PCs while another 17% was explained by PC3 (Figures 3.7 and 3.8). In Figure 3.7 PC1 separates the samples according to attributes: *Floral*, *honey* and *potpourri* grouped on the left hand side of the PC with *green pepper* to the middle and *guava green*, *tomato leaf/stalk*, *grapefruit*, *guava ripe* and *passion fruit* on the right.

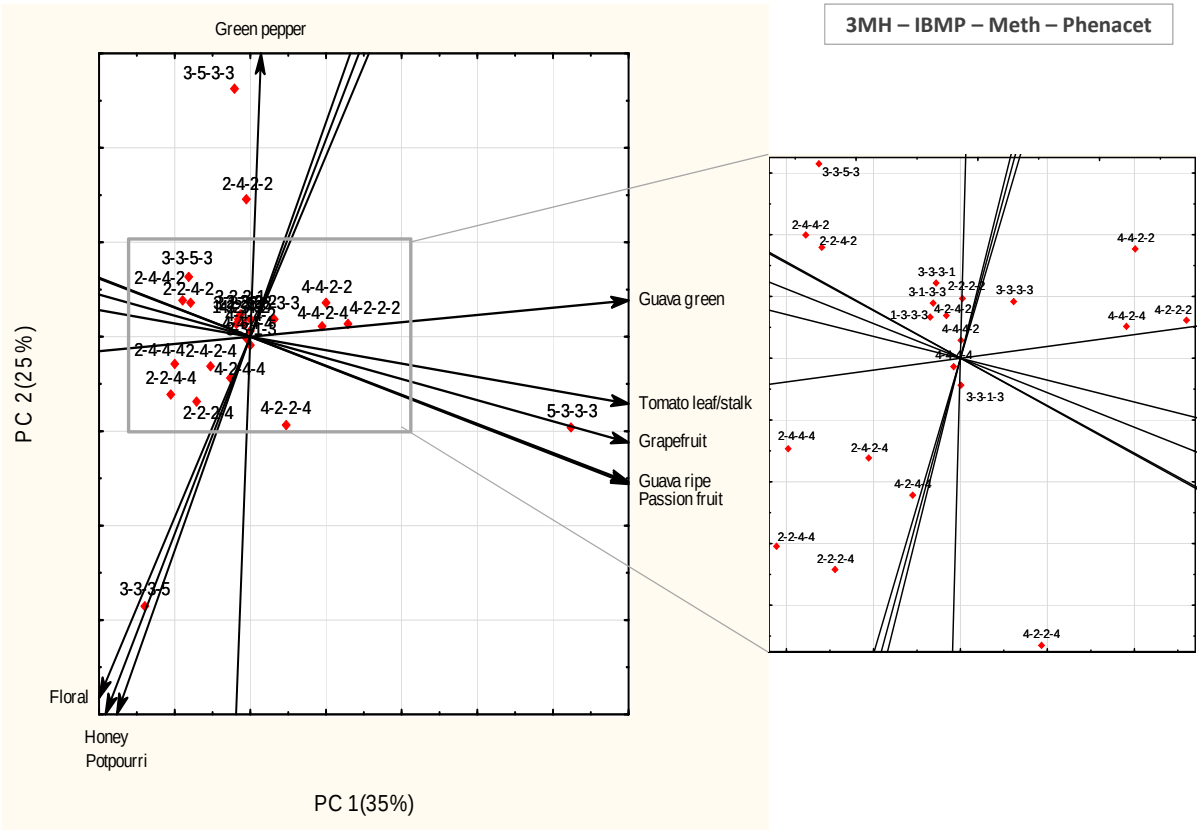

Figure 3.7 PCA biplot showing PC1 and PC2. The selected area is enlarged to better evaluate the clusters in the centre of the PCA.

As expected, sample 3-3-3-5 correlated strongly with *floral*, *honey* and *potpourri*. Samples 2-4-2-2 and 3-5-3-3 correlated strongly with *green pepper*. Sample 5-3-3-3 strongly correlated with the thiol associated attributes while 3-3-5-3 showed a good correlation with *cooked potato* and *cooked beans* in PC3 (Figure 3.8). Of all the samples tested, the sample combinations containing a compound at level 5 correlated very strongly with the associated descriptors as indicated in results from the individual compound tests (Figures 3.3 to 3.6) with the exception of IBMP where the sample 2-4-2-2 also had a strong correlation with *green pepper*. None of the other 3 compounds, when present at level 4 (4-2-2-2; 2-2-4-2; 2-2-2-4), elicited the same strength in correlation with the associated

attributes. This could be an indication of IBMP being a dominating compound when in combination with the other aroma compounds. This tendency was also found in other studies (Parr *et al.*, 2007; Hein *et al.*, 2009; King *et al.*, 2011; Van Wyngaard, 2013).

Samples with combinations of levels 3 and 1 were mostly clustered to the middle of the PC (Figures 3.7 and 3.8). The low level of one compound (level 1) when in combination with all of the other compounds present at level 3, seems to not have a big influence on the perception of the aroma compounds and clustered together with sample 3-3-3-3 which was considered to be the centre sample. Interestingly, samples 2-2-2-2 and 4-4-4-4 also clustered in this group and seem to correspond closely to sample 3-3-3-3 probably due to the fact that no singular attribute defined those samples.

Samples 4-2-2-2, 4-4-2-2, 4-2-2-4 and 4-4-2-4 correlated better with the thiol-related attributes compared to the other samples (Figures 3.7 and 3.8). In these samples 3MH was present at level 4 while methional was present at level 2 (perception threshold). This could be an indication of methional suppressing the perception of the thiol-related descriptors especially when looking at samples 4-4-4-4, 4-4-4-2 and 4-2-4-2 which were located in the central cluster. These samples still contained 3MH at level 4 but with methional also at relatively high concentrations. The only other sample with 3MH at level 4 was 4-2-2-4. This sample was located more towards the centre of the biplot which could indicate suppression of 3MH by phenylacetaldehyde as well, however this suppression was not as severe as with methional. A slight suppression of the thiol-related attributes by IBMP can be seen as the samples containing IBMP at level 4 (4-4-2-2 and 4-4-2-4) did not correlate as strongly with the thiol-associated characteristics when compared to 4-2-2-2.

With the exception of 4-4-2-4 and 4-4-4-4, all other samples containing phenylacetaldehyde at level 4 correlated better to *floral*, *honey* and *potpourri* compared to the other samples. The presence of IBMP at level 4 could suppress phenylacetaldehyde's attribute intensities seeing that samples 2-2-4-4, 2-2-2-4 and 4-2-2-4 correlated best with the attributes associated with phenylacetaldehyde. Interestingly, sample 2-2-4-4 which contained higher concentrations of methional showed a similar correlation to the phenylacetaldehyde attributes than sample 2-2-2-4. This could indicate the non-suppressive effect of methional on phenylacetaldehyde, also considering sample 4-4-2-4 which had a weaker correlation with *floral*, *honey* and *potpourri* when compared to sample 4-4-4-4.

In PC3 sample 2-4-4-2 seemed to correlate well with *cooked beans* and *cooked potato* (Figure 3.8). As mentioned before, the *cooked beans* attribute was used for describing both IBMP and methional. The presence of IBMP as well as methional at relatively high concentrations in the same sample could enhance each other and intensify the *cooked beans* descriptor. This is especially interesting

when locating sample 2-2-4-2, which did not correlate as strongly to the *cooked beans* and *cooked potato* attributes as 2-4-4-2.

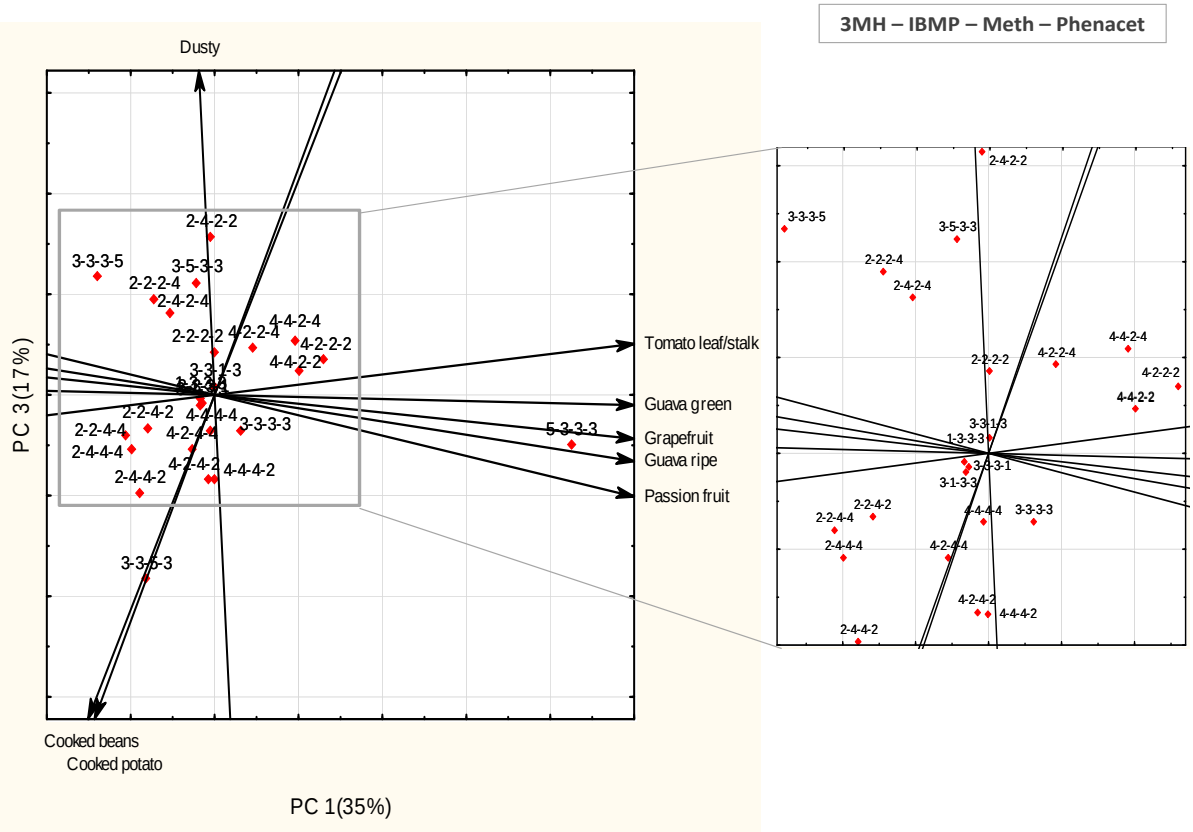

Figure 3.8 PCA biplot PC1 and PC3. The selected area is enlarged to better evaluate the clusters in the centre of the PCA.

The PCA biplots effectively show the correlations between the attributes and thereafter the relationship with the various samples (data points). The grouping of the attributes in this analysis makes it difficult to draw conclusions regarding individual descriptors and their relationship with the samples. Statistical networks were constructed to investigate each attribute separately. Node colours were scaled according to the differences in the average intensity units between the sample in question and the centre sample. The networks provide visual indications of how the various samples influence the intensity of the attribute. Only the attributes that are affected by the samples and the samples that have a statistically significant difference from the centre sample are shown in the network.

As indicated by the biplots, samples 2-2-2-2, 3-3-3-3 and 4-4-4-4 can each be considered centre points (Figure 3.7 and 3.8). Statistical networks were constructed with these three samples as centre samples to investigate the effect of the change in concentrations on the attribute intensities.

Figure 3.9 shows the network containing samples with compound combinations of levels 1, 3 and 5 with sample 3-3-3-3 as centre sample. As expected, the red nodes (increase in attribute intensity) only occurred when the corresponding compound was present at level 5. These increases in intensities were large with *guava ripe, potpourri* and *grapefruit* increasing with 58.6, 42.6 and 40.5 units respectively. Other increases in *green pepper* (31.5 units), *cooked potato* (30.8 units), *floral* (36.1 units) and *passion fruit* (35.9 units) were also high. *Cooked beans* seems to be more influenced by the presence of methional (25.2 units for 3-3-5-3) when compared to IBMP (6.9 units for 3-5-3-3) for the specific concentrations tested.

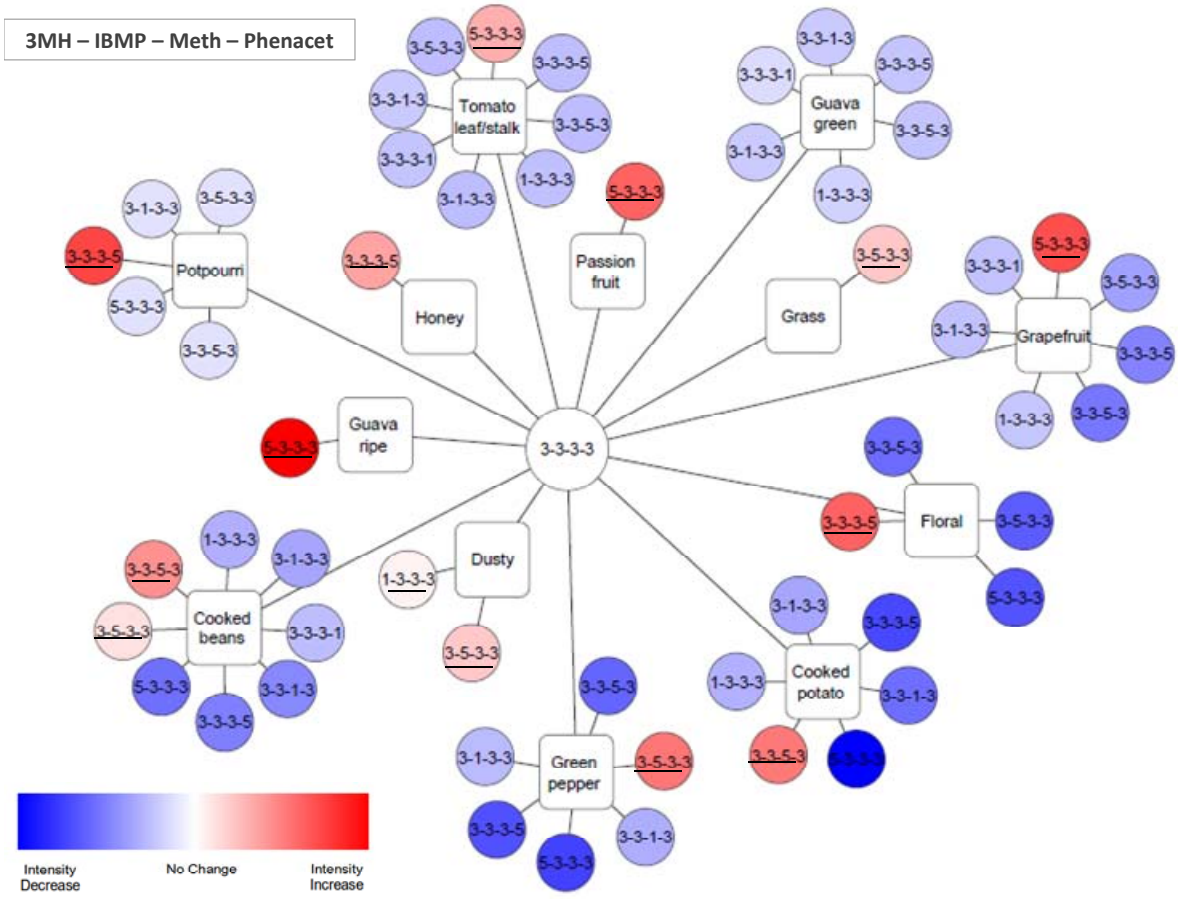

Figure 3.9 A statistical network (centre sample 3-3-3-3) showing the effect of sample combinations on attribute intensity. Edges between attributes and coloured nodes indicate a statistically significant difference from the centre sample. Red and underlined sample nodes indicate attribute intensity increase; blue nodes indicate attribute intensity decrease. Darker colour shows larger increase/decrease.

Sample 2-2-2-2 consisted of all four compounds present at their perception thresholds. Figure 3.10 shows the network constructed with this sample as the centre sample. The network is dominated by red nodes which would indicate a general increase in attribute intensities as the sample composition changed. Decreases in *dusty* were evident with these changes. With the testing of the single compounds, a negative correlation between *dusty* and both 3MH and phenylacetaldehyde was observed, while a positive correlation was observed between *dusty* and IBMP (Figure 3.2). This effect is repeated in combination with the other compounds as there was an increase in intensity (8.1 units) for sample 2-4-2-2.

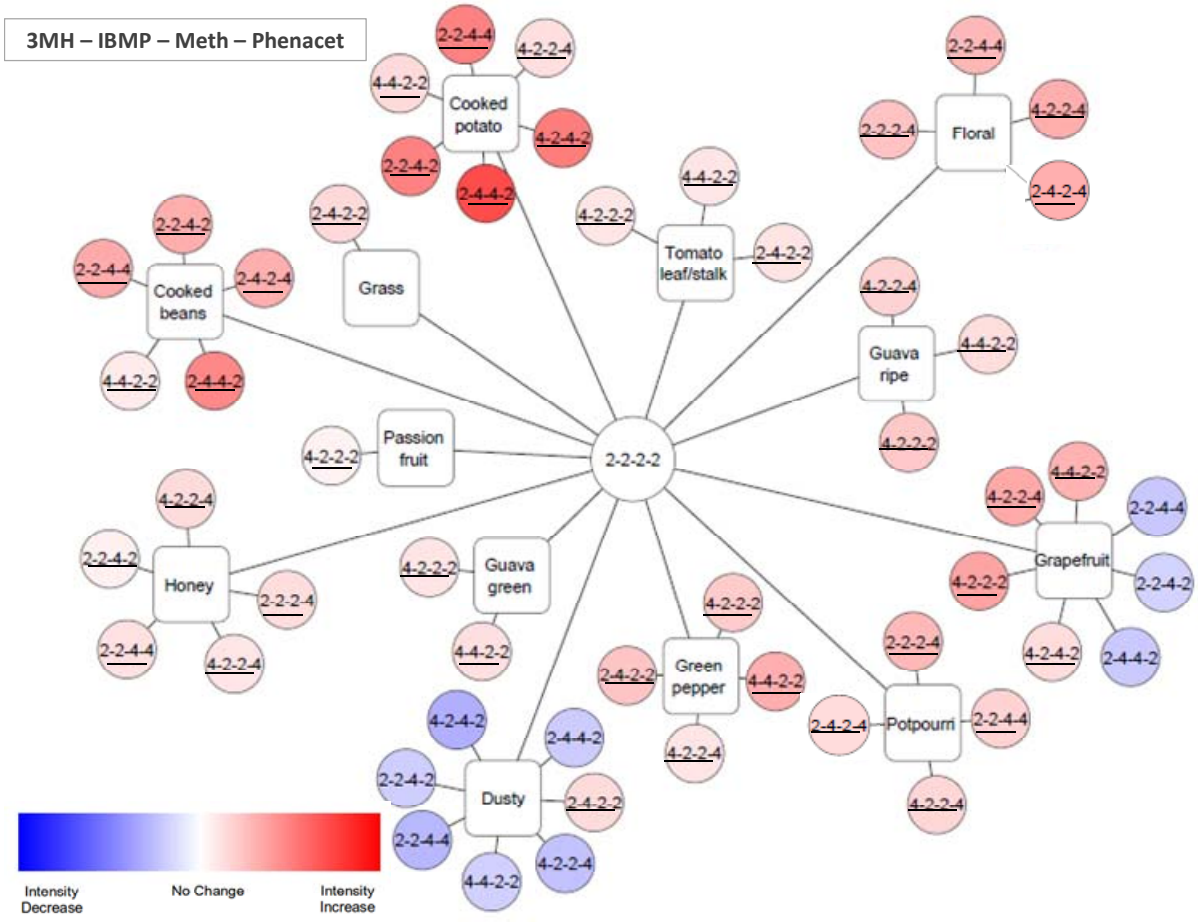

Figure 3.10 A statistical network (centre sample 2-2-2-2) showing the effect of sample combinations on attribute intensity. Edges between attributes and coloured nodes indicate a statistically significant difference from the centre sample. Red and underlined sample nodes indicate attribute intensity increase; blue nodes indicate attribute intensity decrease. Darker colour shows larger increase/decrease.

In all the samples containing 3MH at level 4 there was an increase in *grapefruit,* however, in the presence of methional and 3MH at level 4 (4-2-4-2), the increase was lower (7.7 units) compared to other samples (17.5-20.9 units). *Grapefruit* intensity actually decreased (4.2-5.2 units) when methional was present at level 4 with 3MH at level 2 when compared to sample 2-2-2-2. This could show the suppressive effect of methional on 3MH and the *grapefruit* attribute specifically. *Tomato leaf/stalk* was used to describe the odour of 3MH when testing the individual compounds as previously mentioned, however the addition of IBMP at level 4 in combination with 3MH (4-4-2-2) or without 3MH at level 4 (2-4-2-2) also resulted in an increase in the *tomato leaf/stalk* attribute. In this case the *tomato leaf/stalk* intensity does not seem to be enhanced by these increases as the intensity was about the same for the three samples (5.8, 5.8 and 6.2 units for 4-2-2-2, 4-4-2-2 and 2-4-2-2 respectively).

Guava green also increased with increased 3MH concentrations (6.0 units), however, this increase was the same or slightly higher when IBMP was also present at level 4 (7.2 units). The perception of the *guava green* and *tomato leaf/stalk* can possibly be enhanced by the "green" odours from IBMP. *Guava ripe* increased with increased 3MH concentrations. The increase was less in the presence of phenylacetaldehyde (10.0 units) and IBMP (7.5 units) compared to 3MH only (12.5 units). A slight suppression might thus occur. Interestingly, *green pepper* not only increased with increased IBMP concentrations, but it also increased when 3MH was at level 4. The intensity of *green pepper* increased with 18.4 units in sample 4-4-2-2 compared to 13.3 units in sample 2-4-2-2. This could show the enhancing effect of 3MH on certain characteristics associated with IBMP. Furthermore, an increase in 3MH (4-2-2-2) also increased the *green pepper* intensity with 11.9 units. The contribution of thiols at certain concentrations to the *cooked green vegetal* attribute has been reported in earlier studies, however there was no contribution to the *fresh green* attribute by these compounds (King *et al.*, 2011).

Cooked beans increased whenever one or both IBMP and methional were present at level 4. The smallest increase was observed when 3MH were also at level 4 (4.6 units for 4-4-2-2). The largest increase was when IBMP and methional both were at level 4 in the same sample (27.2 units for sample 2-4-4-2). The presence of phenylacetaldehyde did not seem to have an influence as the increases for 2-2-4-4 and 2-2-4-2 were 19.4 and 18.7 units respectively. As mentioned before, the *cooked beans* attribute was used for both IBMP and methional when evaluated singularly and the increase in this attribute could be an indication of an enhancing effect between these two compounds. *Cooked potato* was significantly increased by increased concentrations of methional. With methional present at level 4 the increases were 28.6, 28.7 and 29.8 units for 2-2-4-4, 2-2-4-2 and 4-2-4-2 respectively. The presence of phenylacetaldehyde and 3MH at level 4 thus did not seem

to suppress the *cooked potato* attribute. To the contrary, when IBMP and methional were present at level 4 in the same sample (2-4-4-2), the increase in *cooked potato* reached 41.0 units. It could be concluded that IBMP could enhance the *cooked potato* descriptor when in the presence of methional.

The *floral* and *potpourri* descriptors increased with phenylacetaldehyde concentrations. *Floral* did not seem to be drastically influenced by the presence of other compounds as the increases ranged from 12.8-18.5 units for samples 2-2-2-4, 2-2-4-4, 4-2-2-4 and 2-4-2-4. *Potpourri* showed the highest increase when phenylacetaldehyde was the only compound present at level 4 (16.2 units for 2-2-2-4). In the other samples (2-2-4-4, 4-2-2-4 and 2-4-2-4), the increase was between 8.1 and 10.1 units. The perception of this attribute might be suppressed by the presence of other compounds.

The statistical network constructed with 4-4-4-4 as the centre sample (Figure 3.11) can be seen as complimentary to the network with 2-2-2-2 as centre sample, however, the network still provides additional information not displayed in the other networks. *Grapefruit* increased when methional concentrations decreased to level 2 (compared to sample 4-4-4-4). This phenomenon does not seem to be influenced by decreases of the other aroma compounds as the increase in intensity was between 6.8 and 9.1 units for samples 4-4-2-4, 4-2-2-4 and 4-4-2-2. The decrease in *grapefruit* also seemed to remain the same (between 14.1 and 15.9 units) when 3MH was lowered to level 2 despite the concentration changes of the other compounds.

There was a slight increase in the *tomato leaf/stalk* intensity (4.9 units) when methional and phenylacetaldehyde levels were lowered to level 2. *Guava ripe* decreased with decreased 3MH concentrations with an increase (7.2 units) when IBMP and methional levels decreased to level 2 (4-2-2-4). *Guava green* intensities decreased (3.1-3.2 units) with lowered 3MH levels and increased (4.9-5.6 units) when methional was present at level 2. The effect of 3MH on the perception of *green pepper* was observed once again. With decreased levels of 3MH or both 3MH and IBMP, the *green pepper* intensity also decreased by 8.4 units (2-4-4-4) and 10.3 units (2-2-4-4) respectively. The decrease in methional and phenylacetaldehyde concentrations led to an increase in *green pepper* intensity (9.2 units for 4-4-2-2) displaying the possible suppression effect of these two compounds on the perception of *green pepper*.

Cooked beans and *cooked potato* intensity increased with 12.1 and 15.2 units respectively with IBMP and methional at level 4 and 3MH and phenylacetaldehyde at level 2 (2-4-4-2). The additive effect of IBMP and methional (in the presence of low concentrations of the other compounds) might play a role in this phenomenon. *Cooked beans* and *cooked potato* intensities decreased when either IBMP or methional concentrations decreased. *Potpourri* might be suppressed by the presence of IBMP as

samples containing IBMP at level 2 (with phenylacetaldehyde remaining at level 4) had an increase in *potpourri* intensity (5.9-6.8 units).

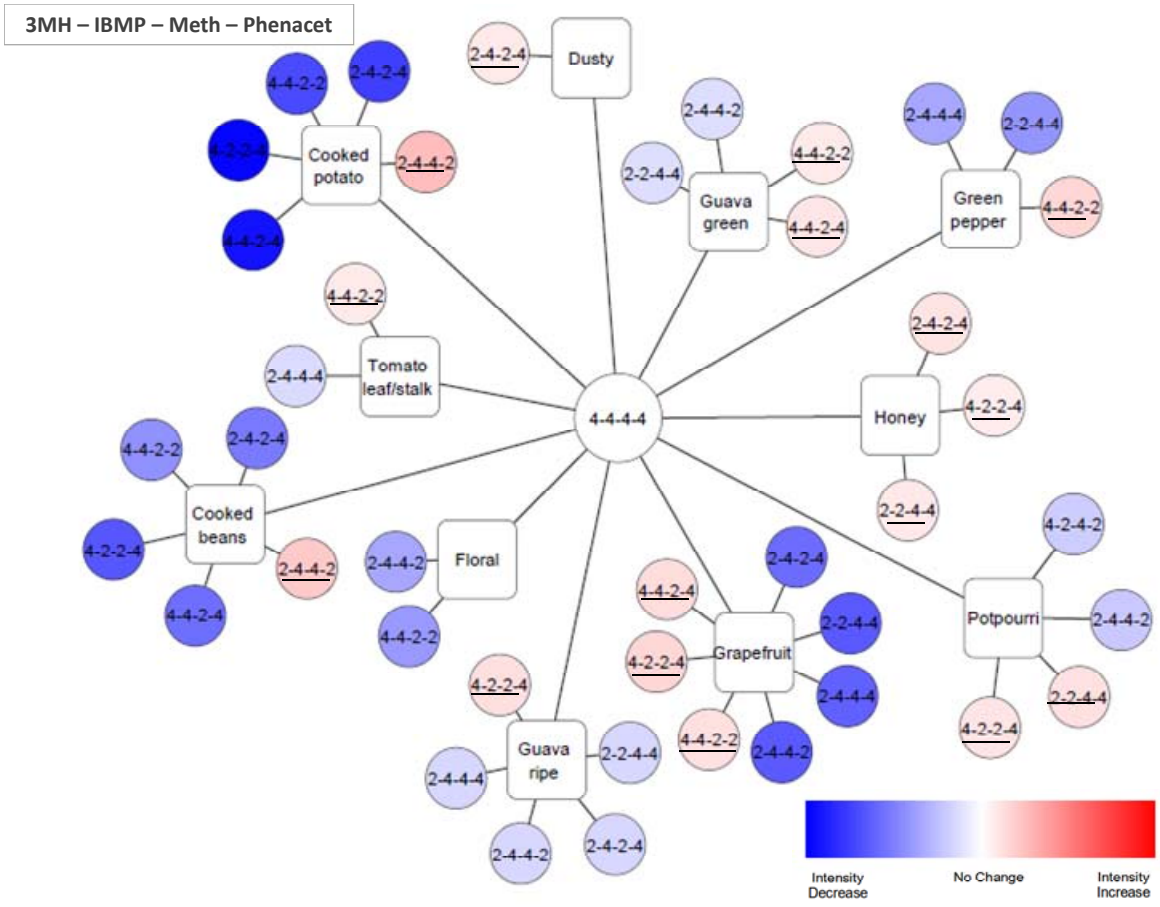

Figure 3.11 A statistical network (centre sample 4-4-4-4) showing the effect of sample combinations on attribute intensity. Edges between attributes and coloured nodes indicate a statistically significant difference from the centre sample. Red and underlined sample nodes indicate attribute intensity increase; blue nodes indicate attribute intensity decrease. Darker colour shows larger increase/decrease.

To show the effect of some of these interactions, response surface graphs were constructed. Response surface graphs are three dimensional representations of the data. Three variables are shown in the graph, two aroma compounds together with the descriptor. The graph thus shows how the intensity of the descriptor changes with the changing concentration of the two aroma compounds. The response surface plot for *grapefruit* (Figure 3.12) clearly showed the suppressive effect of methional. At a low methional concentration, the increase in 3MH concentration will lead to an increase in *grapefruit* intensity. However, as the concentration of methional increases, the *grapefruit* intensity will decrease even at high 3MH concentrations. In Figure 3.13 the suppressive

effect of methional on 3MH can again be observed as the increasing concentration of methional suppressed the *guava ripe* attribute. Overall, methional has a dominant effect over 3MH. This could have important implications during wine aging. Not only are the thiols oxidation sensitive (Blanchard *et al.*, 2004; Nikolantonaki *et al.*, 2010), but the remaining thiols will be suppressed as soon as methional is formed in the wine.

3MH present at relatively low concentrations enhanced the *green pepper* attribute in the presence of IBMP (Figure 3.14). This was seen throughout the IBMP range. However, at higher 3MH levels, the attribute intensity decreased. The suppression of IBMP odours by 3MH has also been observed in other studies (King *et al.*, 2011; Van Wyngaard, 2013). The present study highlighted the importance of testing intensities at various concentrations as the some interactions between compounds changed as the concentrations changed. The attribute c*ooked beans* was also influenced by various compounds. 3MH suppressed *cooked beans* when present at higher concentrations (Figures 3.15 and 3.16). On the other hand, when IBMP and methional were present in the same medium, there was an enhancing effect that can be seen in Figure 3.17.

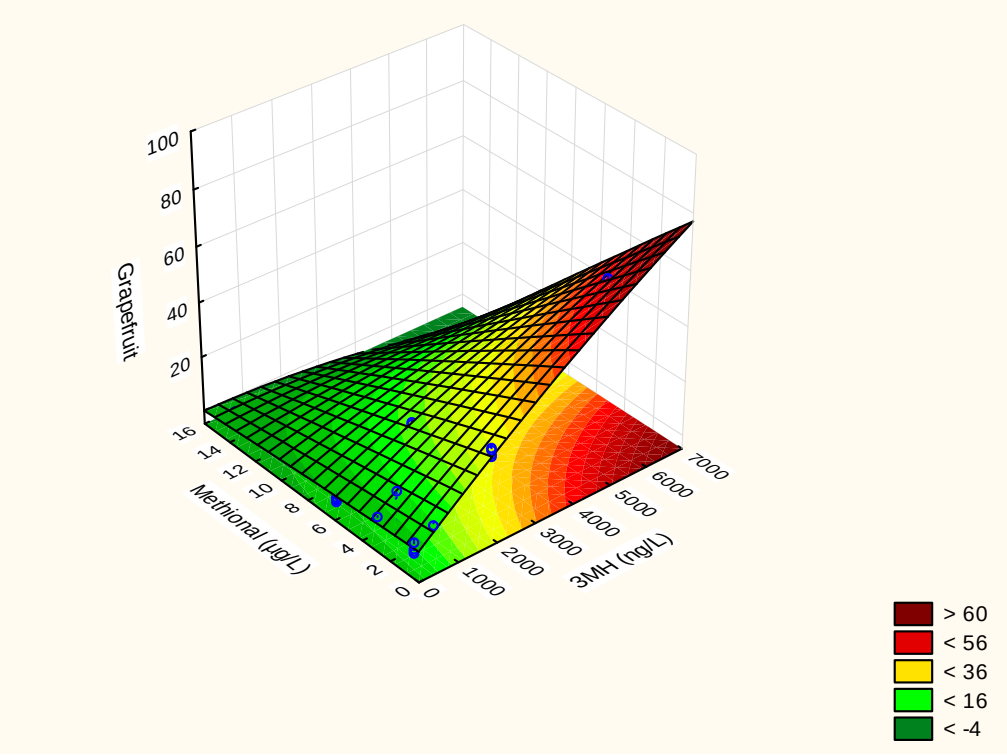

Figure 3.12 Response surface plot of the *grapefruit* attribute as influenced by 3MH and methional.

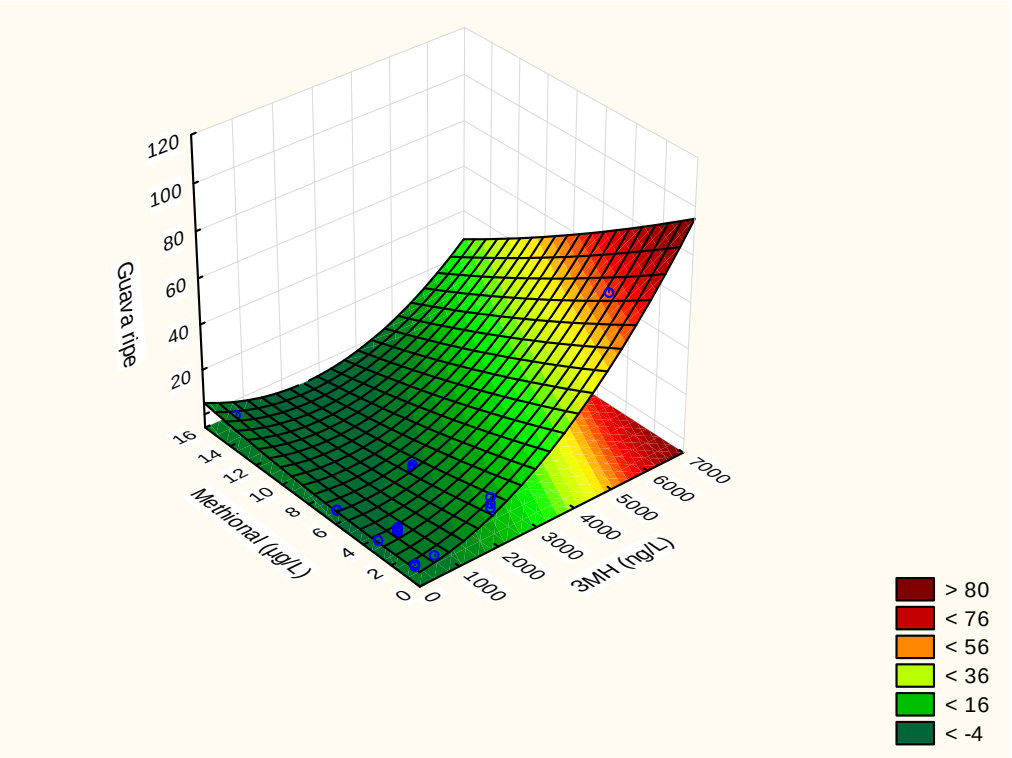

Figure 3.13 Response surface plot of the *guava ripe* attribute as influenced by methional and 3MH.

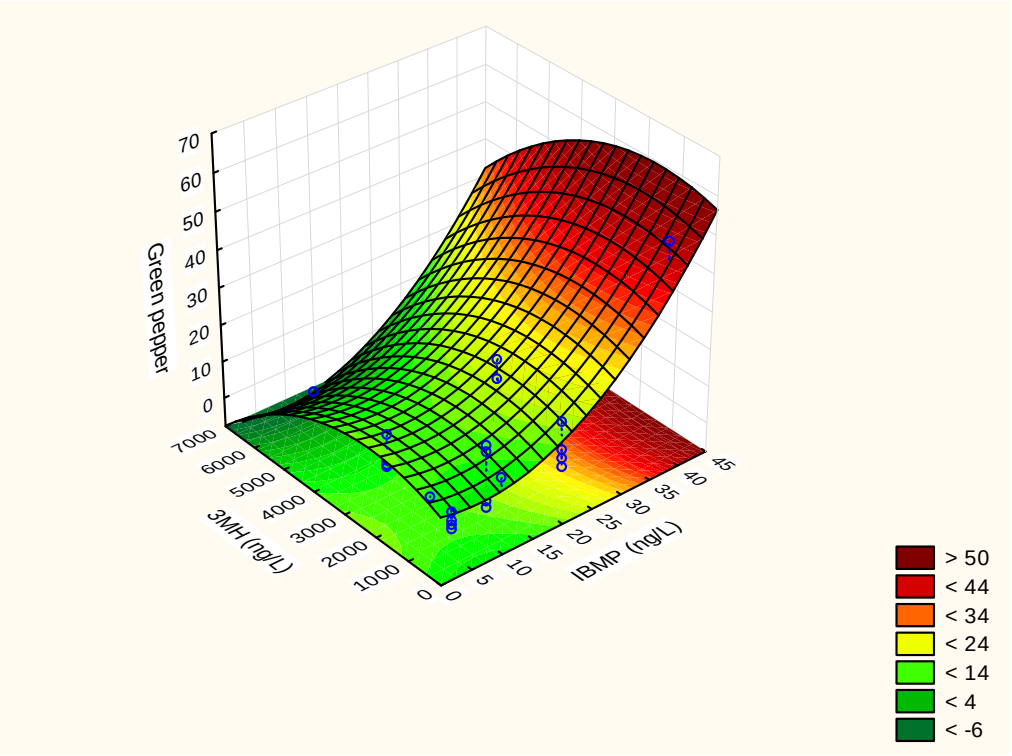

Figure 3.14 Response surface plot of the *green pepper* attribute as influenced by 3MH and IBMP.

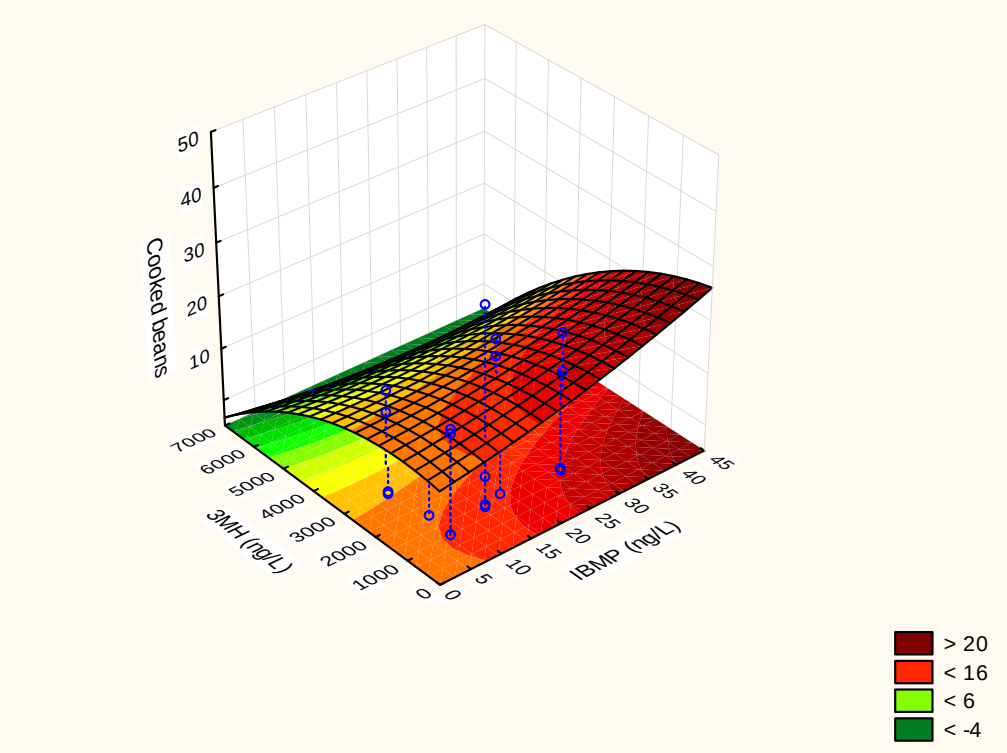

Figure 3.15 Response surface plot of the *cooked beans* attribute as influenced by 3MH and IBMP.

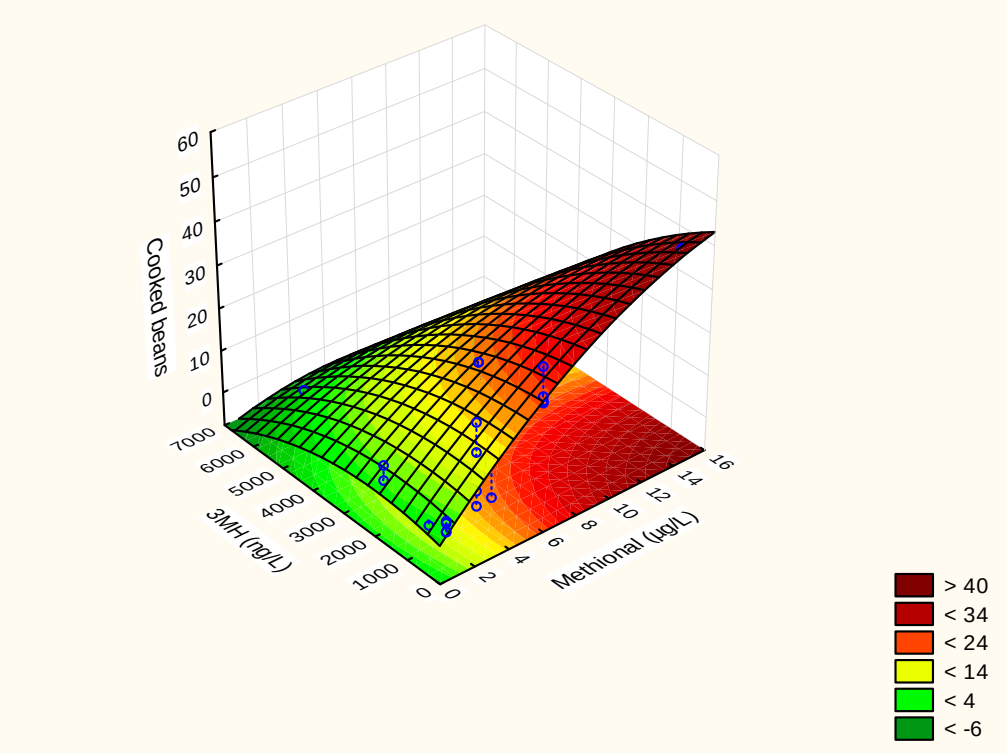

Figure 3.16 Response surface plot of the *cooked beans* attribute as influenced by 3MH and methional.

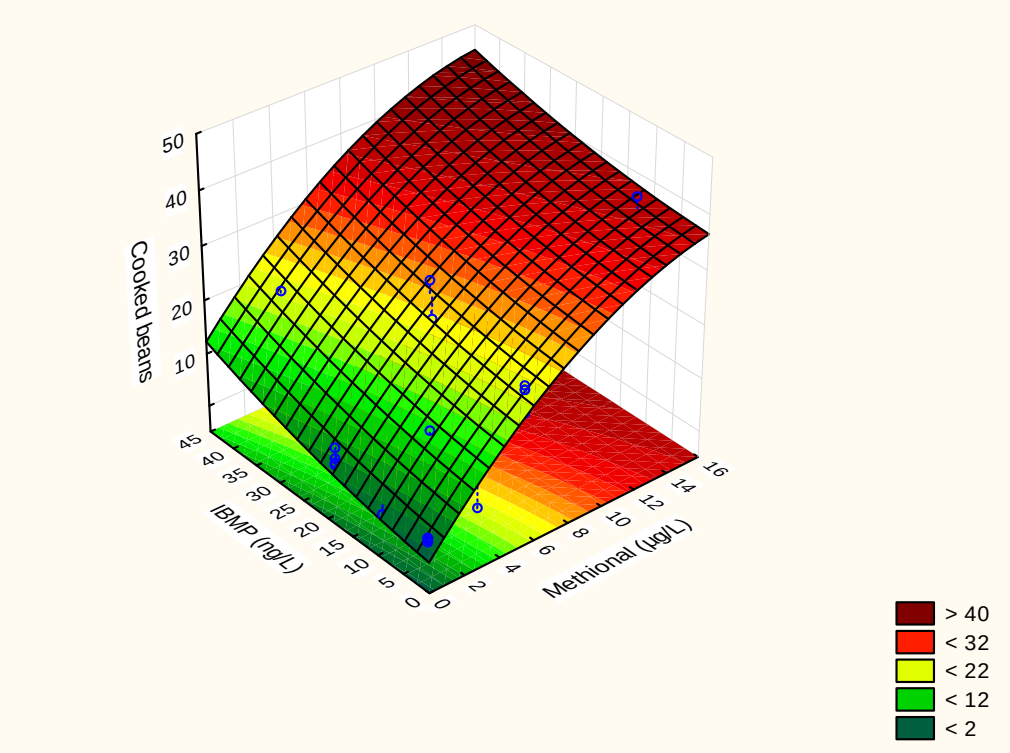

Figure 3.17 Response surface plot of the *cooked beans* attribute as influenced by IBMP and methional.

The contribution of 3MH to "green" characteristics has been reported before (King *et al.*, 2011). Some studies found no correlation between IBMP and IPMP levels in wine and the *vegetal* attribute and it was suggested that compounds other than the methoxypyrazines can be responsible for the perception of this aroma (Ferreira *et al.*, 2002; Preston *et al.*, 2008). The present study confirms the contribution of 3MH to these characteristics. As mentioned before, the enhancing effect of IBMP and methional in the perception of *cooked beans* and *cooked potato* could have important implications during wine aging. As IBMP is not oxidation-sensitive (Marais, 1998), the odours that occur due to this compound remain in the wine for a long period of time. With the formation of methional the enhancing effect could occur, causing odours such as *cooked beans* and *cooked potato* to be perceived at a stronger intensity level causing the wine to smell over-aged even at lower methional concentrations. This should be kept in mind when wines with higher methoxypyrazine content are stored for later consumption.

Certain compounds could suppress individual attributes in a unique way. By using statistical networks, these individual influences can be observed while this is not possible when analysing biplots as all the attributes that arise from one compound remain grouped. For instance, IBMP can enhance 3MH-related compounds such as *guava green* and *tomato leaf stalk*, while suppressing

guava ripe. The concentrations of the compounds involved should also be kept in mind when analysing the data as the interactions observed are dependent on the concentrations.

The analytical measurement of aroma compounds to predict the sensory composition of a wine should be used as an approximate tool as this study clearly showed the complex interactions between various compounds influencing the perception of the overall aroma. The exact mechanisms of the interactions remain unknown. The interactive effects could occur orthonasally or chemically in the medium. Further investigations are needed to elucidate this phenomenon.

3.4 CONCLUSION

The aim of this study was to improve the understanding of interactive effects between aroma compounds. In the present study interactive effects between typical Sauvignon blanc aroma compounds and compounds that occur due to oxidation were investigated.

The perception of flavours in wine is complicated by the vast number and combinations of aroma compounds present. The matrix used to test the interactions between compounds should not be underestimated as various compounds (volatile or non-volatile) present, even at below threshold concentrations, could potentially influence the perception of the compound tested. The challenge thus remains to investigate the sensory interaction between these compounds in real wine.

This study highlighted the importance of protecting a young, fruity Sauvignon blanc wine from forming atypical aging or oxidation-related compounds as these compounds (especially methional) significantly suppressed the fruity flavours. Further studies should include an investigation of other oxidation and aging compounds that could affect the aroma composition of the wine, whether these interactions also occur in real wine and whether consumer studies yield similar results.

3.5 ABBREVIATIONS USED

3-mercaptohexan-1-ol (3MH); 3-mercaptohexan-1-ol acetate (3MHA); 4-mercapto-4-methylpentan-2-one (4MMP); 3-isobutyl-2-methoxypyrazine (IBMP); 3-isopropyl-2-methoxypyrazine (IPMP); 3-*sec*-butyl-2-methoxypyrazine (SBMP); PCA, principle component analysis.

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Chapter 4

Research results

A chemical and sensory study on the evolution of aromatic and non-aromatic compounds during the progressive oxidative storage of a Sauvignon blanc wine

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4.1 INTRODUCTION

The shelf life of especially white wine is of great concern for the wine industry. Oxidation may play an important role in white wine's composition and its sensory characteristics. The shelf life of young white wines and the rate of oxidation in general are influenced by factors such as the composition of the wine (antioxidant content, pH etc.), storage time, storage temperature, type of closure (oxygen ingress and adsorption), colour of the bottle and the storage position (Silva Ferreira *et al.*, 2002b; Skouroumounis *et al.*, 2005; Lopes *et al.*, 2006; Papadopoulou & Roussis, 2008; Maury *et al.*, 2010; Dimkou *et al.*, 2013; He *et al.*, 2013). During oxidation, a decrease in certain aroma compounds occurs followed by a change in wine colour (Singleton & Kramling, 1976; Singleton *et al.*, 1979; Escudero *et al.*, 2000b). The change in aroma will typically result in the wine initially losing some of the varietal character (Blanchard *et al.*, 2004; Nikolantonaki *et al.*, 2010) after which an increase in unwanted oxidation aroma compounds such as aldehydes and sotolon will occur (Wilderandt & Singleton, 1974; Escudero *et al.*, 2000a; Silva Ferreira *et al.*, 2003c; Loscos *et al.*, 2010; Escudero *et al.*, 2002; Silva Ferreira *et al.*, 2003c; Lavigne *et al.*, 2008). Oxygen modulates the oxidation reactions, however other chemical reactions not involving oxygen could also occur during wine aging, thus a certain degree of change in wine composition will occur even in an environment devoid of oxygen (Ugliano *et al.*, 2009).

Sauvignon blanc wines can have a large diversity of flavours such as fruity ("grapefruit", "passion fruit", "gooseberry", "citrus", "tropical") and green characters ("green pepper", "grassy", "asparagus") (Marais, 1994; Tominaga *et al.*, 1998a; Lund *et al.*, 2009; Coetzee & Du Toit, 2012). These descriptors have been attributed to key chemical aroma and flavour compounds occurring in the wine. The volatile thiols (4-mercapto-4-methylpentan-2-one (4MMP), 3-mercaptohexan-1-ol (3MH) and 3-mercaptohexan-1-ol acetate (3MHA)) can contribute to "tropical/fruity" style wines, while the methoxypyrazines (3-isobutyl-2-methoxypyrazine (IBMP), 3-isopropyl-2-methoxypyrazine (IPMP), 3-*sec*-butyl-2-methoxypyrazine (SBMP)) can give the wine a "green pepper", "grassy" and "asparagus" odour (Lacey *et al.*, 1991; Tominaga *et al.*, 1998a; Swiegers *et al.*, 2006; Coetzee & Du Toit, 2012). Both of these odour groups are often sought after for the production of quality Sauvignon blanc wines (Lund *et al.*, 2009; King *et al.*, 2011). The volatile thiols are known to be oxidation sensitive and can also decrease due to acid hydrolysis during aging (Blanchard *et al.*, 2004; Tominaga *et al.*, 2004; Herbst *et al.*, 2008). The methoxypyrazines are not sensitive to oxidation and studies have shown methoxypyrazine concentration to remain the same during oxidative handling of juice and wine (Marais, 1998; Coetzee *et al.*, 2013). These two groups of compounds are considered

to be character impact compounds for Sauvignon blanc wines, however they are not solely responsible for the overall aroma. Various other chemical groups such as esters, higher alcohols, fatty acids and terpenes can also contribute significantly to the aromatic composition (Swiegers *et al.*, 2006). The origin and reaction of the various aroma groups have been discussed in Chapter 2 and a comprehensive review on Sauvignon blanc aroma (with a focus on volatile thiols) and the reaction of various aroma groups to oxidation has been recently published (Coetzee & Du Toit, 2012).

During and after the disappearance of the positive aroma, the formation of unwanted aroma compounds such as acetaldehyde, methional, phenylacetaldehyde and sotolon can occur during wine oxidation and can be detrimental the wine aroma, causing flavours described as “green apple”, “potato”, “honey” and “curry” to dominate the wine aroma bouquet (Frivik & Ebeler, 2003; Silva Ferreira *et al.*, 2003c). The presence of these oxidation compounds could also interact with remaining pleasant aroma compounds leading to the suppression of certain positive attributes as was seen in the case of methional in Chapter 3. The formation of these oxidation-related aromas should be avoided to preserve the fresh and fruity character of the wine.

In some cases, a certain amount of oxygen can significantly improve the wine aroma and quality by preventing the formation of ‘reductive’ off-odours in the bottle. Aroma attributes associated with these off-odours are “rotten egg”, “cabbage” and “garlic” and the compounds, H_2S and methyl mercaptan have been indicated as being primarily responsible for post-bottling reduction (Lopes *et al.*, 2009; O'Brien *et al.*, 2009b; Ugliano *et al.*, 2011). Studies investigating various types of bottle closures (with different oxygen transmission rates) concluded that closures that are less oxygen permeable allow better preservation of fresh and fruity aromas, especially the preservation of volatile thiols (Lopes *et al.*, 2009). However, these types of closures could promote the production of ‘reductive’ off-odours (Godden *et al.*, 2001; Skouroumounis *et al.*, 2005; Kwiatkowski *et al.*, 2007; Lopes *et al.*, 2009; O'Brien *et al.*, 2009b).

The effect of oxygen and aging on various aspects of dry white wines have been investigated (Silva Ferreira *et al.*, 2003c; Brajkovich *et al.*, 2005; Herbst *et al.*, 2008; Lopes *et al.*, 2009; Dimkou *et al.*, 2011; Herbst-Johnstone *et al.*, 2011; Ugliano *et al.*, 2011; Makhotkina *et al.*, 2012; Fracasetti *et al.*, 2013) and the evolution of wine sensory quality is thought to reach a peak after a period in the bottle, however the time period necessary and the amount of oxygen required to reach this peak remain unknown. This identification is complex, as it depends on how the wine was intended to evolve, when it is going to be consumed and how sensitive the wine is towards oxygen. Understanding a wine’s oxygen needs enables the winemaker to control wine evolution in the bottle by choosing the optimal closure in accordance with the desired wine style and shelf life.

In a commercial cellar, oxygen can come into contact with wine during different winemaking processes, such as transfer of wine, filtration and bottling (Castellari *et al.*, 2004; Du Toit, 2006). In the past a number of studies investigated the effect of adding large amounts of oxygen to white wines and/or storage at elevated temperatures (Ferreira *et al.*, 1997; Escudero *et al.*, 2002; Silva Ferreira *et al.*, 2003c; Loscos *et al.*, 2010; Cejudo-Bastante *et al.*, 2013). Although this type of approach enables a more rapid rate of oxidation and yielded valuable data, not much is known about repetitive oxidation of Sauvignon blanc wine over a prolonged period of time at oxygen levels and temperatures which would normally occur in a commercial wine cellar. Furthermore, the collection of a large variety of data from aromatic to non-aromatic chemical compounds as well as sensory assessment including both orthonasal and visual investigations, are a first for white wine aging research.

This is the first study that we know of that monitored Sauvignon blanc wine evolution in a controlled environment after which both chemical and sensory analysis were performed. Successive mild oxygen additions were applied and oxygen concentrations were specifically measured while the wines were kept at a mild temperature over a long period of time. Frequent sampling also allowed the investigation of the evolution of the aromatic and non-aromatic compounds during the time of the study and the inclusion of sensory data at each of these stages provided a comprehensive assessment of wine oxidative aging in terms of chemical composition and sensory effects.

4.2 MATERIALS AND METHODS

4.2.1 Oxidation of the wine

A Sauvignon blanc wine was oxidised at various stages over time to compare chemical content and sensory impact. Wine was collected from a commercial cellar (De Grendel Wine Estate, Tygerberg, South Africa) directly after the 2011 harvest. The wine was transported to the experimental cellar of the University of Stellenbosch after which sulphur dioxide (SO_2) and ascorbic acid content were measured (section 4.2.7). The wine was then divided using nitrogen gas (Afrox South Africa) into 33 x 5 litre glass bottles. Figure 4.1 shows the layout of the study done in triplicate. After distribution of the wine into the bottles, the first treatment (Control 0) was sampled and frozen at -20 °C for analyses. This treatment would serve as the “beginning” of the trail (no time lapse). All of the Ox treatments then received oxygen by using a micro oxygen sparger (Figure 4.2) connected to a

cylinder containing high purity (99.5%) medical oxygen (Afrox South Africa). No oxygen was added to the Control samples. All treatments were stored in the dark at 15 °C. The next oxygenation took place when the dissolved oxygen concentrations reached a level below 1 mg/L. Overall the study proceeded for 7 months.

Figure 4.1 Experimental layout

During sparging, the oxygen concentration was constantly monitored by using oxygen sensor spots (Pst3; PreSens, Regensburg, Germany) fitted inside each bottle allowing a non-invasive measurement (by not opening the vessel) over time (Figure 4.2). Presens uses the oxoluminescense principle to provide reliable measurements of dissolved oxygen in the wine through the technical capability tied up in the oxygen sensor spot. A light-emitting diode (LED) provides a blue light excitation pulse to the oxygen sensor spot, which returns a fluorescent red light signal indicative of the oxygen concentration.

Sparging stopped as soon as the required dissolved oxygen concentration was reached. The bottles were then hermetically sealed using a tight sealing plastic screw cap. As soon as all the oxygen in the Ox samples was consumed, the next phase (T1 Control and T1 Ox) was sampled and frozen at -20 °C. The remaining Ox treatments (T2 Ox to T5 Ox) were oxygenated again to the required level of dissolved oxygen and the process was repeated until all treatments were sampled and frozen for analyses.

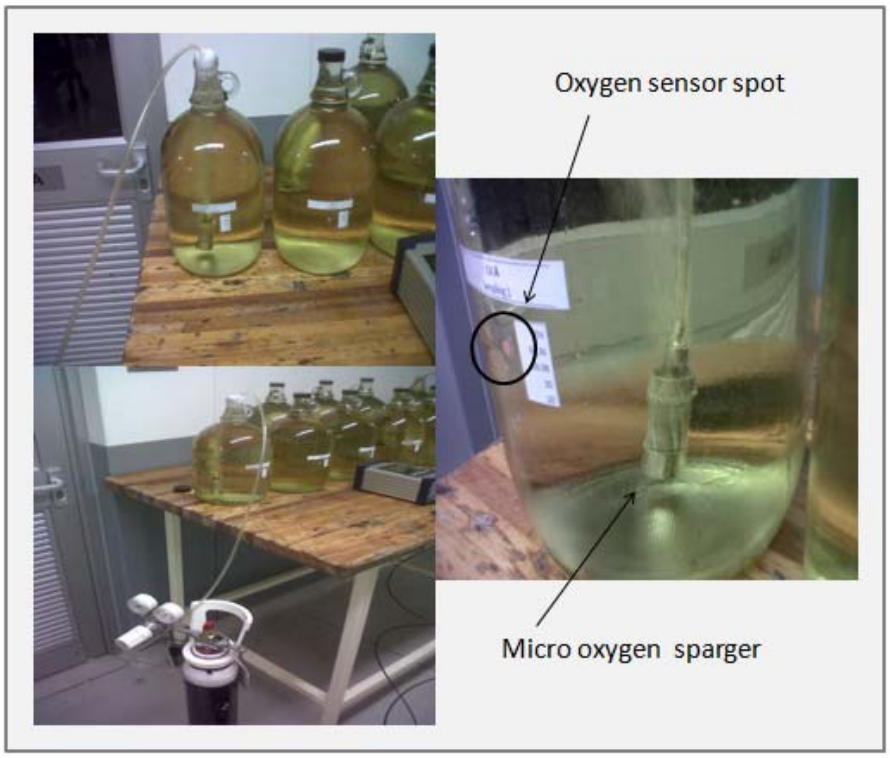


Figure 4.2 Oxygen dosing process

4.2.2 Sampling

Sampling was done at the end of each time frame (T1, T2, T3, T4 and T5) and all samples were frozen until the complete set of 33 samples could be analysed at the same time. To the samples taken for the analyses of volatile thiols, phenols and glutathione (GSH), 100 mg/L sulphur dioxide was added. The remainder of the wine (± 4 litres) were transferred into 5 L plastic containers, sealed under CO_2 gas and frozen at -20˚C for sensory analysis.

4.2.3 Volatile thiol analysis

Analysis was done to quantify three volatile thiols, 3MH, 3MHA and 4MMP. Measurement of volatile thiols were done using sample preparation and quantification as first described by Tominaga *et al.* (1998b). The method was adjusted according to Suklje *et al.* (2012). Some modifications such as the addition of deuterated internal standards were implemented for the quantification of 3MH [3-mercapto(1-$^2H_2$)hexanol] and 3MHA [3-mercapto(1-$^2H_2$)hexyl acetate] (Hebditch *et al.*, 2007). 4-Methoxy-2-methyl-2-mercaptobutane (4MMB) was used as internal standard for 4MMP.

To 50 mL wine, 5 mL of 1mM p-hydroxymercurybenzoate (p-HMB) solution and then 0.5 mL of 2 nM butylated hydroxyanisole (BHA) solution was added. After stirring the sample, deuterated labelled isotopes were added. The wine sample was percolated on an anion exchange column using Dowex resin (Sigma-Aldrich) after which the thiols were eluted with 50 mL of 50 mM cysteine solution and extracted into dichloromethane prior to concentration. Organic phases were evaporated under reduced pressure (250 mbar) to approximately 0.5 mL and transferred into 1.5 mL dark vials. The Soxhlet flask was rinsed with 0.5 mL dichloromethane and then placed in an ultrasonic bath for one minute. Samples were then further concentrated under reduced pressure (100 mbar) to approximately 30 µL.

The concentrated samples were injected onto a gas chromatograph (GC) (Agilent Technologies 7890A) coupled to a mass spectrometric detector (Agilent Technologies 5975C upgraded with a Triple-Axis Detector, Agilent, Santa Clara, CA, USA). The thiols were separated on a HP-Innowax column (60 m x 0.25 mm x 0.25 µm) with helium as carrier gas at a flow rate of 0.6 mL/min. Injector temperature was set at 240˚C. The initial oven temperature (50˚C for 5.0 min) was ramped to 115°C at a rate of 3°C/min, then increased to 150 at 40°C/min (held for 3.0 min), after which it was increased to 205°C at a rate 3°C/min and finally increased to 250°C at 10°C/min (held for 19.6 min) before dropping to 50°C at 40˚C/min (held for 3.0 min). The ion source temperature was 230˚C, the auxiliary temperature at 250˚C and the quadrupole temperature adjusted to 150˚C. For qualitative determination retention time and mass spectrum in selective ion monitoring mode (SIM) were used.

4.2.4 Methoxypyrazine analysis

IBMP and IPMP analyses were outsourced to an accredited laboratory (VinLAB Pty Ltd, Stellenbosch, South Africa). The method used was adjusted according to various published methods (Allen & Lacey, 1998; Kotserides *et al.*, 1999; Roujou de Boubée *et al.*, 2000; Hartmann *et al.*, 2002). Fifty millilitres of wine containing isopropylethoxypyrazine as internal standard were extracted using C18 solid phase extraction (SPE) cartridges (ISOLUTE, International Sorbent Technology). The extract was concentrated under a gentle flow of nitrogen to 500 µL, of which 1 µL was injected into the instrument. Gas chromatography (Varian 3900 GC) coupled to a mass spectrometer (MS) (Varian, Saturn 2100T) was used. The instrument contained a COMBIPAL-xt auto sampler with a split/splitless 1177 injection port. Separation was done using a CPWAX52 column (30 m x 0.25 mm x 0.25 µm) from Agilent J&W. The carrier gas consisted of helium at a flow rate of 1.3 mL/min. The injection port was heated to 220˚C and the MS transfer line to 245˚C. Oven temperature started at 50˚C for

5.0 min which was then increased at 5˚C/min to 110˚C after which it was ramped to 245˚C at 25˚C/min. Each compound was quantified by comparison with a calibration curve constructed using pure standards. The relative peak area and internal standard peak area were then compared to the calibration curve in order to quantify each compound. The limit of quantification (LOQ) and detection limit (LOD) were 2.5 and 1 ng/L respectively.

4.2.5 Ester, acid and alcohol analysis

Chemicals, standards, and model wine matrix have been described (Louw, 2007; Louw *et al.*, 2009). Five millilitres of wine with internal standard, 4-methyl-2-pentanol (100 µL of 0.5 mg/L solution in 12 % v/v ethanol, 2.5 g/L tartaric acid, deionised Milli-Q-Water® (Millipore Filter Corp., Bedford, MA, USA)), pH adjusted to 3.5 using 0.1 M NaOH), was extracted with 1 mL of diethyl ether by sonicating the ether/wine mixture for 5 minutes. The wine/ether mixture was then centrifuged at 3600 g for 3 min. The ether layer was removed and dried on anhydrous Na_2SO_4. The extract was then transferred to a vial containing an insert and capped. Each extract was injected into the instrument in duplicate.
A gas chromatography-flame ionization detector (GC-FID) was used for the analysis. Validation of the method, in terms of selectivity, linearity, LOD, LOQ, recovery, robustness, and repeatability, has been described (Louw, 2007; Malherbe, 2011). A J&W DB-FFAP capillary GC column (Agilent, Little Falls, Wilmington, DE) with dimensions 60 m × 0.32 mm × 0.5 µm and a Hewlett Packard 6890 Plus GC (Agilent, Little Falls, Wilmington, DE) equipped with a split/splitless injector and FID detector were used. Three µL of the diethyl ether extract was injected at 200˚C. The initial oven temperature was 33˚C for 8.0 min, after which the temperature was increased by 21˚C/min to 130˚C, at which it was held for 1.3 min. The temperature was then ramped at 21˚C/min to 170˚C held for 1.0 min then up to 240˚C held for 2.5 min. Post run occurs at 240˚C for 5.0 min. The split ratio was 15:1, and the split flow rate was 49.5 mL/min. The column flow rate was 3.3 mL/min using hydrogen as a carrier gas. The detector temperature was 250˚C and with a column flow of 6.0 mL/min, cleaned the column of high boiling contaminants. Quantification was performed by comparing the ratio of the peak area and internal standard peak area with calibration graphs constructed using pure standards (Louw, 2007; Malherbe, 2011).

4.2.6 Monoterpene analysis

Solid phase extraction was performed in a Visiprep SPE vacuum manifold 20-port model from Supelco, in which there 20 positions are available for performing the SPE simultaneously. Cartridges (Strata SDB-L, Phenomenex, Torrance, CA, USA) were conditioned by rinsing with 4 mL dichloromethane, 4 mL methanol and finally 4 mL of model wine solution (12% ethanol-water mixture). Internal standard, 50 µL of 2,6-dimethyl-6-hepten-2-ol (25 mg/L in ethanol) was added to 50 mL of wine and mixed briefly. The wine was then rinsed through the cartridge by vacuum suction (-0.5 kPa). Clean-up was obtained by flushing the cartridge with 4 mL Milli-Q-Water®. The cartridge was then dried under vacuum (-10 kPa) for 15 minutes. Finally, terpenoids were eluted from the solid phase using 2 mL dichloromethane. The dichloromethane elute was dried on sodium sulphate crystals and injected into the GC-FID. Each extract was injected into the GC-FID in duplicate.

Separation and quantification were performed on a Hewlett-Packard (Palo Alto, CA) 5890 Series II gas chromatograph equipped with a 60 m × 0.32 mm × 0.5 µm fused DB-FFAP capillary column (J&W Scientific, Folsom, CA), and flame ionization detector. Separation conditions were as follows: injector temperature 200˚C; GC column temperature 40˚C (12 min) at 12˚C/min to 190˚C, followed by a temperature ramp of 15˚C/min to a final temperature of 250˚C held for 2 min; carrier gas helium at 40 kPa. Each compound was quantified by comparison with a calibration curve constructed using pure standards. The relative peak area and internal standard peak area were then compared to the calibration curve in order to quantify each compound (Louw, 2007).

4.2.7 Sulphur dioxide and ascorbic acid analysis

Free and total SO_2 were analysed potentiometrically (Ripper method) using the Metrohm titration unit (Metrohm Ltd., Switzerland) (Amerine & Ough, 1974). Ascorbic acid analysis was done by an accredited commercial laboratory (Vinlab, Stellenbosch) using an automated enzymatic procedure.

4.2.8 Glutathione analysis

A 200 µL wine sample was transferred into a 2 mL eppendorf tube (Eppendorf, Hamburg, Germany) after which it was diluted 5 times by adding 800 µL HPLC grade Milli-Q-Water® supplemented with 1000 mg/L SO_2 and a freshly prepared 500 mg/L ascorbic acid solution (Sigma-Aldrich, St. Louis, MO,

USA) to protect the sample against oxidation. The diluted sample was then centrifuged (Centrifuge 5415 D, Eppendorf, Hamburg, Germany) at 12 800 rpm for 5.0 minutes at 20°C after which 950 µL were transferred to an amber coloured vial which was sparged with CO_2 gas before capping.

Ultra performance liquid chromatography-tandem mass spectrometric (UPLC-MS/MS) analysis was performed on a Waters Acquity UPLC (Milford, MA) connected to a Waters Xevo triple-quadrupole MS using electronspray ionization in the positive mode. Separation was achieved on a Waters Acquity BEH Phenyl column (100 x 2.1 mm x 1.7 µm), using a 0.4% trifluoroacetic acid (Solvent A) to acetonitrile (Solvent B) gradient. The injection volume was 3 µL. The MS settings were optimized for best sensitivity, a Cone voltage of 18 V was used for reduced GSH and 20 V for oxidised GSH (GSSG). Data was acquired in multiple reaction monitoring mode (MRM). A MRM transition of 308.1>179.1 at a collision energy of 17 V was used for GSH. A MRM transition of 613.1 > 355.1 at a collision energy of 20 V was used for GSSG (Kritzinger *et al.*, 2013).

4.2.9 Phenolic analysis

Reverse phase-high performance liquid chromatography (RP-HPLC), adapted from the method of Peng *et al.* (2002) was performed on a Agilent 1200 series HPLC system equipped with a diode array detector (Agilent Technologies, Palo Alto, CA, USA). Data processing was done with Chemstation software (Agilent, Waldbronn, Germany). Separations were carried out on a polystyrene/divinylbenzene reversed phase column (PLRP-S, 100Å, 150 × 4.6 mm, 3 µm) from Polymer Laboratories (Ltd) (Shropshire, UK) protected with a guard cartridge (PLRP-S, 10 × 4.6 mm) (Polymer Laboratories (Ltd), Shropshire, UK) with the same packing material. The following mobile phases were used: solvent A, containing 1.5% *o*-phosphoric acid (Merck) and solvent B consisting of acetonitrile (Sigma). A flow rate of 1 mL/min was used and a column temperature of 35˚C. Phenols were quantified using external standards: (+)-catechin hydrate (Fluka), (-)-epicatechin (Sigma) and caffeic acid (Sigma). Monomeric flavanols were quantified at 280 nm as mg/L (+)-catechin units with a quantification limit of 1.5 mg/L, and (-)-epicatechin as (-)-epicatechin with a quantification limit of 1.5 mg/L. *Trans*-caftaric acid and grape reaction product (GRP) have a maximal absorbance at 316 nm and were quantified as mg/L caffeic acid. Wine samples were filtered through a 0.45 µm filter after which the sample was placed in a 1.5 mL dark glass vial and protected with nitrogen gas from oxidation. The LOQ was determined as the smallest area that could be accurately integrated (<3% standard deviation), or expressed as signal to noise ratio of at least 10 (Peng *et al.*, 2002).

4.2.10 Carbonyl compounds and acetal analysis

The extraction procedure was done according to a previous publication by Silva Ferreira *et al.* (2003a). In short, 50 mL of wine sample were spiked with 50 µL of 3-octanol in a hydroalcoholic solution (447 mg/L) as the internal standard. Anhydrous sodium sulphate (5 g) was added to increase the ionic strength of the sample after which the wine was extracted twice with 5 mL of dichloromethane. The two organic phases were then blended and dried with anhydrous sodium sulphate. A total of 2 mL of the organic extract were concentrated to 0.4 mL under a constant nitrogen stream. Extracts were analysed using a Varian 450 GC, equipped with a mass spectral detector, Varian 240-MS, and the Varian MS workstation software version 6.9.1. Separation was done using a Stabilwax-DA column (60 m x 0.25 mm x 0.25 µm) fused silica (Restek, Bellefonte, PA). The injection port was heated to 220˚C. The injection volume was 2 µL in splitless mode. Helium was used as carrier gas (Gasin, Portugal) at a constant flow rate of 1.0 mL/min.

Oven temperature started at 40˚C for 1.0 min which was then increased at 2˚C/min to 220˚C and held for 20 min. Mass spectra were acquired in the electron impact mode (ionization energy, 70 eV; source temperature, 180˚C). Ion trap temperatures were set at 230, 45 and 170˚C respectively. Mass range was *m/z* 33-350, with scan rate of 6 scans per second in full scan mode. The emission current was 50 µA, and the electron multiplier was set in relative mode to the autotune procedure. Maximum ionization time was 25 000 µs, with an ionization storage level of *m/z* 35. Compound identification was achieved by comparing retention times and mass spectra obtained from a sample containing pure, authentic standards. Kovats indices were calculated and also used as validation when compared to mass spectra, as reported in the National Institute of Standards and Technology (NIST) 05 MS library database. Quantification was done on the basis of standard calibration curves. No standard curves were constructed for benzaldehyde, *cis*- and *trans*-dioxane, *cis*- and *trans*-dioxolane, methionol, furfural and 5-hydroxymethylfurfural. For these compounds, the peak area to internal standard ratio was used to compare the various treatments.

Total acetaldehyde was analysed using an Arena 20XT enzyme robot (Thermo Electron Oy, Finland). The kit used for the analysis is the Boehringer Mannheim / R-Biopharm Succinic acid (Roche catalogue number 10668613035 manufactured by R-Biopharm AG, Darmstadt). Acetaldehyde is quantitatively oxidised to acetic acid in the presence of aldehyde dehydrogenase (Al-DH) and nicotinamide-adenine dinucleotide (NAD). This enzyme buffer is at pH 9 which releases all bound acetaldehyde from the sulphite. The amount of NADH formed by this reaction is stoichiometric to the amount of acetaldehyde and NADH is determined by means of its light absorbance at 340 nm.

The calibration range for the analysis ranged from 0-216 mg/L and an r^2 value of 0.998 was obtained. Analysis and automation were done according to the manufacturer's instructions.

4.2.11 Spectrophotometric measurement

Two millilitres of wine sample was placed in 10 mm optical path length cuvette. The colour of the wines were measured at 420 and 440 nm against the blank (Milli-Q-Water®) using a Thermo Spectronic GENESYSTM 10 Ultraviolet (UV) spectrophotometer (Analytic Jena Specord 50 UV/VIS Spectrophotometer; Jena, Germany). The absorbance at 420 nm is commonly used in the wine industry to measure brown colour in white wines (Singleton & Kramling, 1976; Zoecklein *et al.*, 1995; Iland *et al.*, 2004) while 440 nm corresponds to the maximum absorbance of coloured pigments in a model wine containing (+)-catechin (Bradshaw *et al.*, 2001).

4.2.12 Sensory aromatic descriptive analysis

The wines were subjected to descriptive analysis for aroma profiling. Twelve judges participated in the evaluation. Four training sessions of two hours each were conducted to obtain consensus between the judges. At each training session the panel was presented with all eleven wines. The first section of the training was aimed at generating suitable attributes for all the wines. The wines were then scored on a 100 mm unstructured line scale according to the attribute intensity. Scaling the attribute at 0 indicated the absence of the descriptor while scaling at 100 indicated to a very high intensity. Reference standards were made in order for the panel to familiarize themselves with the natural scent. After training, the wines were subjected to testing (no discussion) where the results were collected on paper ballots. Each replication was tested in duplicate. This would mean that the sample set was subjected to 6 sessions of testing (each biological repetition tested twice). The evaluation was done in booths with artificial daylight lighting and temperature control at 20±2˚C. Black ISO wine tasting glasses were used to avoid any potential bias on the panel due to visual cues, with the risk that if carried out under natural lighting darker wines may have been rated as higher in attributes related to oxidation. Sample glasses were marked with a random three-digit code (unique for each judge) and glasses were covered with a plastic lid prior to sensory assessment to prevent the aroma of contaminating the laboratory environment. The order of the samples was random and balanced across the assessors. Panellists evaluated the samples orthonasally only and regular breaks were taken between samples to prevent fatigue.

4.2.13 Sensory colour analysis

Sorting was done to classify the wines according to colour. Each biological repetition was sorted by 17 judges which resulted in 51 answers (3 biological repetitions). Judges consisted of winemakers, final year and post graduate viticulture and oenology students and wine researchers. Judges were chosen according to their knowledge of wine and were asked to arrange the 11 wines from least oxidised to most oxidised. The sorting sheet also stipulated the wines be arranged according to colour only (no sniffing). The sorting was done in booths with standard artificial daylight lighting and 40 mL of the wine sample were presented in clear ISO wine tasting glasses. Sample glasses were marked with a random three-digit code (unique for each judge) and glasses were covered with a plastic lid.

4.2.14 Data analysis

For statistical analysis of the chemical data, one-way analysis of variance (ANOVA) was conducted to investigate differences between treatments. For the sensory data, assessor performance was evaluated using PanelCheck (Version V1.4.0, Nofima, Tromsø, Norway) according to the workflow as described by Tomic *et al.* (2010). ANOVA, correlation analysis, partial least squares regression (PLS) and cluster analysis (CA) were performed using Statistica software (Statistica 10, Statsoft Inc., Tulsa, USA), while principle component analysis (PCA) was done using "UBbipl" package (Gower *et al.*, 2011). The PCA biplots were created using the correlation matrix of the mean data and in the PLS, the chemical data are presented as x variables (predictor variables) and sensory descriptive data as y variables. Post-hoc Fisher's least significant difference (LSD) tests were used to test for significance and a p value threshold of 0.05 ($p<0.05$) was used for the determination of statistical significance.

4.3 RESULTS AND DISCUSSION

4.3.1 Oxygen concentration

Oxygen was added to the Ox treatments at five different intervals, while the Control did not receive any oxygen. Table 4.1 shows the total amount of oxygen consumed by the treatments as well as the time lapse between each oxygenation.

Table 4.1 Total amount of oxygen consumed (mg/L) and time between sampling of each stage. Oxygen concentration values are means of triplicate analysis with standard deviation; different letters indicate significant differences at $p<0.05$.

		Total consumed oxygen concentration (mg/L)	
Treatment and days aged		Control	Ox
T0	0 days	0	
T1	64 days	0	6.59^{e} ±0.33
T2	148 days	0	11.93^{d} ±0.06
T3	190 days	0	16.99^{c} ±1.04
T4	204 days	0	22.18^{b} ±1.04
T5	218 days	0	29.99^{a} ±0.41

Total dissolved oxygen concentrations administered during this study varied from an average of 6.59 mg/L up to 29.99 mg/L. After alcoholic fermentation, oxygen can dissolve at various concentrations due to winemaking practices such as racking, filtration, centrifugation, wine transfers, cold stabilization and closure types (Castellari *et al.*, 2004), while working conditions such as the temperature of the wine, the level of the wine in the tank and the protection with inert gas can also have a significant influence (Lisjak, 2007).

4.3.2 Chemical analyses

Chemical analyses were conducted on aromatic as well as non-aromatic compounds. Table 4.2 shows the concentration of all compounds as well as the colour absorbance values at each sampling stage. Table 4.3 shows various aromatic compounds, the perception thresholds (as reported in literature) as well as the medium in which the thresholds were tested along with the sensory attributes associated with each compound. Usually more than one aroma detection threshold has been published and where possible, the value determined in a synthetic wine medium was used for consistency.

As mentioned earlier, the volatile thiols and the methoxypyrazines are considered to be aroma impact compounds for Sauvignon blanc wines specifically. These compounds are often sought after in commercial Sauvignon blanc wines and winemakers will attempt to produce wines with high concentrations of these compounds, while trying to preserve them as best possible (Lund *et al.*, 2009). Other aroma groups such as the esters are also important in the aromatic bouquet of the wine, while acids and alcohols are not always considered as being major contributors, however their role as an aromatic "buffer" has been reported (Ferreira *et al.*, 2008). Monoterpenes are usually associated with the Muscat family of grapes. However, these compounds could be present in significant amounts in Sauvignon blanc wines and could thus influence the aroma (Benkwitz *et al.*, 2012a). The aldehydes especially can influence the overall aromatic bouquet during oxidation, while other compound groups such as the acetals might not alter the composition (Silva Ferreira *et al.*, 2002a; Silva Ferreira *et al.*, 2003c).

Table 4.2 Concentration and absorbance values of all chemical compounds measured. Values are means of triplicate analysis; different letters in a row indicate significant differences at $p<0.05$.

Compound	unit	T0 Control	T1 Control	T2 Control	T3 Control	T4 Control	T5 Control	T1 Ox	T2 Ox	T3 Ox	T4 Ox	T5 Ox
Oxygen												
Total amount of O_2 consumed	mg/L							6.59^{e}	11.93^{d}	16.99^{c}	22.18^{b}	29.99^{a}
Volatile Thiols												
4-Mercapto-4-methylpentan-2-one (4MMP)	ng/L	37.34^{a}	28.92^{b}	15.40^{cde}	19.47^{c}	9.61^{f}	12.02^{fd}	25.57^{b}	16.15^{cd}	10.22^{fe}	9.71^{f}	9.49^{f}
3-Mercaptohexylacetate (3MHA)	ng/L	97.61^{a}	83.23^{b}	76.41^{bc}	69.15^{c}	62.45^{c}	62.66^{c}	76.14^{bc}	48.15^{d}	41.46^{de}	24.38^{f}	29.58^{fe}
3-Mercaptohexanol (3MH)	ng/L	647.91^{c}	640.33^{c}	807.1^{a}	824.44^{a}	790.71^{a}	709.00^{b}	575.46^{d}	516.31^{e}	397.37^{f}	370.29^{f}	294.90^{g}
Esters												
Isoamyl acetate	mg/L	7.52^{a}	6.36^{b}	5.60^{c}	5.08^{cd}	4.78^{ed}	4.67^{efd}	6.27^{b}	4.93^{ed}	4.44^{ef}	4.40^{ef}	4.13^{f}
Hexyl acetate	mg/L	0.90^{a}	0.87^{b}	0.85^{c}	0.83^{cd}	0.83^{ed}	0.82^{efd}	0.87^{b}	0.83^{ed}	0.81^{ef}	0.81^{ef}	0.80^{f}
2-Phenylethyl acetate	mg/L	2.74^{a}	2.44^{b}	2.26^{bc}	2.17^{dc}	2.09^{dec}	2.02^{de}	2.43^{b}	2.11^{dec}	1.99^{de}	1.99^{de}	1.92^{c}
Ethyl acetate	mg/L	87.09^{a}	81.33^{ab}	82.36^{ab}	82.07^{ab}	73.04^{b}	82.73^{ab}	81.61^{ab}	74.22^{b}	77.47^{ab}	82.23^{ab}	77.73^{ab}
Ethyl butyrate	mg/L	0.57^{a}	0.55^{ac}	0.55^{ab}	0.53^{ac}	0.51^{ac}	0.54^{ac}	0.53^{ac}	0.50^{c}	0.52^{ac}	0.51^{cb}	0.49^{c}
Ethyl lactate	mg/L	5.00^{c}	5.43^{c}	8.05^{ab}	9.05^{a}	7.42^{b}	9.37^{a}	5.68^{c}	7.18^{b}	8.35^{ab}	9.40^{a}	9.41^{a}
Ethyl hexanoate	mg/L	1.50^{a}	1.52^{a}	1.54^{a}	1.54^{a}	1.51^{a}	1.51^{a}	1.52^{a}	1.44^{a}	1.48^{a}	1.56^{a}	1.53^{a}
Ethyl octanoate	mg/L	1.36^{d}	1.87^{b}	1.62^{bc}	1.61^{bd}	1.56^{dc}	1.36^{dc}	1.86^{b}	1.48^{dc}	1.86^{b}	2.27^{a}	1.86^{b}
Ethyl decanoate	mg/L	2.12^{de}	3.91^{a}	2.64^{db}	2.45^{dc}	2.33^{dc}	1.56^{e}	3.40^{ab}	2.27^{de}	3.31^{ab}	3.82^{a}	3.13^{abc}
Diethyl succinate	mg/L	0.45^{g}	0.67^{f}	1.03^{e}	1.28^{d}	1.30^{d}	1.44^{c}	0.72^{f}	1.09^{e}	1.51^{c}	1.77^{b}	1.94^{a}
Acids												
Acetic acid	mg/L	446.88^{a}	415.44^{ac}	438.25^{ab}	433.74^{ab}	371.12^{c}	429.15^{ab}	413.92^{ac}	390.24^{cb}	402.02^{ac}	422.57^{ac}	432.95^{ab}
Propionic acid	mg/L	1.85^{a}	1.74^{a}	1.88^{a}	1.93^{a}	1.68^{a}	1.90^{a}	1.79^{a}	1.70^{a}	1.80^{a}	1.86^{a}	1.86^{a}
Butyric acid	mg/L	0.29^{a}	0.28^{a}	0.29^{a}	0.30^{a}	0.28^{a}	0.29^{a}	0.28^{a}	0.28^{a}	0.28^{a}	0.29^{a}	0.28^{a}
Isobutyric acid	mg/L	0.78^{a}	0.73^{ab}	0.76^{ab}	0.76^{ab}	0.70^{b}	0.74^{ab}	0.74^{ab}	0.71^{ab}	0.71^{ab}	0.76^{ab}	0.74^{ab}
Isovaleric acid	mg/L	0.63^{a}	0.58^{ab}	0.60^{ab}	0.61^{ab}	0.58^{ab}	0.60^{ab}	0.59^{ab}	0.56^{b}	0.57^{b}	0.60^{ab}	0.57_{ab}
Hexanoic acid	mg/L	5.51^{a}	5.22^{a}	5.40^{a}	5.44^{a}	5.30^{a}	5.32^{a}	5.23^{a}	5.03^{a}	5.09^{a}	5.32^{a}	5.32^{a}
Octanoic acid	mg/L	8.02^{a}	7.34^{ac}	7.82^{ac}	7.94^{ab}	7.84^{ac}	7.85^{ac}	7.27^{ac}	7.24^{ac}	7.05^{cb}	6.98^{c}	7.46^{ac}
Decanoic acid	mg/L	2.97^{a}	2.25^{ce}	2.54^{cb}	2.54^{cb}	2.53^{cbd}	2.66^{ab}	2.18^{ce}	2.28^{ceb}	2.02^{e}	2.07^{e}	2.14^{ed}
Alcohols												
Methanol	mg/L	107.93^{ab}	97.44^{ab}	107.83^{ab}	90.69^{ab}	83.25^{ab}	116.69^{a}	96.79^{ab}	69.46^{b}	83.93^{ab}	88.58^{ab}	103.24^{ab}
Propanol	mg/L	61.62^{a}	57.05^{ab}	58.88^{ab}	57.88^{ab}	50.21^{b}	56.40^{ab}	57.12^{ab}	52.55^{ab}	54.81^{ab}	57.32^{ab}	56.47^{ab}
Butanol	mg/L	1.70^{a}	1.59^{ab}	1.66^{a}	1.59^{ab}	1.38^{b}	1.56^{ab}	1.55^{ab}	1.47^{ab}	1.49^{ab}	1.57^{ab}	1.56^{ab}
Isobutanol	mg/L	19.25^{a}	18.14^{ab}	18.53^{ab}	18.13^{ab}	15.90^{b}	17.84^{ab}	17.72^{ab}	16.74^{ab}	17.30^{ab}	17.91^{ab}	17.50^{ab}
Isoamyl alcohol	mg/L	161.59^{a}	155.43^{a}	160.90^{a}	159.98^{a}	149.95^{a}	157.98^{a}	153.75^{a}	148.48^{a}	150.57^{a}	157.03^{a}	158.67^{a}
Hexanol	mg/L	0.91^{cb}	0.91^{c}	0.97^{ac}	0.99^{ab}	0.96^{ac}	0.99^{ac}	0.91^{cb}	0.91^{cb}	0.95^{ac}	1.00^{a}	1.03^{a}
Phenylethanol	mg/L	13.49^{a}	13.00^{a}	13.39^{a}	13.59^{a}	12.71^{a}	13.10^{a}	13.02^{a}	12.57^{a}	12.75^{a}	13.43^{a}	13.57^{a}
3-Ethoxy-1-propanol	mg/L	2.06^{a}	1.92^{ab}	2.09^{a}	2.06^{a}	1.59^{b}	2.01^{a}	2.00^{a}	1.67^{ab}	1.78^{ab}	1.95^{ab}	1.95^{ab}
Methionol	peak/IS ratio	247.41^{a}	243.31^{a}	244.31^{a}	256.05^{a}	236.03^{a}	248.98^{a}	246.16^{a}	240.95^{a}	236.73^{a}	235.56^{a}	238.04^{a}

Table 4.2 Continued.

Compound	unit	T0 Control	T1 Control	T2 Control	T3 Control	T4 Control	T5 Control	T1 Ox	T2 Ox	T3 Ox	T4 Ox	T5 Ox
Monoterpenes												
Linalool	mg/L	10.46^{e}	11.50^{cd}	12.86^{ab}	13.07^{ab}	11.44^{cd}	11.13^{ed}	11.59^{cd}	12.08^{cb}	13.10^{a}	10.68^{ed}	10.88^{ed}
Geraniol	mg/L	1484.25^{a}	1480.22^{a}	1431.77^{a}	1551.61^{a}	1131.57^{b}	984.73^{bc}	1606.67^{a}	1442.44^{a}	1640.41^{a}	864.74^{c}	937.26^{bc}
Farnesol	mg/L	32.84^{a}	39.00^{a}	34.25^{a}	34.03^{a}	18.77^{b}	16.93^{b}	41.99^{a}	34.02^{a}	39.84^{a}	21.26^{b}	20.08^{b}
Antioxidants												
Free SO_2	mg/L	43.33^{a}	31.67^{b}	32.00^{b}	31.33^{bc}	29.67^{dc}	28.33^{d}	19.67^{e}	10.00^{f}	7.00^{g}	6.67^{gh}	5.00^{h}
Total SO_2	mg/L	100.33^{a}	93.33^{b}	90.67^{b}	93.67^{b}	93.67^{b}	92.33^{b}	82.00^{c}	60.67^{d}	59.67^{d}	55.67^{de}	51.33^{e}
Reduced glutathione	mg/L	16.79^{a}	10.27^{b}	7.78^{c}	6.12^{d}	5.55^{d}	5.42^{d}	5.39^{d}	1.52^{e}	0.56^{e}	0.62^{e}	0.57^{e}
Oxidised glutathione	mg/L	0.09^{f}	0.27^{dce}	0.16^{fe}	0.15^{fe}	0.23^{de}	0.15^{fe}	0.21^{df}	0.33^{dc}	0.38^{bc}	0.48^{b}	0.70^{a}
Grape Reaction Procuct	Caftaric acid units	2.04^{c}	3.45^{b}	3.94^{b}	3.86^{b}	5.51^{a}	5.20^{a}	3.62^{b}	3.82^{b}	4.13^{b}	5.74^{a}	4.23^{b}
Phenols												
Gallic acid	mg/L	0.29^{b}	0.52^{a}	0.51^{a}	0.49^{a}	0.51^{ac}	0.50^{a}	0.50^{a}	0.49^{a}	0.52^{a}	0.49^{a}	0.49^{a}
***(+)*- Catechin**	mg/L	2.62^{c}	3.51^{a}	3.47^{a}	3.08^{ac}	3.30^{ab}	3.25^{ab}	3.12^{ac}	2.83^{cb}	2.86^{cb}	2.83^{cb}	2.64^{c}
Caffeic acid	mg/L	6.00^{b}	6.83^{a}	6.43^{ab}	6.39^{ab}	6.64^{ab}	6.64^{ab}	6.35^{ab}	5.93^{b}	6.38^{ab}	6.34^{ab}	6.46^{ab}
***trans*- Caftaric acid**	Caffeic acid equivalents	11.26^{dc}	12.63^{a}	11.90^{ad}	11.97^{ad}	12.40^{ab}	12.46^{ab}	12.01^{abc}	11.13^{d}	11.69^{db}	12.08^{abc}	11.45^{dc}
***p*- Coumaric acid**	mg/L	3.96^{ab}	4.44^{a}	4.17^{ab}	4.17^{ab}	4.29^{a}	4.30^{a}	4.08^{ab}	3.78^{b}	4.07^{ab}	4.03^{ab}	4.11^{b}
***p*- Coutaric acid**	Coumaric acid equivalents	0.75^{e}	0.85^{bc}	0.84^{bc}	0.88^{b}	0.95^{a}	0.95^{a}	0.77^{ed}	0.77^{e}	0.80^{ec}	0.83^{bcd}	0.76^{e}
Polymeric phenols	Catechin equivalents	20.06^{ab}	20.81^{a}	17.89^{cd}	17.62^{cf}	16.52^{fc}	15.92^{fe}	18.62^{cb}	17.19^{cf}	17.67^{cde}	16.65^{fd}	15.72^{f}
Carbonyl compounds												
Acetaldehyde	mg/L	42.61^{d}	41.67^{d}	41.42^{d}	40.43^{d}	40.95^{d}	40.60^{d}	42.84^{d}	42.76^{d}	51.81^{c}	78.44^{b}	94.62^{a}
Methional	ug/L	0.00	0.00	0.00	0.00	0.00	0.00	0.58^{d}	1.31^{c}	2.64^{b}	3.8^{a}	4.1^{a}
Phenylacetaldehyde	ug/L	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Benzaldehyde	peak/IS ratio	12.53^{d}	12.95^{dc}	14.64^{dc}	17.91^{bc}	17.02^{bd}	16.36^{bd}	20.46^{b}	31.00^{a}	29.43^{a}	15.37^{dc}	6.78^{e}
Furfural	peak/IS ratio	10.91^{e}	18.60^{d}	25.57^{d}	34.61^{c}	34.98^{c}	35.88^{c}	23.32^{d}	51.81^{b}	79.29^{a}	75.77^{a}	81.89^{a}
5-Hydroxymethylfurfural	peak/IS ratio	4.32^{d}	6.91^{bd}	6.07^{dc}	6.82^{bd}	9.29^{bc}	8.80^{bc}	7.41b^{d}	10.55^{b}	16.68^{a}	15.81^{a}	17.42^{a}
Sotolon	ug/L	0.00	0.00	0.00	0.00	0.00	0.00	0.13^{a}	0.15^{a}	0.13^{a}	0.27^{a}	0.41^{a}
Acetals												
***Cis*- dioxane**	peak/IS ratio	14.31^{c}	13.54^{c}	13.78^{c}	15.11^{c}	13.27^{c}	13.31^{c}	16.79^{c}	16.51^{c}	26.04^{c}	41.98^{b}	94.10^{a}
Cis-dioxolane	peak/IS ratio	6.82^{c}	2.82^{c}	2.02^{c}	2.00^{c}	1.86^{c}	1.78^{c}	7.30^{c}	5.34^{c}	28.08^{c}	71.49^{b}	182.00^{a}
***Trans*- dioxane**	peak/IS ratio	3.36^{cd}	3.07^{d}	3.08^{cd}	3.18^{cd}	3.04^{d}	2.97^{d}	3.84^{cd}	3.28^{cd}	5.97^{c}	9.75^{b}	21.61^{a}
***Trans*- dioxolane**	peak/IS ratio	4.91^{c}	2.12^{c}	1.40^{c}	1.34^{c}	1.14^{c}	1.07^{c}	4.25^{c}	3.01^{c}	11.96^{c}	31.23^{b}	82.79^{a}
Spectrophotometric measurements												
420 nm	absorbance units	0.053^{g}	0.055^{gf}	0.059^{ef}	0.058^{ef}	0.059^{ef}	0.061^{e}	0.062^{e}	0.077^{d}	0.085^{c}	0.093^{b}	0.098^{a}
440 nm	absorbance units	0.039^{g}	0.040^{gf}	0.043^{fe}	0.042^{efg}	0.042^{fe}	0.044^{de}	0.047^{d}	0.059^{c}	0.064^{b}	0.070^{a}	0.073^{a}

IS = Internal standard

Table 4.3 List of the aromatic compounds found in the wine, aroma perception thresholds and attributes used to describe the various odours.

Compound	Perception threshold	Threshold determined in	Descriptors	Reference
Volatile Thiols				
4-Mercapto-4-methylpentan-2-one (4MMP)	0.8 ng/L	12% ethanol, pH 3.5	Passion fruit, broom, black current	Darriet *et al.,* 1995
3-Mercaptohexylacetate (3MHA)	4.2 ng/L	12% ethanol, pH 3.5	Passion fruit, grapefruit, gooseberry, guava	Tominaga *et al.,* 1996
3-Mercaptohexanol (3MH)	60 ng/L	12% ethanol, pH 3.5	Passion fruit, grapefruit, gooseberry, guava	Tominaga *et al.,* 1998a
Methoxypyrazines				
3-Isobutyl-2-methoxypyrazine (IBMP)	2 ng/L	water	Green pepper	Buttery *et al.,* 1969
3-Isopropyl-2-methoxypyrazine (IPMP)	2 ng/L	water	Asparagus	Buttery *et al.,* 1969; Allen *et al.,* 1998
Esters				
Isoamyl acetate	0.05 mg/L	12.5% ethanol, pH3.2	Banana, fruity, sweet	Benkwitz *et al.,* 2012b
Hexyl acetate	0.4 mg/L	12.5% ethanol, pH3.2	Apple, cherry, pear, flower	Benkwitz *et al.,* 2012b
2-Phenylethyl acetate	0.25 mg/L	10% ethanol	Rose, honey, tobacco, flower	Guth, 1997
Ethyl acetate	12.3 mg/L	10% ethanol, pH 3.2	Pineapple, fruity, varnish, solvent	Escudero *et al.,* 2004
Ethyl butyrate	0.02 mg/L	10% ethanol	Acidic, fruity, apple	Guth, 1997
Ethyl lactate	146 mg/L	14% ethanol, pH3.5	Lactic, buttery, fruity	Moyano *et al.,* 2002
Ethyl hexanoate	0.014 mg/L	11% ethanol, pH3.4	Green apple peel, fruit, banana, brandy	Ferreira *et al.,* 2000
Ethyl octanoate	0.005 mg/L	11% ethanol, pH3.4	Sweet, ripe banana, pear, soapy	Ferreira *et al.,* 2000
Ethyl decanoate	0.2 mg/L	11% ethanol, pH3.4	Fruity, floral, grape, soapy, brandy	Ferreira *et al.,* 2000
Diethyl succinate	1.2 mg/L	10% ethanol, pH 3.5	Fruity, melon	Peinado *et al.,* 2004
Acids				
Acetic acid	300 mg/L	10% ethanol, pH 3.2	Vinegar	Escudero *et al.,* 2004
Propionic acid	8.1 mg/L	11% ethanol, pH3.4	Rancid, pungent, soy	Ferreira *et al.,* 2000
Butyric acid	0.173 mg/L	11% ethanol, pH3.4	Rancid, cheese, sweat	Ferreira *et al.,* 2000
Isobutyric acid	2.3 mg/L	11% ethanol, pH3.4	Acidic	Ferreira *et al.,* 2000
Isovaleric acid	0.033 mg/L	11% ethanol, pH3.4	Blue cheese	Ferreira *et al.,* 2000
Hexanoic acid	0.42 mg/L	11% ethanol, pH3.4	Sweat, cheesy, fatty	Ferreira *et al.,* 2000
Octanoic acid	0.50 mg/L	11% ethanol, pH3.4	Sweaty, rancid, harsh, fatty	Ferreira *et al.,* 2000
Decanoic acid	1.00 mg/L	11% ethanol, pH3.4	Rancid, fatty	Ferreira *et al.,* 2000

Table 4.3 Continued.

Compound	Perception threshold	Threshold determined in	Descriptors	Reference
Alcohols				
Propanol	306 mg/L	10% ethanol, pH 3.5	Alcohol, ripe fruit	Peinado *et al.,* 2004
Butanol	150 mg/L	10% ethanol, pH 3.5	Fusel odour, medicinal	Peinado *et al.,* 2004
Isobutanol	40 mg/L	10% ethanol	Fusel, alcohol, nail polish	Guth, 1997
Isoamyl alcohol	30 mg/L	10% ethanol	Whiskey, malt, burnt	Guth, 1997
Hexanol	8 mg/L	10% ethanol	Grassy, green, resin, flower, woody	Guth, 1997
Phenylethanol	14 mg/L	10% ethanol, pH3.5	Honey, spice, rose, lilac	Peinado *et al.,* 2004
Methionol	1 mg/L	10% ethanol, pH3.5	Cauliflower, cooked cabbage, sweet, potato	Peinado *et al.,* 2004
Monoterpenes				
Linalool	25.2 µg/L	11% ethanol, pH3.4	Fruity, citrus, floral, lavender	Ferreira *et al.,* 2000
Geraniol	300 µg/L	10% ethanol	Rose, geranium	Guth, 1997
Farnesol	1 mg/L	10% ethanol, pH3.5	Floral, oily	Peinado *et al.,* 2004
Carbonyl compounds				
Acetaldehyde	0.5 mg/L	10% ethanol	Sherry, nutty, bruised apple	Guth, 1997
Methional	0.5 µg/L	11% ethanol,pH 3.4	Baked potatoes	Escudero *et al.,* 2000b
Phenylacetaldehyde	1 µg/L	10% ethanol, pH 3.2	Honey	Culleré *et al.,* 2007
Benzaldehyde	2 mg/L	10% ethanol, pH3.5	Liquor, checmical, bitter cherry	Escudero *et al.,* 2002; Peinado *et al.,* 2004
Furfural	150 mg/L	beer	Cooked vegetables, woody, paper, green fruits	Câmara *et al.,* 2004
5-Hydroxymethylfurfural	100 mg/L	beer	Aldehyde, chemical, woody	Câmara *et al.,* 2004
Sotolon	2 µg/L	12% ethanol, pH 3.5	Spice, curry, nutty	Pons *et al.,* 2004
Acetals				
***Cis*- dioxane, *Cis*- dioxolane, *Trans*- dioxane, *Trans*- dioxolane**	100 mg/L (mixture of 4 isomers)	Estimated by sniffing from GC-O	sweet, old port-like	Silva Ferreira *et al.,* 2002a

4.3.2.1 Volatile Thiols

The volatile thiols contribute to a large extent to the fruity aroma in Sauvignon blanc wines, often described as “tropical”, “passion fruit”, “guava” and “grapefruit”. These compounds form an important part of the overall aroma bouquet of Sauvignon blanc wine and the retention of these compounds is important during aging, especially due to the thiols being oxidation sensitive (Blanchard *et al.*, 2004; Nikolantonaki *et al.*, 2010). The decrease of these compounds during oxygen exposure and aging can be due to the direct reaction with hydrogen peroxide in the presence of a metal ion (Jocelyn, 1972); the thiols can react with polyphenolic compounds via a nucleophilic, acid-catalysed substitution reactions (Ribéreau-Gayon, 1998, 2004b) and they can degrade at a fast rate due to the reaction with phenolic oxidation products such as the very reactive *o*-quinones (Nikolantonaki *et al.*, 2010). Any of these reactions will result in a loss of thiol-derived varietal character. Recent observations reported the *o*-quinone trapping as the main mechanism accounting for 3MH loss in wine under oxidative conditions, while other reactions seem to contribute marginally (Kreitman *et al.*, 2013).

In this study, the values obtained for 3MHA falls within the range of concentrations found in Sauvignon blanc wines originating from around the world, however 3MH concentrations are lower when considering wines from New Zealand, but were on par with concentrations found in general in South African Sauvignon blanc wines (Table 4.2) (Lund *et al.*, 2009; Van Wyngaard, 2013). After about 7 months of storage at 15˚C in the dark, a 30% decrease in 3MHA concentration was seen for the Control samples while the Ox samples’ concentration decreased by about 75% (Figure 4.3).

A decrease of up to 46% has also been observed in New Zealand Sauvignon blanc wines that have been stored for 7 months at 15˚C (Herbst-Johnstone *et al.*, 2011). Another study done in French rosé wines reported a 60% loss in 3MHA within 3 months of storage (Murat, 2005). The loss in 3MHA can be due to oxidation of the volatile thiol with previously described mechanisms (probably the reaction taking place in the Ox samples) and/or due to a hydrolysis reaction. 3MHA, being an acetate ester, can hydrolyse to form 3MH and acetic acid (Figure 4.4). The oxidation of 3MH could also accelerate this reaction by removal of the hydrolysis product. This hydrolysis reaction is probably responsible for the decrease in 3MHA concentration especially in the Control samples.

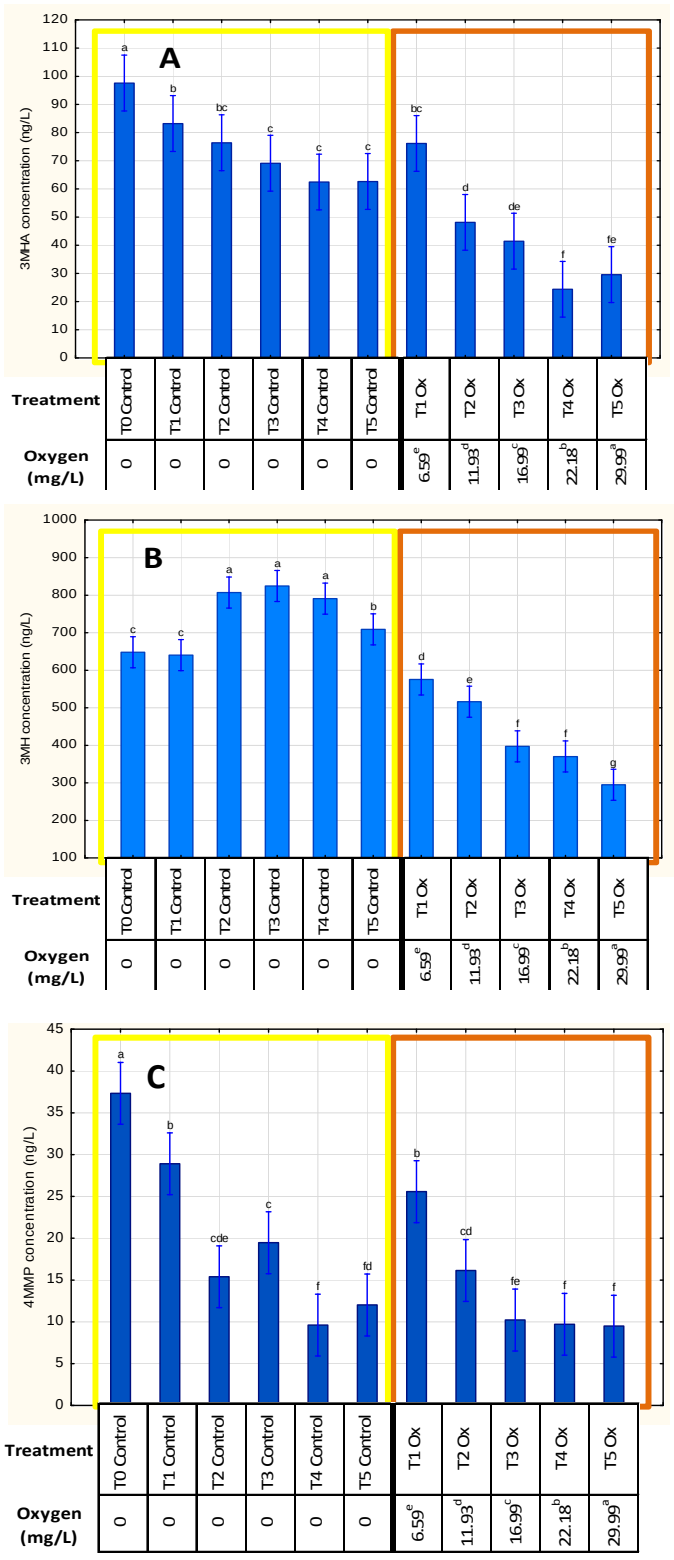


Figure 4.3 3MHA (A), 3MH (B), 4MMP (C) concentration (ng/L) of the respective treatments. Values are means of triplicate analysis; different letters indicate significant differences at $p<0.05$.

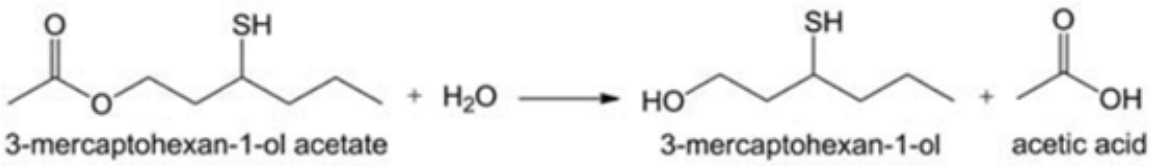


Figure 4.4 Hydrolysis of 3-mercaptohexan-1-ol acetate to 3-mercaptohexan-1-ol and acetic acid (Herbst-Johnstone *et al.*, 2011).

To the contrary, 3MH concentrations remained stable (Table 4.2, Figure 4.3) from T0 Control to T1 Control (up to 64 days) then started to increase with the maximum of 824.44 ng/L at T3 Control after which it decreased to a level of 709.00 ng/L at T5 Control which, interestingly, was still significantly higher than the initial concentration of 647.91 ng/L at T0 Control. This increase is likely due to the hydrolysis of 3MHA to 3MH and similar results have been reported in previous studies (Herbst *et al.*, 2008; Herbst-Johnstone *et al.*, 2011). Herbst-Johnstone *et al*. (2011) found a slight decrease (5%) in 3MH concentrations during the first 3 months of storage after which the concentration increased up to 7 months of storage. Another study by the same author reported an increase in 3MH levels of up to 63% during the first 3 months of storage after which 3MH levels decreased (Herbst *et al.*, 2008).

Other than the hydrolysis of 3MHA, the possibility of the release of 3MH from remaining precursors or any 3MH disulphide present in the wine during aging exists, however, the thiol precursors appear to be stable at wine pH, so that the free form should not be able to form via this mechanism (Ugliano *et al.*, 2011). The role of known precursors is still to be clarified and the possibility of other not yet identified precursors which could also contribute to the increase in 3MH concentrations during aging needs to be elucidated (Capone *et al.*, 2010; Sarrazin *et al.*, 2010; Coetzee & Du Toit, 2012). These mechanisms should be investigated especially considering that the 3MHA quantitative loss does not make up for the gain in 3MH concentration on a 1:1 ratio. In the Ox samples, a 54% decrease in 3MH concentration was observed from T0 Control to T5 Ox. The reactivity of 3MH and the oxidation thereof has been reported (Blanchard *et al.*, 2004; Nikolantonaki *et al.*, 2010) and is probably the cause of this decrease.

Given a perception threshold of 4.2 ng/L (Table 4.3), 3MHA may play an important role in especially young Sauvignon blanc wines. The perception threshold of 3MH is also relatively low (60 ng/L; Table 4.3) and the sensory impact may be considerable in young wines. The degradation of 3MHA and the subsequent production of 3MH by hydrolysis, is expected to lower the intensity of some tropical aromas such as “passion fruit” and “sweaty” aromas (Coetzee *et al.,* 2012).

The values obtained for 4MMP (Table 4.2, Figure 4.3) fall within the range of concentrations found in South African and French Sauvignon blanc wines (Tominaga *et al.*, 1998b; Van Wyngaard, 2013). In

the Control samples, 4MMP content decreased by 74% from T0 to T5. The addition of oxygen in the Ox samples did not seem to accelerate this decrease (as is the case with 3MH and 3MHA), but rather decreased to a similar degree when compared to the Control samples. 4MMP has been shown not to be as sensitive to oxidation when compared to the other thiols (Nikolantonaki *et al.*, 2010; Coetzee *et al.*, 2013). Reaction rates between the *o*-quinone and the volatile thiols have been reported to increase as steric hindrance declines (Nikolantonaki *et al.*, 2010; Nikolantonaki & Waterhouse, 2012). 4MMP is a tertiary thiol, therefore it is not as oxidation sensitive when compared to 3MH, which is a secondary thiol. The decrease observed in this study could be due to natural degradation of the molecule over time. A decrease of 74% due to natural degradation is significant and should be kept in mind when storing a wine.

Overall the loss in volatile thiols could change the aromatic composition of the wine substantially.

4.3.2.2 Methoxypyrazines

Methoxypyrazines have been shown not to be sensitive to oxidation as even hyperoxidation (using H_2O_2) of an IBMP-spiked Chenin blanc wine did not have any effect on the IBMP concentration (Marais, 1998). Oxidation of Sauvignon blanc juice also had no effect on the methoxypyrazine content in the corresponding wines (Coetzee *et al.*, 2013). Methoxypyrazine analysis was thus done on a select few samples to verify whether any differences between extreme samples existed. T0 Control had an IBMP concentration of 12.90 ng/L, while T5 Control and T5 Ox contained 13.90 and 13.00 ng/L respectively. This would suggest that there was no treatment effect on the concentration of this compound. Small differences observed would not have an effect on the sensorial perception and the differences seen here could also be considered as small variations in measurements at these low concentrations. No IPMP was detected in these samples. The continuous presence of IBMP would indicate the presence of the “green” aroma characters in all the wine samples. Sensory profiling will be discussed in section 4.3.3, however it is interesting to note that a correlation between pyrazine concentration and the “green” attributes in wines does not always exist, suggesting the contribution of other aroma compounds to these attributes (Ferreira *et al.*, 2002; Preston *et al.*, 2008).

4.3.2.3 Esters, acids and alcohols

Three acetate esters, isoamyl acetate, hexyl acetate and 2-phenylethyl acetate (Table 4.2, Figure 4.5) were significantly degraded during the 7 month storage. Aging time thus seems to be the main driver of degradation in most cases as the Control treatment did not differ significantly from its Ox counterpart. The process for the loss of acetate esters during aging is again expected to be hydrolysis of the ester to acetic acid and an alcohol, which occurs readily at wine pH. The decline in acetate esters during storage has been reported in literature previously (Marais & Pool, 1980; Ramey & Ough, 1980; Ferreira *et al.*, 1997; Moio *et al.*, 2004; Lambropoulos & Roussis, 2007; Makhotkina & Kilmartin, 2012; Patrianakou & Roussis, 2013). The preservation of these esters is important as they contribute to the pleasant aroma of white wines often described as “banana”, “fruity”, “pear”, “apple”, “rose” and “flower” (Francis & Newton, 2005; Swiegers *et al.*, 2005; Benkwitz *et al.*, 2012b).

Ethyl lactate and diethyl succinate concentrations increased during the study (Table 4.2, Figure 4.6). This occurred in both the Control and Ox samples. Diethyl succinate concentrations increased to a higher concentration in the Ox samples when compared to the Control counterparts especially in the last three sampling stages. These two compounds are usually associated with malolactic fermentation (Louw *et al.*, 2010) and contribute odours such as “fruity”, “floral” and “brandy”. Their occurrence could also arise due to the transformation of lactic and succinic acids to form ethyl lactate and diethyl succinate during fermentation and maturation (De Villiers *et al.*, 2003). The increase of these compounds during aging have been reported in literature previously (Rapp, 1988; Ferreira *et al.*, 1997; Pérez-Coello *et al.*, 2003; Hernanz *et al.*, 2009) and interestingly these compounds have been identified as being the main discriminant variables in 90 white wines with various ages (Pérez-Coello *et al.*, 2003).

No significant change in concentration was observed for ethyl hexanoate, while ethyl octanoate and ethyl decanoate concentrations exhibited inconsistency during the storage time (Table 4.2, Figure 4.7). There seems to be a slight decrease in ethyl octanoate and ethyl decanoate when considering the Control samples only (T1 Control to T5 Control). The disappearance of the ethyl esters of some higher alcohols during storage has been reported before (Marais & Pool, 1980; Ramey & Ough, 1980; Ferreira *et al.*, 1997; Moio *et al.*, 2004; Ugliano *et al.*, 2008; Patrianakou & Roussis, 2013), while the stable concentration of ethyl hexanoate during aging of Riesling and Cabernet franc wines has also been reported elsewhere (Blake *et al.*, 2010). In the Ox samples, an increase in these ethyl esters was seen when comparing the Control samples with the Ox counterparts, especially at sampling stages T4 and T5. A change in the hydrolysis-esterification equilibria could account for this

observation (Ramey & Ough, 1980), especially if a change in acetic acid concentration occurred over the storage period (Escalona *et al.*, 2002). An increase in ethyl octanoate during a 12 month storage period has also been reported previously (Marais & Pool, 1980; Blake *et al.*, 2010).

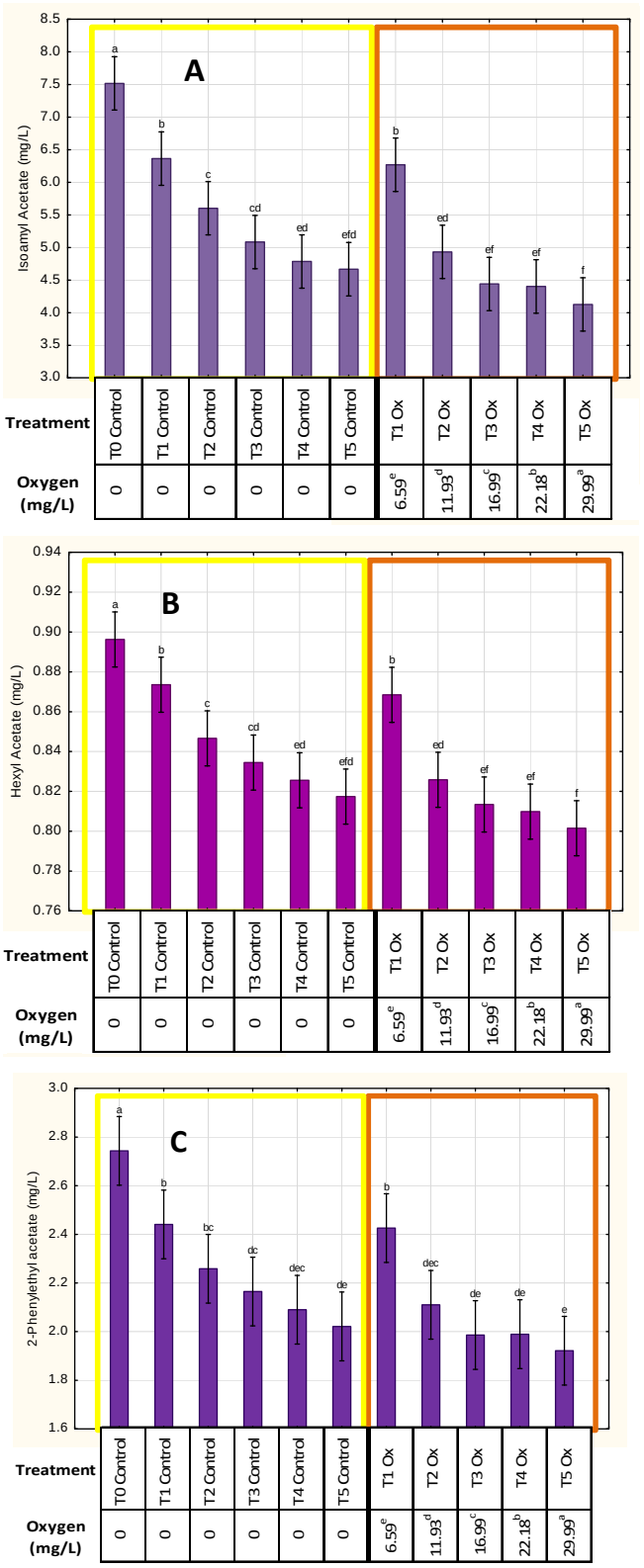


Figure 4.5 Isoamyl acetate (A), hexyl acetate (B) and 2-phenylethyl acetate (C) concentration (mg/L) of the respective treatments. Values are means of triplicate analysis; different letters indicate significant differences at $p<0.05$.

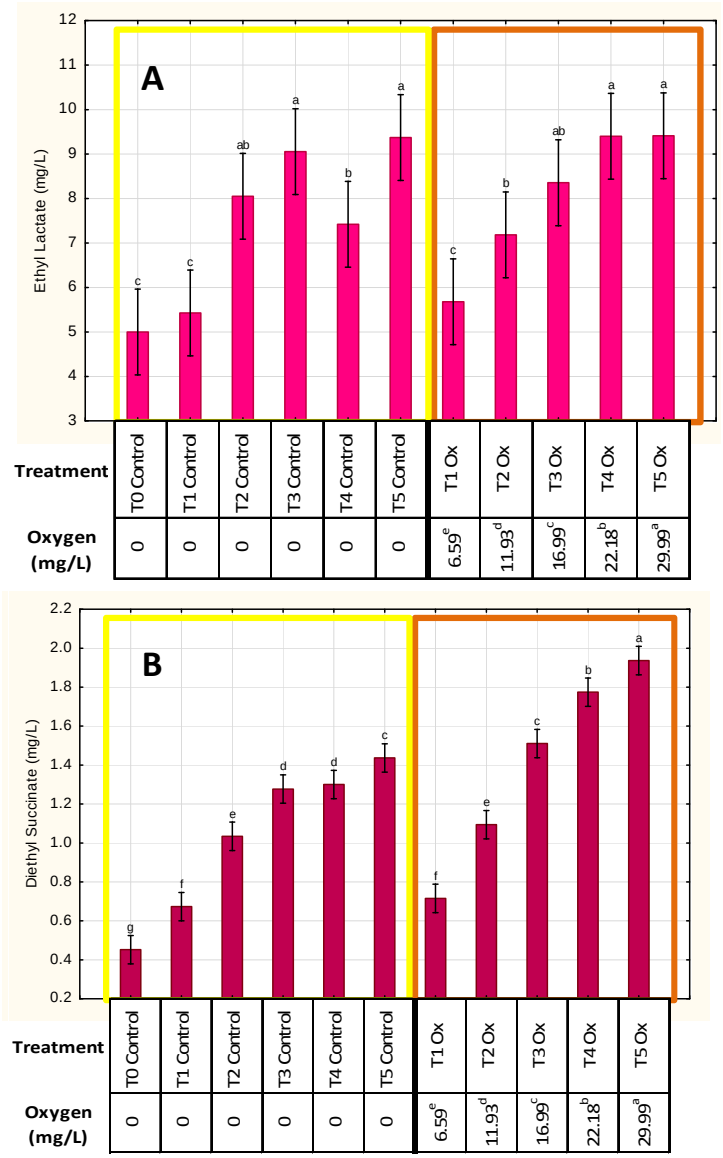


Figure 4.6 Ethyl lactate (A) and diethyl succinate (B) concentration (mg/L) of the respective treatments. Values are means of triplicate analysis; different letters indicate significant differences at $p<0.05$.

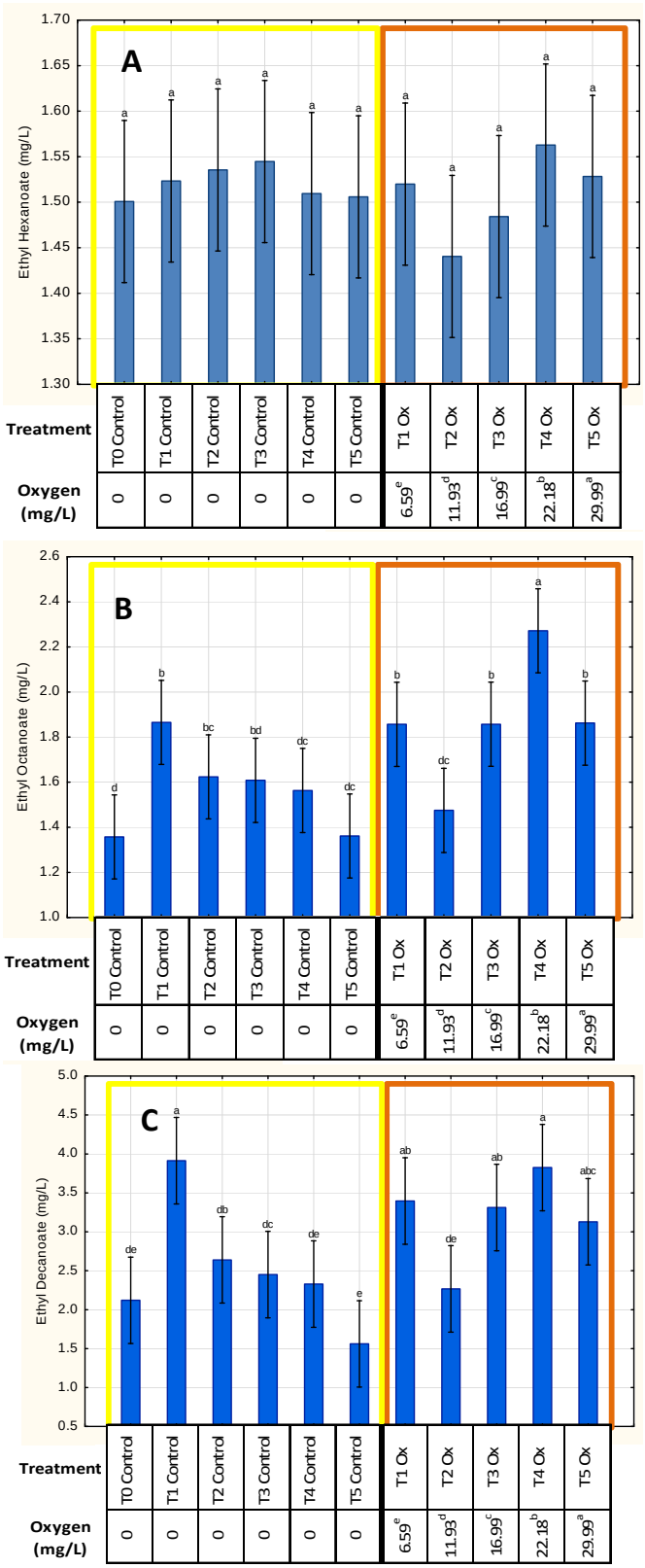


Figure 4.7 Ethyl hexanoate (A), ethyl octanoate (B) and ethyl decanoate (C) concentration (mg/L) of the respective treatments. Values are means of triplicate analysis; different letters indicate significant differences at $p<0.05$.

Even though there were no significant differences in hexanoic acid concentrations, a slight but not significant decrease in concentration was observed for octanoic acid, while there was a significant decrease in decanoic acid when comparing the Control samples to their Ox counterparts especially during the late oxidative stages (Table 4.2, Figure 4.8). The same tendency was observed in other studies (Marais & Pool, 1980; Ferreira *et al.*, 1997; Câmara *et al.*, 2006; Blake *et al.*, 2010; Lee *et al.*, 2011) and reported the results as surprising due to the expectation of these compounds to increase as a result of the hydrolysis of the corresponding ethyl ester. As mentioned earlier, the ethyl ester concentration did not decrease, to the contrary, in some cases the concentration increased and would thus not result in the formation of the corresponding acid. The possibility of fatty acid autoxidation to yield aldehydes has been contemplated (Nykänen, 1986) and can possibly explain the slight decrease in acid concentration observed. Other short chain fatty acids, such as acetic acid, propionic acid, butyric acid, isobutyric acid and isovaleric acid, were not significantly affected by the treatment (Table 4.2). Fatty acids have been reported not to oxidise easily since the oxidation of these compounds under laboratory conditions required the use of oxidants stronger than H_2O_2 (Ferreira *et al.,* 1997).

With the exception of hexanol (Figure 4.9), the alcohols did not seem to be affected by oxidation (Table 4.2). This has also been observed by other studies (Ferreira *et al.*, 1997), which means that the oxidation of the alcohols to the corresponding aldehydes (Wilderandt & Singleton, 1974) could only account for a small fraction as the concentrations did not decrease significantly. Hexanol contributes to the “grassy” and “green” odours of a wine and can be formed due to oxidation of linoleic and linolenic acids and its formation during aging has been reported previously (Marais & Pool, 1980; Oliveira *et al.*, 2006), however in the present study the hexanol concentrations did not exceed the perception threshold (Table 4.3) and probably did not play an important role in the sensory perception of the wine. Methionol concentrations has been reported to decrease during aging (Ferreira *et al.*, 1997), however in this study, there was no significant difference in methionol concentrations (Table 4.2).

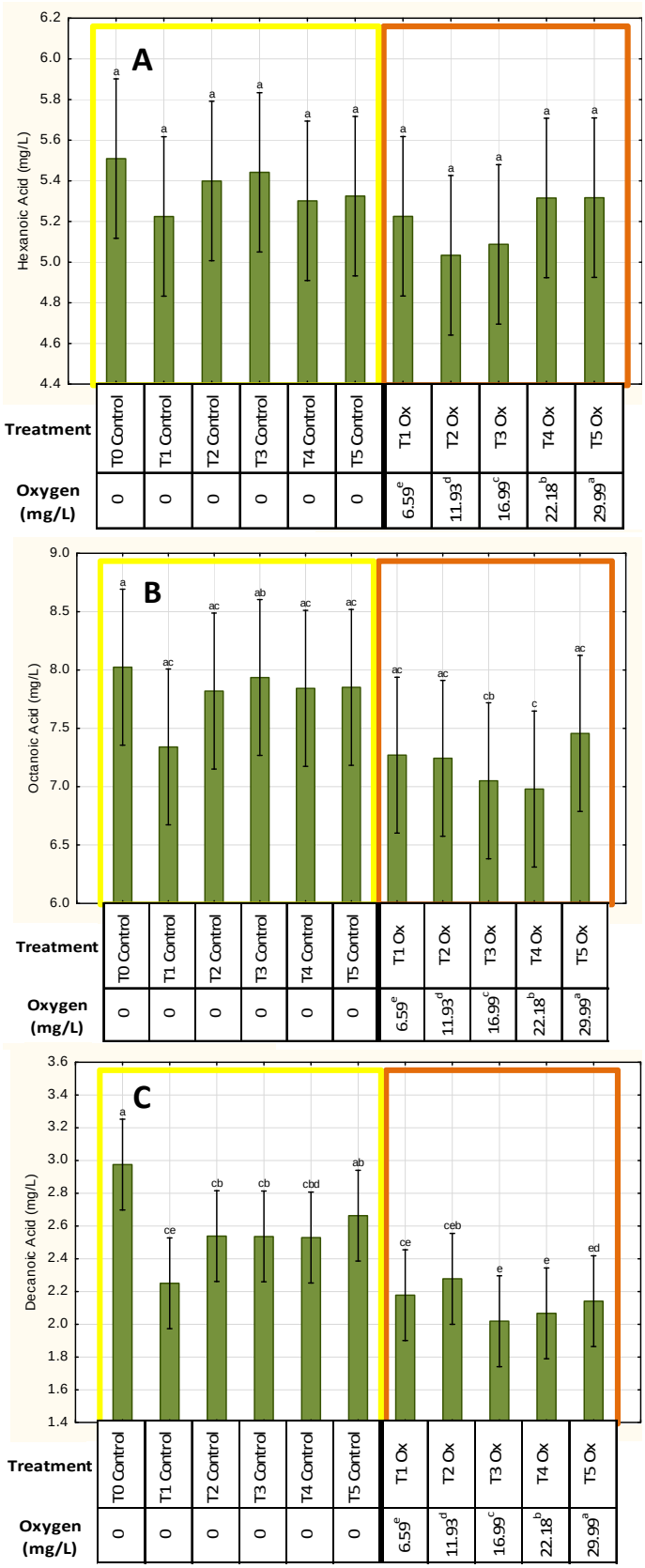


Figure 4.8 Hexanoic acid (A), octanoic acid (B) and decanoic acid (C) concentration (mg/L) of the respective treatments. Values are means of triplicate analysis; different letters indicate significant differences at $p<0.05$.

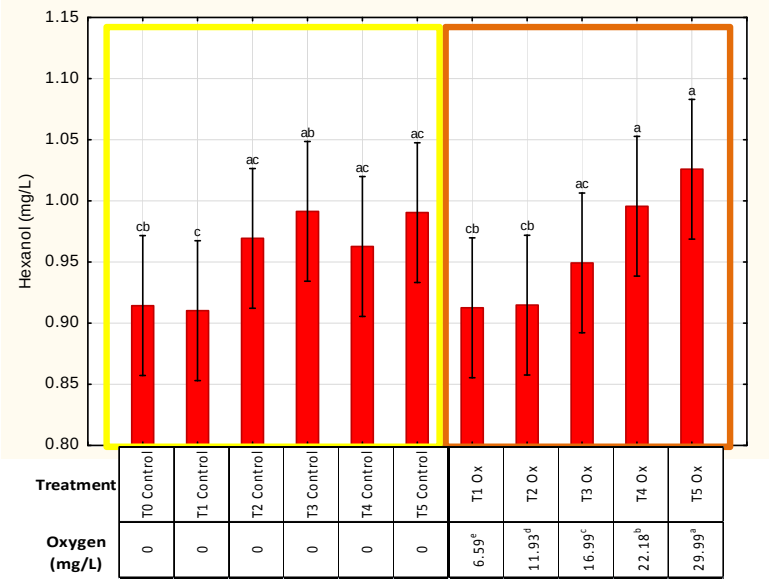


Figure 4.9 Hexanol concentration (mg/L) of the respective treatments. Values are means of triplicate analysis; different letters indicate significant differences at $p<0.05$.

4.3.2.4 Monoterpenes

Monoterpene concentrations are known to change considerably during wine aging (Rapp & Güntert, 1986) and the loss in aroma may occur due to oxidation or transformation of the terpene compounds (Versini *et al.*, 1981; Papadopoulou & Roussis, 2001). These compounds are sensitive to acidic conditions and storage time (Marais, 1983) and a remarkable loss of monoterpene alcohols occurs due to the formation of terpene oxides that have sensory thresholds about 10 times higher than the precursors (Papadopoulou & Roussis, 2001).

In this study, linalool concentration initially increased during the aging period after which it started to decrease (Table 4.2, Figure 4.10). The decrease in linalool concentration during wine oxidation and aging has been reported previously (Papadopoulou & Roussis, 2001, 2008; Loscos *et al.*, 2010; Cejudo-Bastante *et al.*, 2013) and may be due to oxidation or the progressive replacement of linalool by α-terpineol (Rodopulo *et al.*, 1970; Marais, 1983; Rapp & Güntert, 1986), however the amount of α-terpineol detected in these samples was under the quantification limit. A substantial amount of bound terpenols still remain in the wine after fermentation and aging could induce the hydrolysis of these bound terpenols to produce the free, aromatic terpenes (Gunata *et al.*, 1986). The extent of the hydrolysis will depend on the specific type and nature of the aglycone (Gunata *et al.*, 1986). This could explain the initial increase in linalool concentration at the beginning of the aging period. There were no significant differences between the Control samples and their Ox counterparts for linalool

and geraniol which could indicate the minor role the addition of oxygen played. Geraniol and farnesol concentration remained the same for the first three sampling stages (Table 4.2, Figure 4.10) after which it started to decrease probably due to degradation of the terpene alcohol.

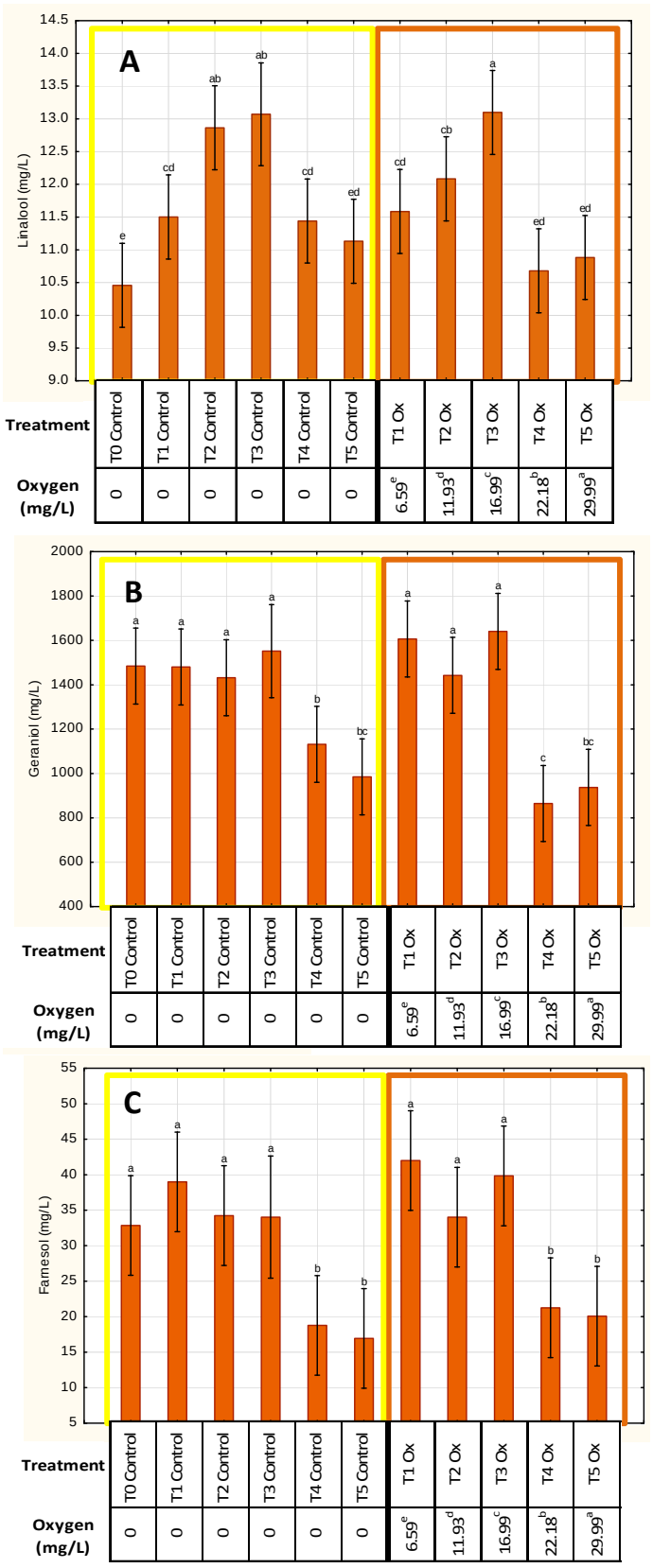


Figure 4.10 Linalool (A), geraniol, (B), farnesol (C) concentration (mg/L) of the respective treatments. Values are means of triplicate analysis; different letters indicate significant differences at $p<0.05$.

4.3.2.5 Sulphur dioxide and Glutathione

No ascorbic acid was detected in the wines. The evolution of free and total SO_2 is shown in Table 4.2 and Figure 4.11. At the beginning of the study, the wine contained adequate levels (according to commercial standards) of both free and total SO_2. In the Control samples, the free SO_2 concentration initially decreased from T0 Control (43.33 mg/L) to T1 Control (31.67 mg/L) (64 days). Thereafter the concentration remained stable with a slight decrease towards the end of the aging period (to 28.33 mg/L in T5 Control). From T0 Control to T5 Control, the total SO_2 concentration decreased from 100.33 mg/L to 92.33 mg/L. This resulted in a 35% and 8% decrease in free and total SO_2 concentrations respectively. This decrease in SO_2 concentration agrees with previous studies (Brajkovich *et al.*, 2005; Lopes *et al.*, 2009; Herbst-Johnstone *et al.*, 2011) and even though care was taken during bottling to achieve minimum oxygen exposure (in the Control samples), some oxygen could have dissolved during transfer and a small amount of oxygen could have remained in the headspace after bottling.

The most important loss in SO_2 has been reported to occur within the first month of bottling, after which the SO_2 undergoes a slower rate of decrease or remains stable, which is consistent with the results from this study (Brajkovich *et al.*, 2005; Lopes *et al.*, 2006; Kwiatkowski *et al.*, 2007; Dimkou *et al.*, 2011; Herbst-Johnstone *et al.*, 2011). With the addition of oxygen, a significant decrease in SO_2 concentration was observed. Free SO_2 concentrations decreased dramatically to reach 10.00 mg/L at T2 Ox after which it decreased further to reach 5 mg/L in T5 Ox resulting in an 88% loss. Godden *et al.* (2001) reported the considerably higher "oxidised" character in Semillon wines with free SO_2 content below 10 mg/L. Total SO_2 decreased with 49% reaching a minimum of 51.33 mg/L in the T5 Ox sample. With the SO_2 reaching these low concentrations, very little antioxidant protection remains in the wine.

The potentiometric method used to analyse the free and total SO_2 content could perhaps lead to a slight over-estimation of the SO_2 content in the sample due to the ability of phenols and sugars to be oxidised by the iodide. The smaller quantities measured in the later Ox stages could indicate that in reality very little to no SO_2 was present in these samples leaving the wine with effectively no SO_2 antioxidant protection.

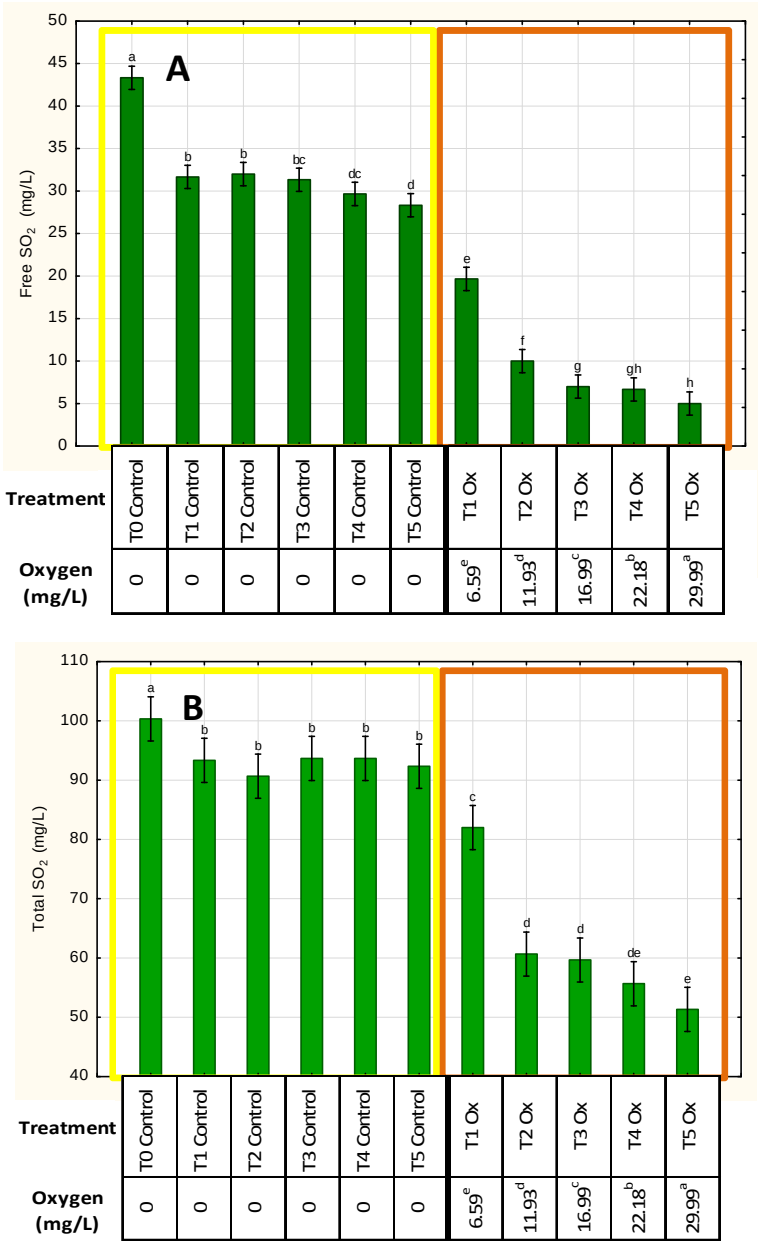


Figure 4.11 Free (A) and Total (B) SO_2 concentration (mg/L) of the respective treatments. Values are means of triplicate analysis; different letters indicate significant differences at $p<0.05$.

Another antioxidant that helps protect a wine from oxidation is GSH (Kritzinger *et al.*, 2013). GSH is very oxidation sensitive and diminishes rapidly during storage of wines (Lavigne *et al.*, 2007). In this study, GSH concentration ranged from 0.56 mg/L to 16.79 mg/L (Table 4.2, Figure 4.12). The initial concentration agrees with a previous study that found the average GSH concentration in 28 young Sauvignon blanc wines to be around 12.5 mg/L (Janes *et al.*, 2010).

In the Control samples, GSH concentrations decreased with 68% while it almost completely disappeared in the Ox samples, decreasing with 97% and correlated well with free SO_2 concentrations (r=0.9095). As mentioned before, the decrease in GSH content from T0 Control to T1 Control could be due to oxygen exposure during the bottling phase. However, unlike the free SO_2 content which remained stable thereafter, the GSH content continued to decline to the end of the study (T5 Control). GSH content in the Ox samples decreased more dramatically, with T1 Ox still containing some residual GSH (5.39 mg/L), while further oxidation led to levels of 1.52 mg/L and lower.

Together with the decrease in reduced GSH, there was an increase in oxidised GSH and GRP (Table 4.2, Figure 4.12). The increase in GRP was slight and not consistent. This phenomenon has also been reported in bottled Sauvignon blanc wine stored for two years showing fairly similar values compared to concentrations found prior to bottling (Herbst *et al.*, 2008). The increase in the oxidation products does not account for the total amount of reduced GSH lost. This has also been seen in other studies (Fracasetti *et al.*, 2013) and could indicate to the reaction of GSH with other substrates such as hydroxycinnamic acids or other compounds not measured. Sonni *et al*. (2011) reported the formation of methyl-glutathionyl-methine-*(t)*-catechin complex in model wine due to the reaction of GSH with (+)-catechin. Furthermore, GRP has been reported to undergo hydrolysis in model and real wine and could explain the low percentage of accounted oxidation products (Cejudo-Bastante *et al.*, 2010). As a thiol, GSH is able to reduce *o*-quinones and H_2O_2. Therefore, it can be hypothesised that GSH competes in these reactions with volatile thiols, thus preventing the loss of the unique aroma brought by these compounds.

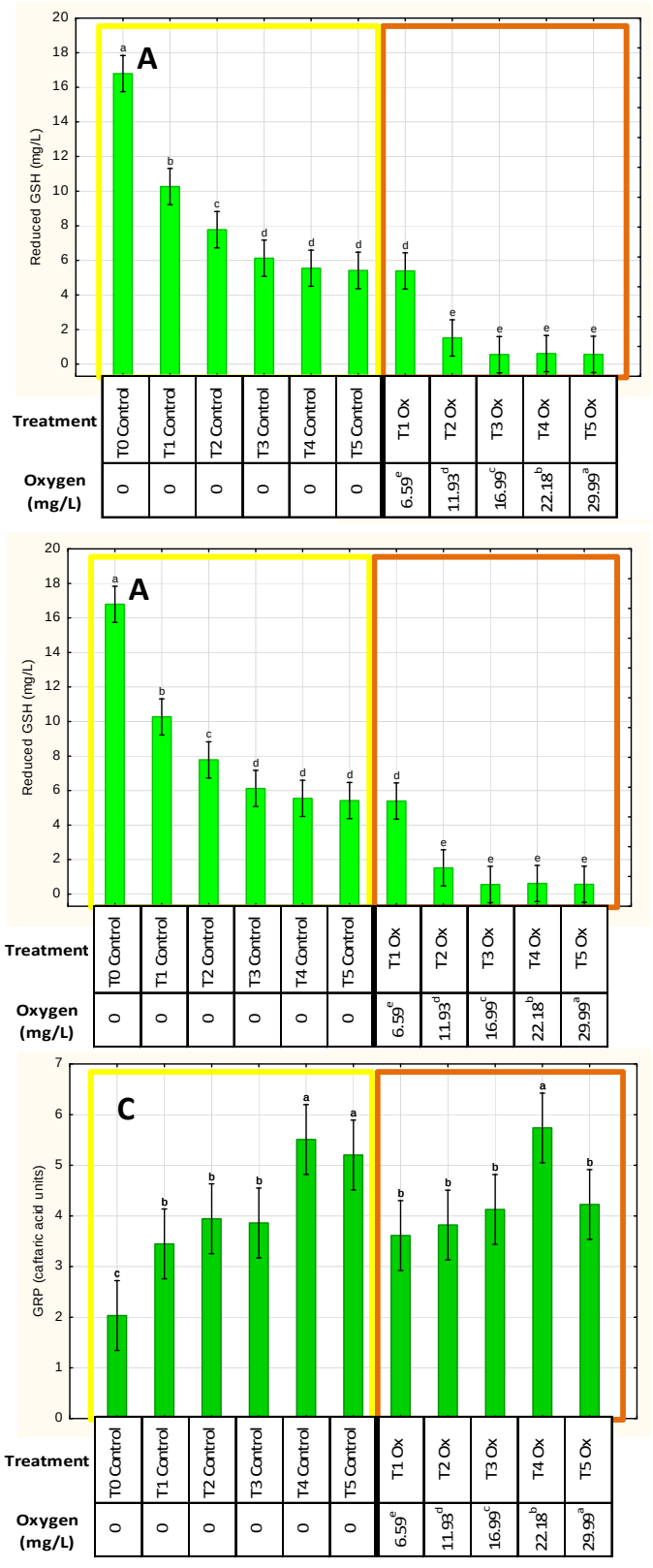


Figure 4.12 Reduced glutathione (mg/L) (A), oxidised glutathione (mg/L) (B) and grape reaction product (caftaric acid units) (C) of the respective treatments. Values are means of triplicate analysis; different letters indicate significant differences at $p<0.05$.

4.3.2.6 Polyphenols

Phenol oxidation can have detrimental effects on the aroma of a wine due to the formation of reactive *o*-quinones that can then in turn react with other wine constituents such as the aromatic volatile thiols.

Seven phenolic compounds were identified in the wines of this study (Table 4.2). Gallic acid was present in very low concentrations (<1 mg/L) when compared to concentrations found in white wines in general, which was found to average around 10 mg/L (Waterhouse & Teissedre, 1997). The concentration of this compound has been reported to remain relatively stable during aging (Waterhouse, 2002), which has also been observed in the current study. Only from T0 Control to T1 Control was a significant increase in gallic acid concentration observed (from 0.29 mg/L to 0.52 mg/L). This could be due to the hydrolysis of gallate esters during the first periods of aging (Waterhouse, 2002).

(+)-Catechin, *trans*-caftaric acid, *p*-coutaric acid, caffeic acid and *p*-coumaric acid concentrations were also quantified in the wines (Table 4.2, Figures 4.13 and 4.14). (+)-Catechin concentrations decreased in the Ox samples when compared to the Control counterparts (Figure 4.13), however in some cases the decrease was not significant. The flavan-3-ols participate in oxidation and polymerization reactions and have been linked to the susceptibility of white wines to browning (Simpson, 1982). (-)-Epicatechin was not detected in any of the samples.

The treatment had little effect on caffeic acid or *p*-coumaric acid concentrations, while a decrease was observed in *trans*-caftaric acid and *p*-coutaric acid concentrations (comparing the Control to Ox counterparts); however these decreases (especially *trans*-caftaric) acid were not always significant.

The loss of flavan-3-ols during aging and the more stable responses of the hydroxycinnamates have been reported previously (Recamales *et al.*, 2006; Herbst *et al.*, 2008; Hernanz *et al.*, 2009; Herbst-Johnstone *et al.*, 2011). This is likely due to the greater susceptibility of the flavan-3-ols to participate in slow oxidative or other degradative processes during bottle storage. In contrast, an increase in the free hydroxycinnamic acids, caffeic acid and *p*-coumaric acid, was reported by Herbst *et al.* (2008) ascribing it to the hydrolysis of their corresponding tartrate esters. In the current study, coutaric acid concentrations increased from T0 Control to T5 Control (0.75 to 0.95 *p*-coumaric acid equivalents) and a slight increase was also observed from T1 Ox to T4 Ox (0.77 to 0.83 *p*-coumaric acid equivalents).

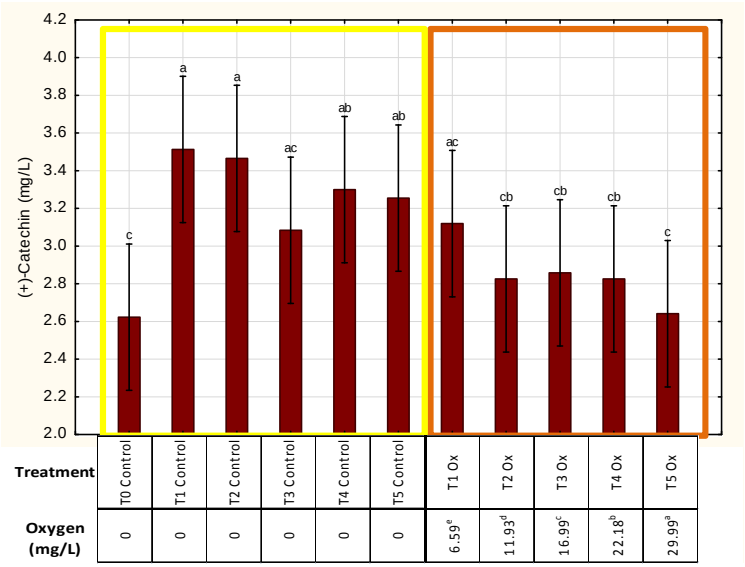
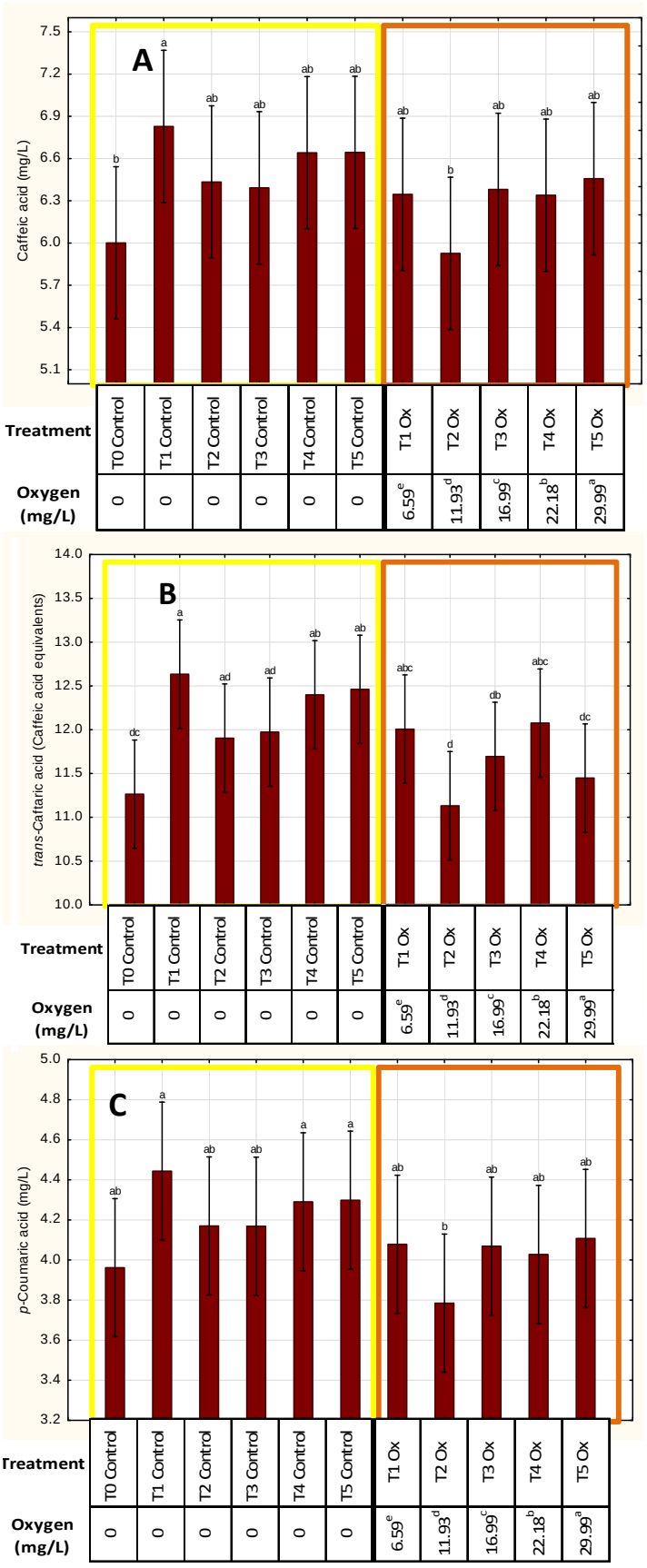


Figure 4.13 Flavan-3-ol: (+)-Catechin concentration (mg/L) of the respective treatments. Values are means of triplicate analysis; different letters indicate significant differences at $p<0.05$.

Similar observations were found for other white wine varieties during 12 months of storage (Recamales *et al.*, 2006; Hernanz *et al.*, 2009), however the decrease was still evident comparing the Control samples with Ox samples. Other studies also reported minor changes in phenolic composition after a 60 day storage period of oxygenated wines (Fracassetti *et al.*, 2013). Sulphur dioxide reacts with the *o*-quinone, reducing it back to the catechol. The concentration of the specific phenol would thus not appear to decrease as it is oxidised. It was therefore proposed that oxidation involves an equilibrium, which is driven forward by removal of the *o*-quinone (Danilewicz, 2012). In the case of (+)-catechin, 96% of the *o*-quinone was reduced back to the phenol in an oxidised model wine medium containing SO_2 (Danilewicz, 2010), however this has not been tested in real wine situations and needs further investigation.

No flavonols (e.g. quercetin derivatives) were detected in these wines even though they have been detected in heavier pressed Sauvignon blanc juice fractions (Patel *et al.*, 2010). The polymeric phenol content of the wines decreased during the study (Figure 4.15), however the difference between the Control samples and the Ox counterparts were mostly not significant. Fracassetti *et al.* (2013) also found decreases in oxygen levels in white wines to not always correlate well with decreases in phenolic compounds.



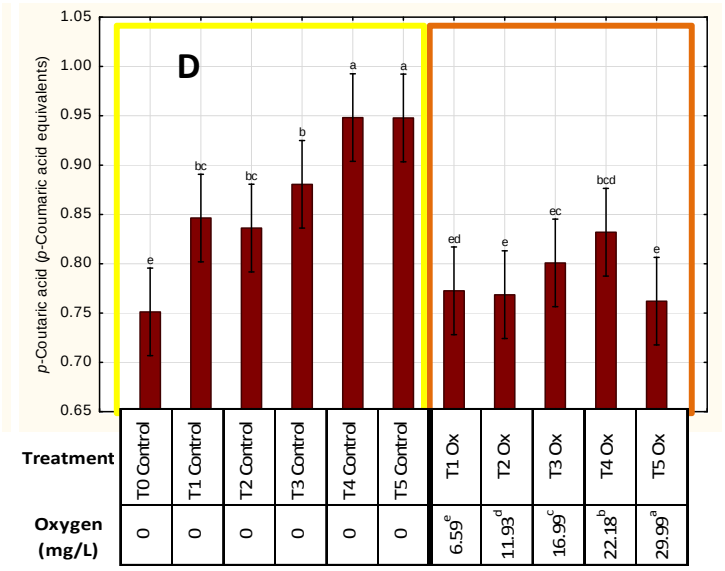


Figure 4.14 Hydroxycinnamic acids: Caffeic acid (mg/L) (A), *trans*-caftaric acid (caffeic acid equivalents) (B), *p*-coumaric acid (mg/L) (C) and *p*-coutaric acid (p-coumaric acid equivalents) (D). Values are means of triplicate analysis; different letters indicate significant differences at p<0.05.

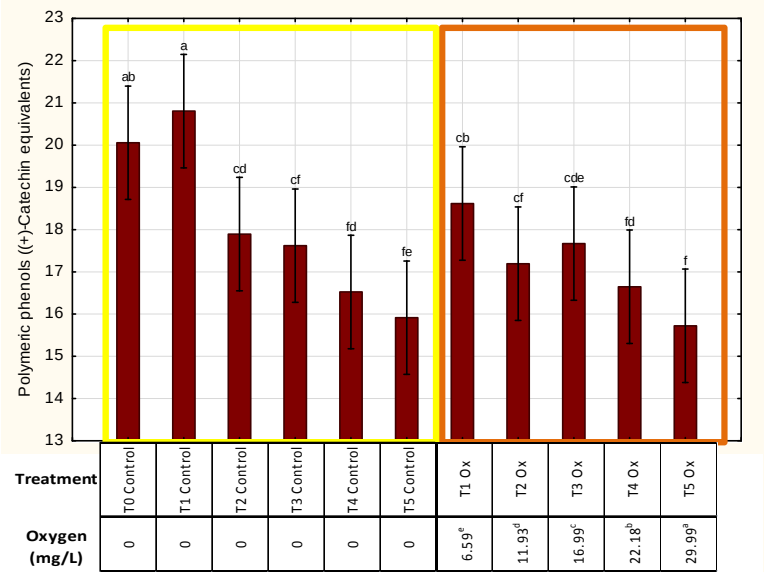


Figure 4.15 Polymeric phenols concentration ((+)-catechin equivalents) of the respective treatments. Values are means of triplicate analysis; different letters indicate significant differences at p<0.05.

4.3.2.7 Aldehydes

In a wine that was not exposed to oxygen, the acetaldehyde present is considered to originate from alcoholic fermentation by yeasts (Margalith, 1981). Acetaldehyde levels produced by yeast can range from 0.5-286 mg/L for *Saccharomyces cerevisiae* yeast strains (Liu & Pilone, 2000). In the presence of free SO_2, acetaldehyde will immediately bind to form hydroxysulphonate, contributing to the bound acetaldehyde present in wine (Liu & Pilone, 2000). In this study, the acetaldehyde present in the Control samples probably originated from alcoholic fermentation and is most likely in the bound form considering the free SO_2 content averaging at 32.72 mg/L for all the Control samples. The average concentration of total acetaldehyde (41.28 mg/L) of the wine at T0 falls within range of acetaldehyde concentration found in dry white wines (Table 4.2, Figure 4.16) (Jackowetz & De Orduña, 2013). This bound form of acetaldehyde is thought to be odourless (Peynaud, 1984; Somers, 1998), however the contribution of the odourless product to the aroma of a wine, either directly or by way of interactions, has not been elucidated.

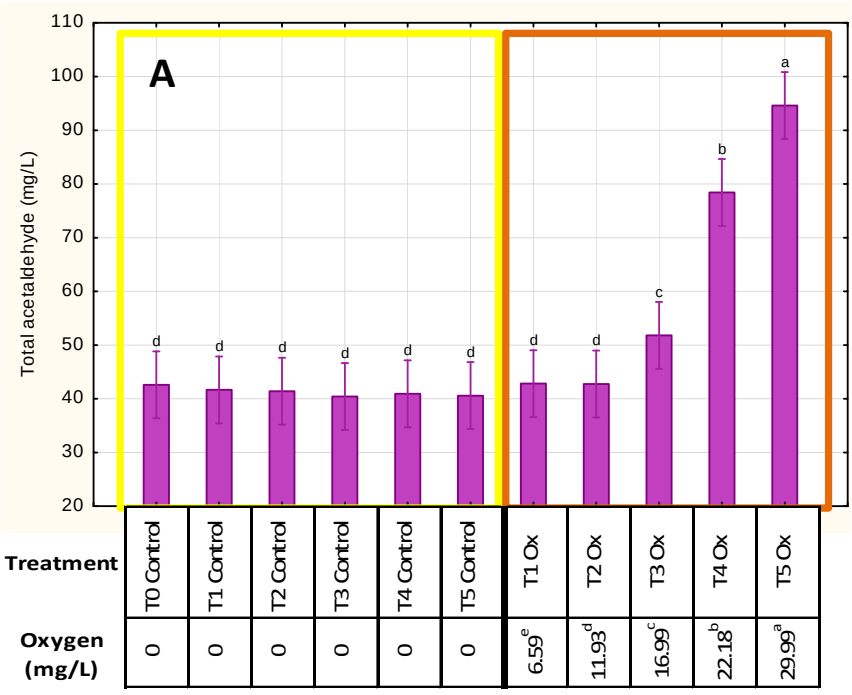


Figure 4.16 Total acetaldehyde concentration (mg/L) of the respective treatments. Values are means of triplicate analysis; different letters indicate significant differences at $p<0.05$.

During the oxidative aging, the total acetaldehyde concentration increased especially during the last three sampling stages of the Ox treatment and reached a maximum of 94.62 mg/L at T5 Ox. This increase is probably due to the oxidation of ethanol (Wilderandt & Singleton, 1974; Ribéreau-Gayon, 1998, 2004b). The increase in total acetaldehyde will initially be due to the increase in bound acetaldehyde as the free SO_2 is consumed. Once all free SO_2 is consumed, an increase in free acetaldehyde will occur. Free acetaldehyde concentration was not determined as there seems to be confusion in literature about the measurement of this compound especially in terms of the measurement of either "total" or "free" acetaldehyde. The different forms of acetaldehyde complicate the analysis.

Total acetaldehyde can be determined chemically (iodimetry) or enzymatically (aldehyde dehydrogenase). However, the chemical method has proven to give results 1-20% higher than the enzymatic method (Ough & Amerine, 1988; Liu & Pilone, 2000). Furthermore, the enzymatic method is considered as one of the best methods for this analysis as it has been reported to be more accurate and specific (Liu & Pilone, 2000). Free acetaldehyde can reportedly be measured by gas chromatography, however the results obtained by these measurements seem to be overestimated at times as most of the wines measured contain a significant amount of "free" acetaldehyde in conjunction with free SO_2. These contradictory results have to be investigated further. Nevertheless, it has been reported that bound acetaldehyde can account for 68.8% of the total SO_2 (Liu & Pilone, 2000; Bakker *et al.*, 1993). The bound acetaldehyde could thus be calculated theoretically. The value obtained by this calculation should be confirmed using more advanced analytical techniques as the percentage bound acetaldehyde can be dependant on various other factors such as the concentration of phenolics, presence of other antioxidants as well as competition for the reaction with sulphur dioxide. The percantage could also change over time as the composition of the wine changes. Nevertheless, the theoretical value of the bound acetaldehyde was calculated using the specified percentage in order to roughly estimate the free acetaldehyde content by subtracting the bound acetaldehyde from the total acetaldehyde measurement. The stepwise calculation of molar conversion followed by the 0.688 ratio calculation and conversion back to mg/L can be seen in Table 4.4 together with free SO_2 concentration for interpretation.

Table 4.4 Calculation of free acetaldehyde from average total SO_2, assuming 68.8% of the total SO_2 in all the samples are bound to acetaldehyde.

Compound	unit	T0 Control	T1 Control	T2 Control	T3 Control	T4 Control	T5 Control	T1 Ox	T2 Ox	T3 Ox	T4 Ox	T5 Ox
Free SO_2	mg/L	43.33^{a}	31.67^{b}	32.00^{b}	31.33^{bc}	29.67^{dc}	28.33^{d}	19.67^{e}	10.00^{f}	7.00^{g}	6.67^{gh}	5.00^{h}
Total SO_2	mg/L	100.33^{a}	93.33^{b}	90.67^{b}	93.67^{b}	93.67^{b}	92.33^{b}	82.00^{c}	60.67^{d}	59.67^{d}	55.67^{de}	51.33^{e}
Total SO_2	mol	0.0070	0.0066	0.0064	0.0066	0.0066	0.0065	0.0058	0.0043	0.0042	0.0039	0.0036
SO_2 : Acet 0.688												
Bound Acetaldehyde	mol	0.0048	0.0045	0.0044	0.0045	0.0045	0.0045	0.0040	0.0029	0.0029	0.0027	0.0025
Bound Acetaldehyde	mg/L	47.46	44.15	42.89	44.31	44.31	43.68	38.79	28.70	28.23	26.34	24.28
Total Acetaldehyde	mg/L	42.61^{d}	41.67^{d}	41.42^{d}	40.43^{d}	40.95^{d}	40.60^{d}	42.84^{d}	42.76^{d}	51.81^{c}	78.44^{b}	94.62^{a}
Bound Acetaldehyde	mg/L	47.46	44.15	42.89	44.31	44.31	43.68	38.79	28.70	28.23	26.34	24.28
Free acetetaldehyde = Total - Bound	mg/L	-4.85	-2.48	-1.47	-3.88	-3.36	-3.08	4.05	14.06	23.58	52.10	70.34

In the Control samples, the bound acetaldehyde concentration accounts for 100% of the total acetaldehyde measured using the enzymatic method. This observation coincides with the fact that sufficient free SO_2 was available in the Control samples to bind any formed acetaldehyde at this stage. This would indicate no free acetaldehyde present in the wine, which is confirmed by the theoretical value calculated for these samples. During the oxidative storage, there was an increase in the theoretical free acetaldehyde concentration. Figure 4.17 shows the theoretical free acetaldehyde concentration together with the total acetaldehyde as well as the free and total SO_2 content. T1 Ox had very low concentrations of free acetaldehyde, while concentrations increased at larger intervals from T2 Ox onwards. The low free acetaldehyde observed in T1 Ox is probably due to the presence of sufficient free SO_2 in the sample, thereby binding excessive acetaldehyde. The rest of the Ox samples experienced a further decrease in free SO_2 with the continued increase in free acetaldehyde.

As mentioned previously the low SO_2 content observed from T2 Ox onwards could be due to the overestimation of this compound concentration when using the Ripper method as the concentration is expected to be zero at this stage. The increase in free acetaldehyde should have important sensorial effects on the wine aroma. The odour perception threshold of acetaldehyde in a synthetic wine solution has been reported as 0.5 mg/L (Guth, 1997). At low levels this compound can contribute pleasant fruity aromas while at higher concentrations it is described as “green apple”, “overripe bruised apple”, “grassy”, “pungent”, “nutty” and “sherry” (Table 4.3) (Henschke & Jiranek, 1993; Miyake & Shibamoto, 1993; Frivik & Ebeler, 2003). The sensory results of these wines will be discussed in section 4.3.3.

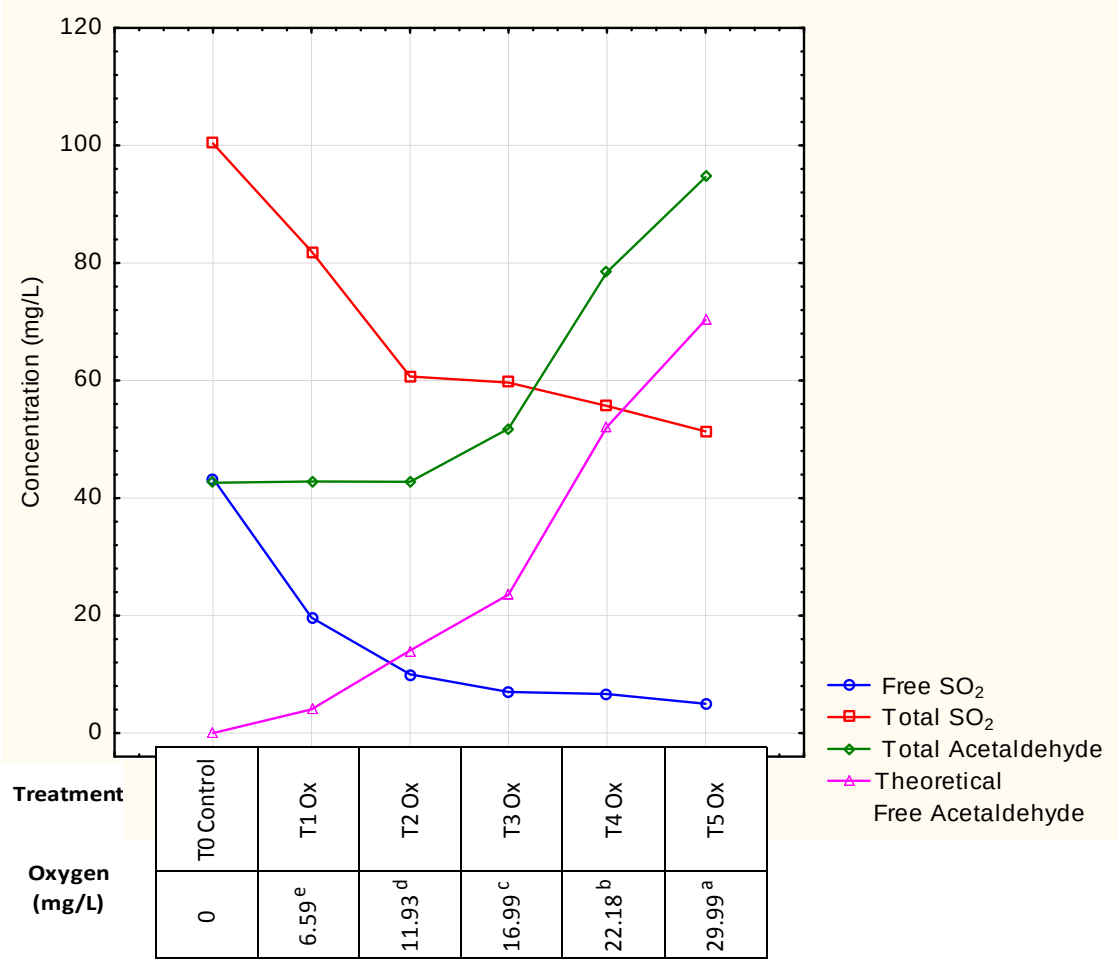


Figure 4.17 Concentration changes of total SO_2, free SO_2, total acetaldehyde and theoretical free acetaldehyde (all in mg/L) due to various treatments.

Methional and phenylacetaldehyde are related to the typical aroma of oxidative spoiled white wine (Silva Ferreira *et al.*, 2002b). These two compounds can be formed due to the oxidation of the respective alcohol (methionol and phenylethanol) (Marchand *et al.*, 2000; Jarauta *et al.*, 2005) or via the reaction of a dicarbonyl (such as the *o*-quinone formed from the oxidation of phenols) with the respective amino acids (methionine and phenylalanine) (Silva Ferreira *et al.*, 2002b; Rizzi, 2006). In this study, no methional was detected in the Control samples, however the methional content increased significantly from T1 Ox (0.58 µg/L) to T5 Ox (4.1 µg/L) (Table 4.2, Figure 4.18).

As mentioned previously, this increase is probably due to the Strecker degradation reaction, especially seeing that methionol concentrations remained the same throughout the treatment (Table 4.2). Sufficient methionine concentrations were present in the T0 Control samples (>4 mg/L; results not shown), making this pathway feasible. Surprisingly, no phenylacetaldehyde was detected in any of the samples, even in the presence of phenylalanine concentrations exceeding 13.0 mg/L in

the T0 Control sample (results not shown). The formation of Strecker aldehydes by the reaction of amino acids with *o*-quinones at 10˚C has been described as "essentially zero" by (Nikolantonaki *et al.*, 2012) and could be an indication of the inhibition of the formation of especially phenylacetaldehyde at lower temperatures as is the case in this study. Methional can contribute "boiled vegetable" and "rotten potato" aroma notes and could significantly influence the aroma of a wine considering the low perception threshold of 0.5 µg/L (Table 4.3). Further sensory contributions will be discussed in section 4.3.3.

Benzaldehyde concentration mostly did not differ between the Control treatments (Table 4.2, Figure 4.18), however the concentration initially increased during the oxidative storage (T1 Ox to T2 Ox), after which it significantly decreased. The increase in benzaldehyde content in white wines has been reported previously (Ferreira *et al.*, 1997), however the decrease thereafter has not been observed. This phenomenon is not easy to explain. The formation of benzaldehyde has been attributed to phenylalanine oxidation (Loyaux *et al.*, 1981), while other authors reported its origin from amygdaline (Nykänen & Suomalainen, 1982). Thereafter, benzaldehyde can react with H_2S in the process forming methyl mercaptan which is responsible for the "smoky/empyreumatic" aroma in wine. However, this mechanism has only been suggested and not conclusively demonstrated (Tominaga, 2003; Tominaga *et al.*, 2003).

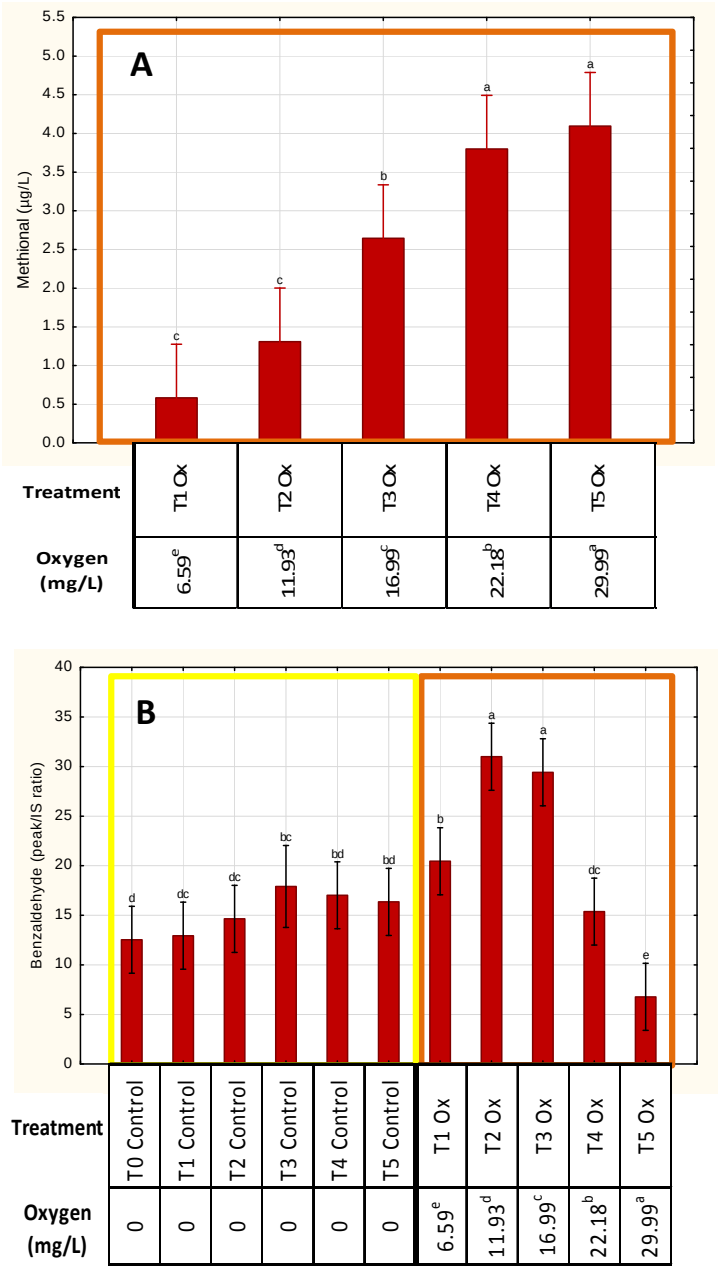


Figure 4.18 Methional (A) concentration (µg/L) and benzaldehyde (B) (peak/IS ratio) of the respective treatments. Values are means of triplicate analysis; different letters indicate significant differences at $p<0.05$.

Furfural and other furanic aldehydes usually have their origin in heating oak during barrel toasting (Ribéreau-Gayon *et al.*, 2006). However, their occurrence has also been suggested to originate from the degradation of carbohydrates during wine aging (Câmara *et al.*, 2004) and a good correlation between time aged and the concentration of these furanic compounds were found in Port and Madeira wines (Silva Ferreira *et al.*, 2003a; Câmara *et al.*, 2004). Furfural has also been identified as being one of the compounds linked with the tendency of wine to brown (Ferreira *et al.*, 1997).

Furfural correlated with the “cooked vegetables” descriptor and could also contribute to the “woody note” of aged wines (Escudero *et al.*, 2002), while hydroxymethylfurfural (5-HMF) gives odours such as “aldehyde” and “caramel” (Meilgaard, 1975; Câmara *et al.*, 2004). In the current study, furfural and 5-HMF concentrations increased during the storage of the Control samples, however this increase was even more prominent in the Ox samples, especially for the last three sampling stages (Table 4.2, Figure 4.19).

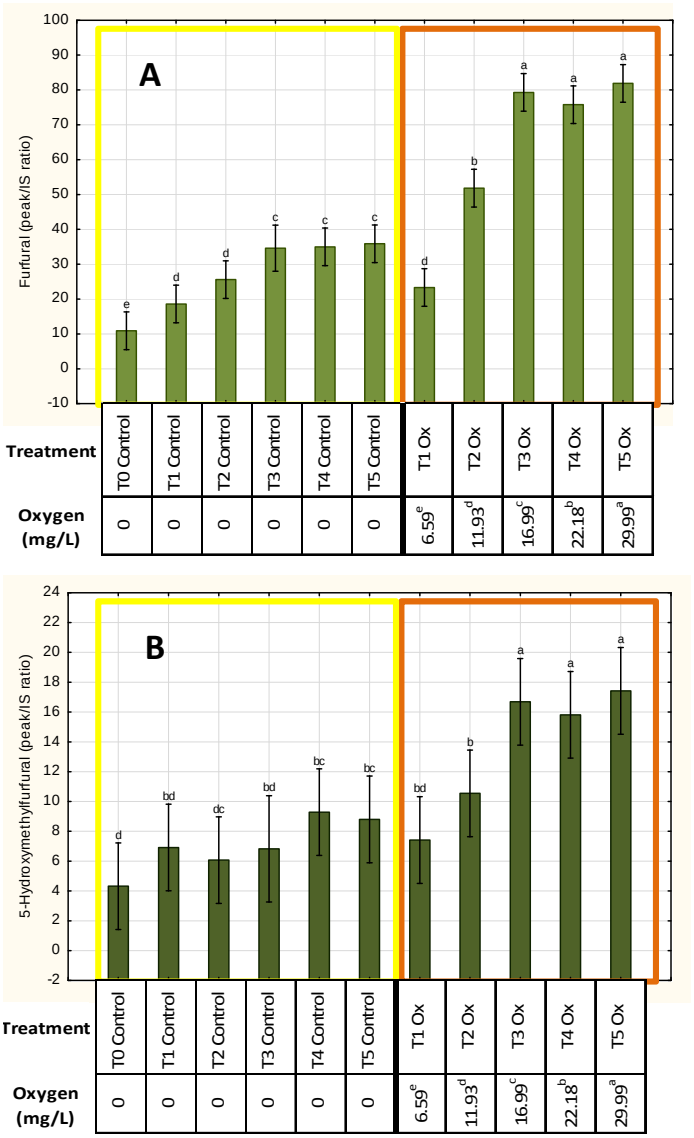


Figure 4.19 Furfural (A) and hydroxymethylfurfural (B) (peak/IS ratio) of the respective treatments. Values are means of triplicate analysis; different letters indicate significant differences at $p<0.05$.

4.3.2.8 Sotolon

Sotolon is a very powerful odourant that smells of "curry" and "myrrh" at high concentrations with "roasting", "maple syrup", "burnt sugar" and "caramel" at lower concentrations (Escudero *et al.*, 2000a; Ghidossi *et al.*, 2012). Young Sauvignon blanc wines were found to contain below 0.5 µg/L sotolon while Sauvignon blanc wines stored under various closures during a 24 month period contained a sotolon content ranging from 0.1-1.1 µg/L (Lopes *et al.*, 2009). The aroma detection threshold for this compound has been reported to be 2 µg/L in model wine (Table 4.3) (Pons *et al.*, 2010) and 8 µg/L in dry white wines (Lavigne *et al.*, 2008).

In this study, no sotolon was detected in any of the Control samples. Sotolon was already detected in the T1 Ox sample and increased up to T5 Ox (although this increase was not significant) (Table 4.2, Figure 4.20). The occurrence of sotolon could be due to the condensation reaction between α-ketobutyric acid and acetaldehyde followed by lactonisation (Takahashi *et al.*, 1976; Pham *et al.*, 1995; Cutzach *et al.*, 1998), especially considering the production of acetaldehyde in the Ox samples.

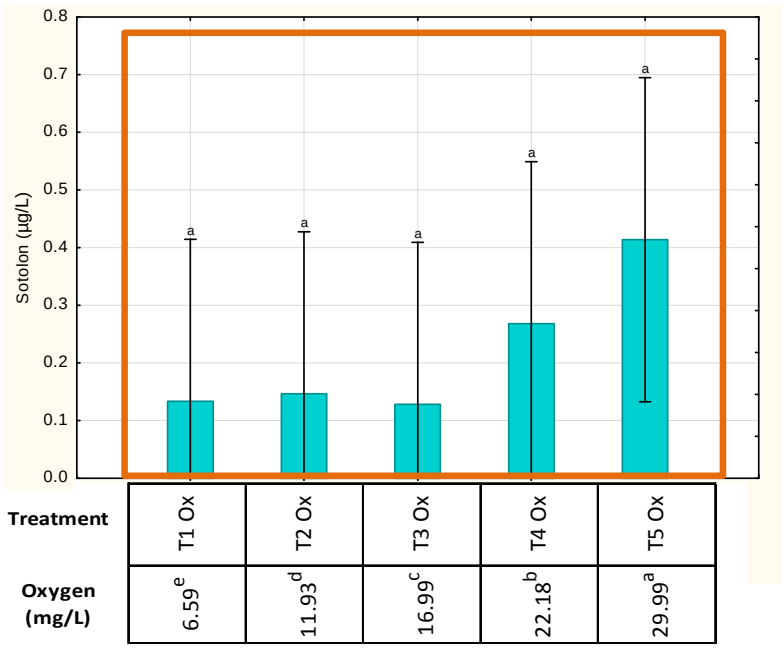


Figure 4.20 Sotolon concentration (µg/L) of the respective treatments. Values are means of triplicate analysis; different letters indicate significant differences at $p<0.05$.

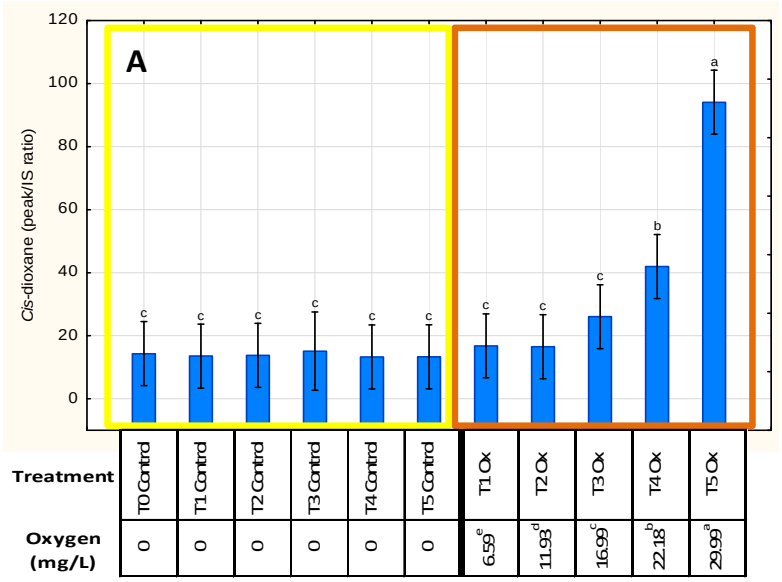
Other pathways seem less likely due to the fact that the wine was fermented dry, leaving minimal sugar content for the Maillard reaction between cysteine and various sugars (Hofmann & Schieberle,

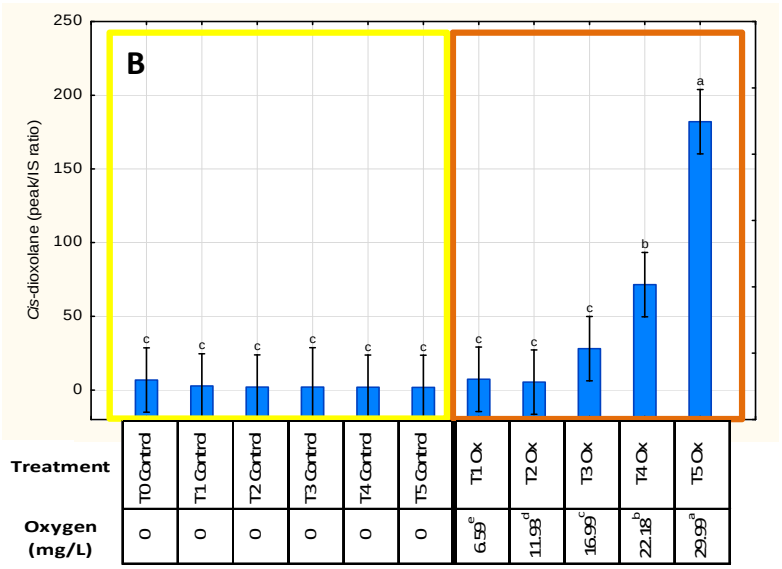
1995, 1997) to occur, while the fact that no ascorbic acid was detected in the samples ruled out the oxidative degradation of ascorbic acid as a pathway for sotolon formation.

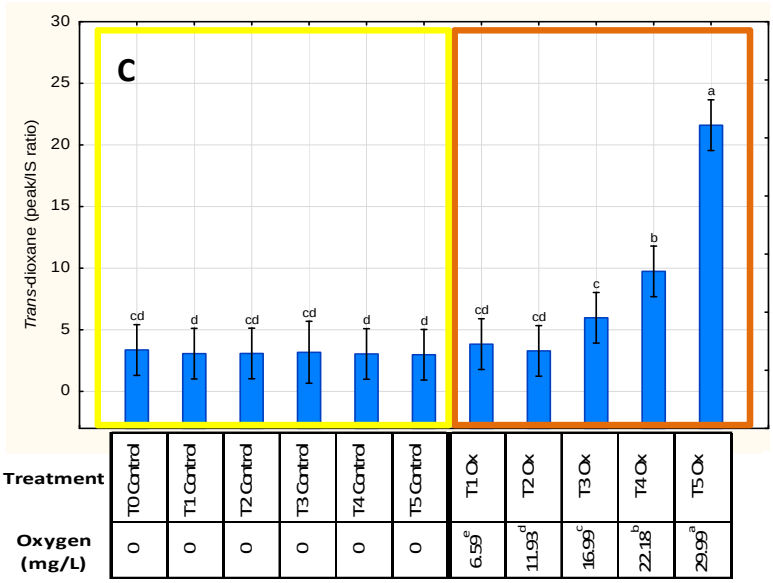
The maximum level quantified in the samples was in T5 Ox, reaching 0.41 µg/L, which is below the detection threshold in both model wine as well as dry white wine. It is thus unlikely that sotolon contributed to the aromatic composition of the wine, however sensory interactions between various aroma compounds can occur and the role of sotolon in these interactions (especially in dry white wines) needs to be investigated. The formation of this compound in white wines seems to be somewhat dependant on temperature and a concentration of around 8 µg/L were obtained during a forced aged experiment conducted at 60˚C (Silva Ferreira *et al.*, 2003b). In a study where South African white wines were assessed for sotolon levels, only those exposed to prolonged high temperatures had sotolon levels higher than the sensory threshold reported white wine (Lavigne *et al.*, 2008; Gabrielli *et al.*, 2013). It would seem as if the conditions of this study, especially the temperature, were not high enough to produce substantial amounts of sotolon.

4.3.2.9 Acetals

Dioxanes and dioxolanes are formed by the condensation reaction between glycerol and acetaldehyde in an acid medium. During oxidation there is a significant formation of acetaldehyde through the oxidation of ethanol and large amounts of the dioxanes and dioxolanes are expected to be produced seeing that sufficient quantity of reagents are available. These compounds serve as oxidation markers in aging Port wines and could possibly also serve as oxidation markers for white wines with low SO_2 content. The odour is described as “sweet” and “old port-like” (Silva Ferreira *et al.*, 2002a). In this study, dioxane formation mainly occurred during the last three sampling stages of the Ox samples (Table 4.2, Figure 4.21), increasing in significant amounts. This corresponds to the increase in acetaldehyde concentration which started to increase in significant amounts from the T2 Ox stage and correlation coefficients of 0.9457 to 0.9519 were calculated between the different acetals and acetaldehyde.







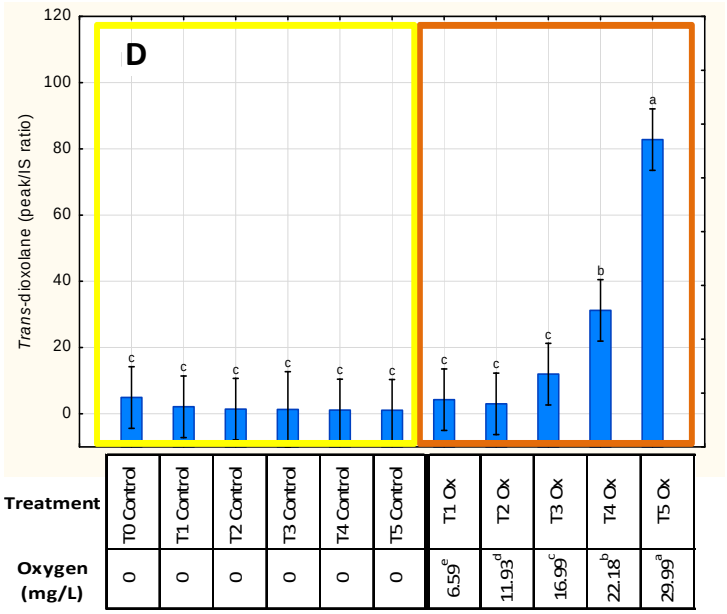


Figure 4.21 *Cis*-dioxane (A), *cis*-dioxolane (B), *trans*-dioxane (C) and *trans*-dioxolane (D) peak/IS ratios of the respective treatments. Values are means of triplicate analysis; different letters indicate significant differences at $p<0.05$.

4.3.2.10 Colour

The optical density at 420 nm (A_{420}) is commonly used in the wine industry to measure yellow (or brown) colour development due to oxidation of white wines (Singleton & Kramling, 1976; Zoecklein *et al.*, 1995; Iland, 2004). In this study, the measurement at 440 nm was also included as this wavelength corresponded to the maximum absorbance of coloured pigments in a model wine containing (+)-catechin (Bradshaw *et al.*, 2001). The absorbance values at both wavelengths can be seen in Table 4.2. A very high correlation (r=0.9973) was found between the absorbances, thus only the results from A_{420} will be discussed as a marker of oxidation.

In the Control samples, an increase in A_{420} can be seen from T0 Control which was 0.053 absorbance units (AU) to T5 Control which was measured as 0.061 AU (Figure 4.22). A steady increase was also recorded for the oxidised samples ending with an absorbance of 0.098 AU for T5 Ox, corresponding to a 46% increase. High risk of advanced oxidation colour formation in wines containing free SO_2 content less than 10 mg/L has been reported (Godden *et al.*, 2001). In the present study, a significant increase in yellow/brown colour was measured already at T2 Ox which corresponded with 10 mg/L free SO_2.

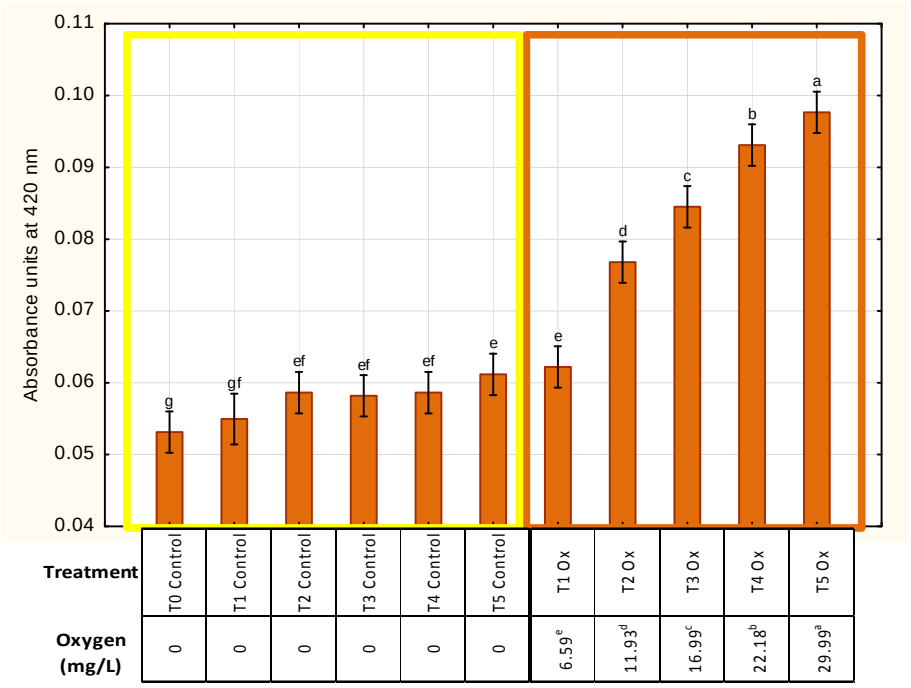


Figure 4.22 Absorbance measurements at 420 nm. Values are means of triplicate analysis; different letters indicate significant differences at $p<0.05$.

Browning in white wines can be due to three mechanisms. The first is the polymerization reactions occurring during oxidation of the polyphenols that could lead to the formation of more intensely coloured products thus leading to the browning of the wine. The second mechanism is the oxidation of tartaric acid forming glyoxylic acid, which mediates the condensation of phenolic molecules which can lead to varying degrees of polymerisation and thus contribute to the development of a yellow/brown colour in the wine. The production of acetaldehyde can also enhance oxidative colouration by inducing condensation of phenolic compounds (Es-Safi *et al.*, 1999; Lopez-Toledano *et al.*, 2004; Monagas *et al.*, 2005). A sensory panel also evaluated the wines according to colour. These results are discussed in section 4.3.3.

4.3.2.11 Combined chemical content

Figure 4.23 shows the PCA biplot constructed of all the chemical data obtained throughout the study. The first two principle components account for 58% of the variation with the most of the variation being explained by PC1 (37%). Located on the left of PC1 a group of positively correlated compounds can be seen which include the volatile thiols, SO_2, reduced GSH and certain acetate esters. Correlating negatively with this group are compounds located to the right of PC1. This group

includes oxidised GSH, furfural, 5-HMF, A_{420}, the acetals, acetaldehyde, some ethyl esters, methional and sotolon. The latter group are all mostly unpleasant smelling oxidation-related compounds. In the middle of PC1 a group of compounds including various acids and alcohols can be seen and is located slightly toward the left side of the PC. For this group of compounds, there seems to be a greater variation in the measurements as replications (data points) are located in a wider range. Some points are located to the high end of the compound concentration, while others are located at the lower end. This variation in repeats was not seen in the other two groups (located on the left and right of PC1). This could indicate the middle group not significantly contributing to the fresh and fruity or oxidative characteristics of the wines.

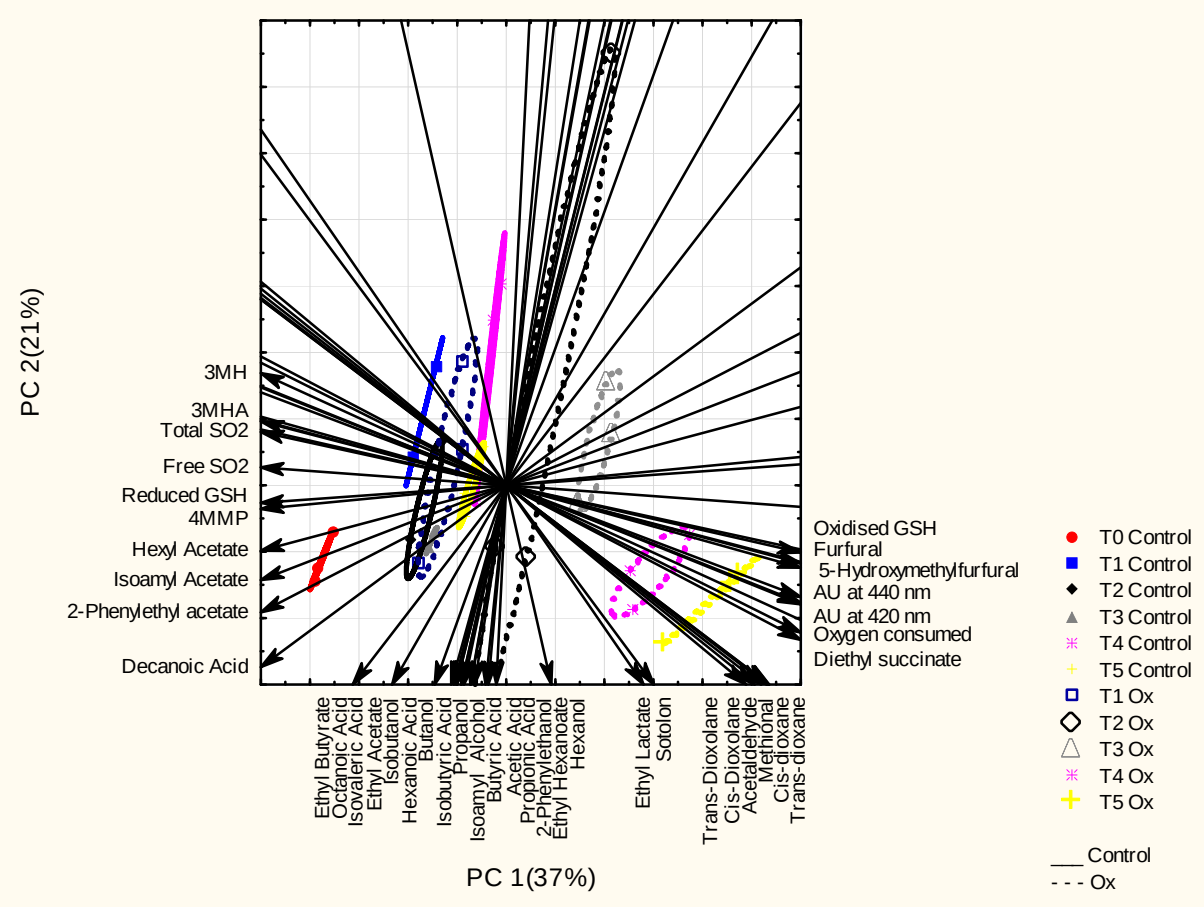


Figure 4.23 PCA biplot of chemical data of various treatments.

T0 Control is separated from the rest of the treatments and is associated with the highest concentration of fresh and fruity aroma compounds (located on the left of PC1). The rest of the Control samples were located towards the middle of PC1, indicating a decrease in the fruity aroma

compounds and antioxidants as time passed (T0-T5). According to the chemical data, T1 Ox contained higher concentrations of the fresh and fruity compounds when compared to T4 Control and T5 Control and seems to correspond well with T2 and T3 Control even though it received oxygen at the beginning of the trial. The rest of the treatments that received oxygen (T2-T5 Ox) experienced a decrease in concentration of fruity aroma compounds and antioxidants as time passed with a corresponding increase in oxidation-related compounds. Most of the phenols, some ethyl esters and the terpenes were not included in the PCA biplot as they did not contribute to the variation between the treatments.

4.3.3 Sensory evaluation

4.3.3.1 Aromatic descriptive analysis

Panel performance was analysed using PanelCheck. Tucker plots of the sensory data obtained from the three tests showed all descriptors to be significant (P<0.001) (Figure 4.24).

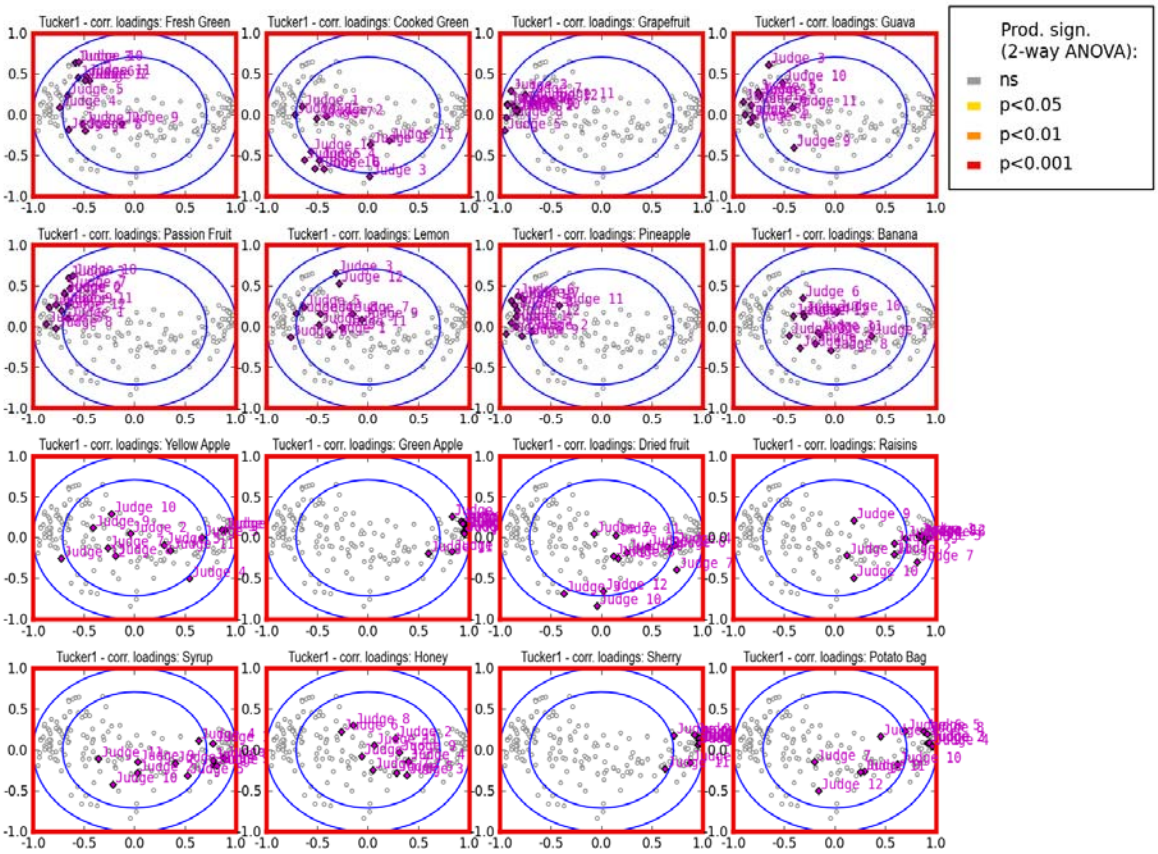


Figure 4.24 Tucker plot showing significance of all the attributes generated by the panel.

PCA was applied to the data obtained from the descriptive analysis performed on the wines (Figure 4.25). The first two principle components accounted for 96.8% of the variation with most of the variation being explained by PC1 (90.5%).

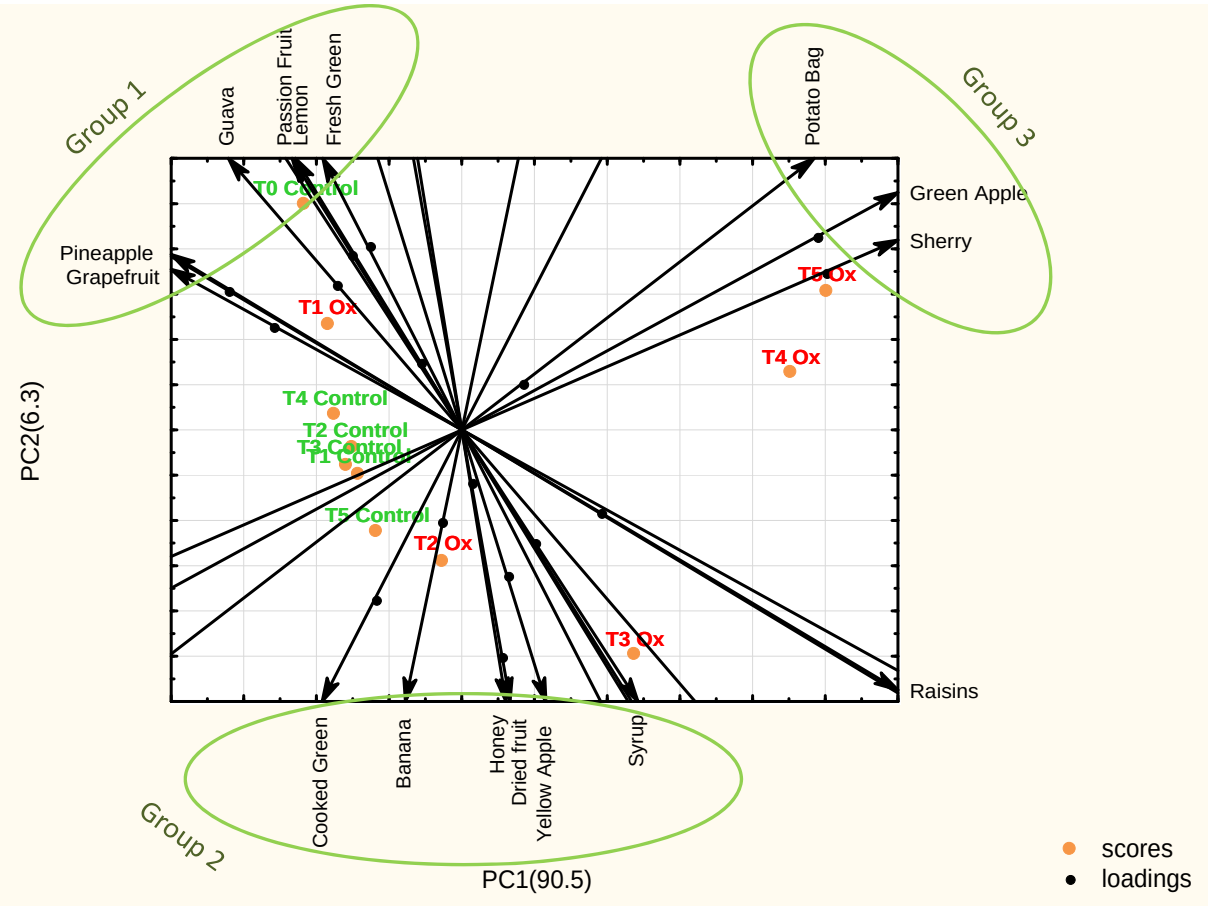


Figure 4.25 PCA biplot of data obtained from descriptive analysis of wines oxidised to various degrees over time.

Descriptors can be divided into 3 main groups. The first group showed positive correlation between the attributes *grapefruit*, *pineapple*, *guava*, *passion fruit*, *lemon* and *fresh green*. These attributes are probably a result of various aromatic compounds such as sulphur-containing compounds like 3MH, 3MHA and 4MMP (*grapefruit*, *guava* and *passion fruit*), esters (*pineapple*, *lemon*) and methoxypyrazines (*fresh green*) and are often used to profile young, fresh and fruity Sauvignon blanc wines (Lund *et al.*, 2009). The second group of attributes (at the bottom of the plot) are *cooked green*, *banana*, *honey*, *dried fruit*, *yellow apple* and *syrup*. These attributes correlate positively to each other and negatively to the first group of descriptors (*raisins* has a weaker correlation with the

rest of the attributes). These attributes are often used to describe wines gradually forming the typical aged and oxidised character (especially *honey*, *dried fruit*, *apple*, *syrup* and *raisins*). The third group of attributes includes *potato*, *green apple* and *sherry*. The latter group of attributes are mostly related to advanced wine oxidation and are often used when profiling Port or sherry wines where oxidation aromas are a signature trait of that style of wine (Margalith, 1981; Henschke & Jiranek, 1993; Miyake & Shibamoto, 1993; Frivik & Ebeler, 2003).

The Control samples are grouped on the left of PC1. T0 Control is separated from the rest of the group due to high intensities of the group 1 descriptors. T5 Control is also separated from T1-T4 Control samples due to higher intensities of group 2 descriptors. There are no clear separation between T1, T2, T3 and T4 Control samples indicating little sensory difference between them. T0 Control has a high intensity of fruity aromas while T1-T4 has less of the group 1 attributes and developed more towards the group 2 attributes. T5 Control has even less of the group 1 attributes when compared to the rest of the Control group. There was no development towards the group 3 attributes for the Control wines.

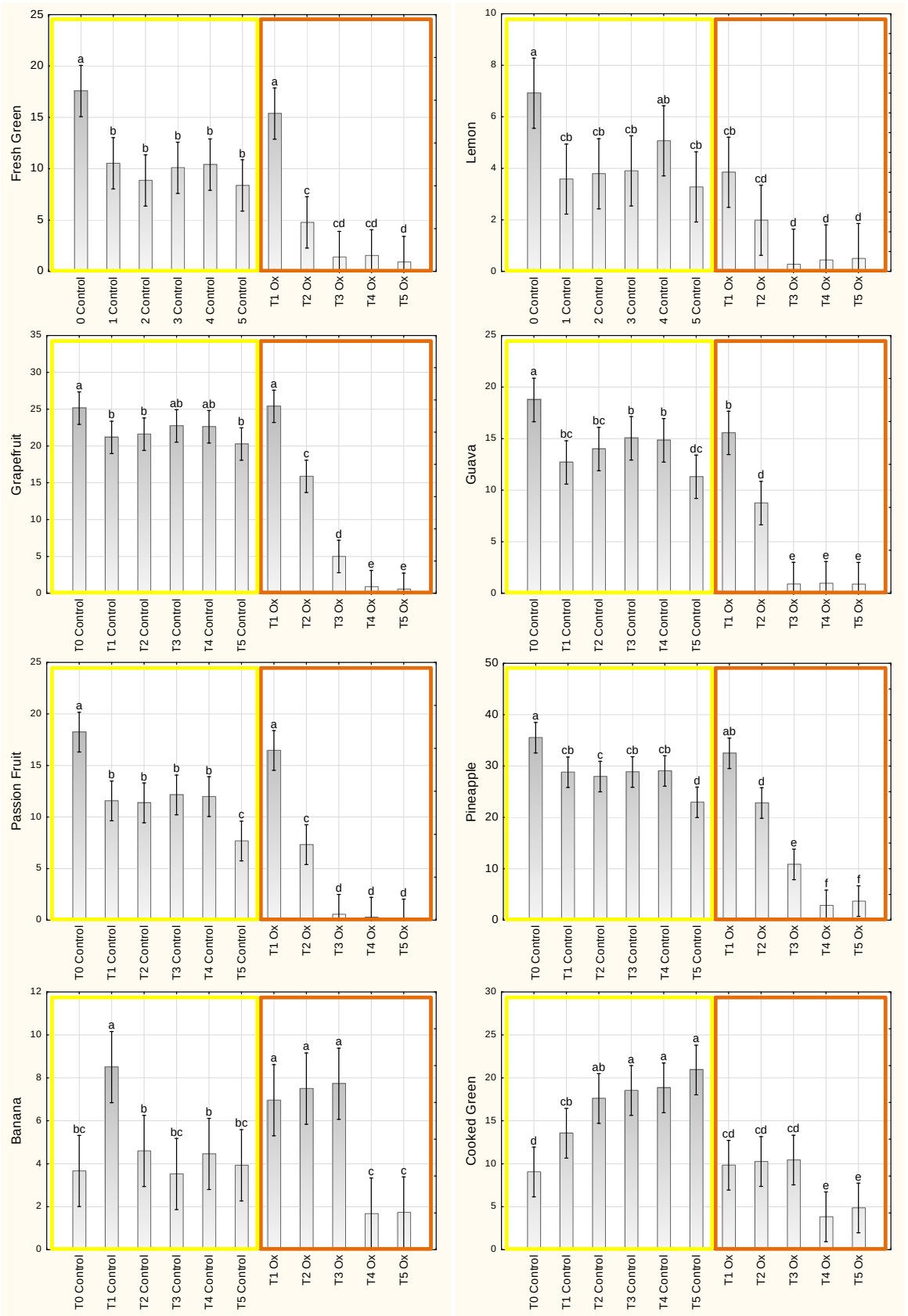
The development over time is clearly defined for the oxidised wines on PC1. T1 Ox is associated with group 1 attributes while T2 and T3 Ox evolved towards the group 2 attributes, consequently losing the fresh characters of group 1 and obtaining aged characters such as *honey*, *syrup* and *raisins*. Only T3 Ox seems to start showing some group 3 attributes. T4 and T5 Ox have a very high intensity of the group 3 attributes and none of the other descriptor groups.

Interestingly, even though T1 Ox received a significant amount of oxygen, it was perceived to be even more fresh and fruity when compared to T1-T5 Control. This result is interesting as T1 Control and T1 Ox both underwent the same time lapse before sampling, however T1 Ox maintained a more fresh and fruity aroma compared to T1 Control despite the fact that it consumed oxygen at the beginning of the study. Considering the chemical content of T1 Ox (Table 4.2, Figure 4.23), it would have been expected for T1 Ox to be positioned more towards the group 2 attributes which is not the case. To the contrary, not only does it not associate strongly with T2 and T3 Control (as suggested by the chemical data in Figure 4.23), but it is actually perceived to be more fresh and fruity correlating better with T0 Control.

Another interesting observation is that T1 Ox contained significantly lower concentrations of free and total SO_2 when compared to all the Control samples (about 10 mg/L less free SO_2; Table 4.2) however, this is not reflected in the PCA biplot constructed from the chemical data (Figure 4.23) as overall concentrations of other compounds drove the data points of T1 Ox to the left of PC1. This could mean that the SO_2 reacted with oxidative species and in this way preserved the positive and oxidation-sensitive aroma compounds. The dissolved oxygen concentration in the T1 Ox sample was

thus low enough not to deteriorate the aroma profile but still high enough to affect the antioxidant content (both SO_2 and GSH). The presence of other aromatic compounds, such as negative sulphur-containing compounds (e.g. methyl mercaptan) which may mask positive fruity-related attributes could also have a significant impact on the overall aromatic composition of the wine and needs to be investigated further. These compounds could potentially mask the fresh and fruity attributes.

Figure 4.26 shows the mean intensity scores for each individual attribute, from which the differences in treatments can be seen in more detail. Attributes which reached the highest intensities are *pineapple* (maximum of 35.6 units for T0 Control), *green apple* (maximum of 49.1 units for T5 Ox) and *sherry* (maximum of 48.9 units for T5 Ox). The overall aroma of the Control samples seems to be dominated by attributes such as *guava*, *pineapple*, *grapefruit* and *cooked green* when considering attribute intensities.



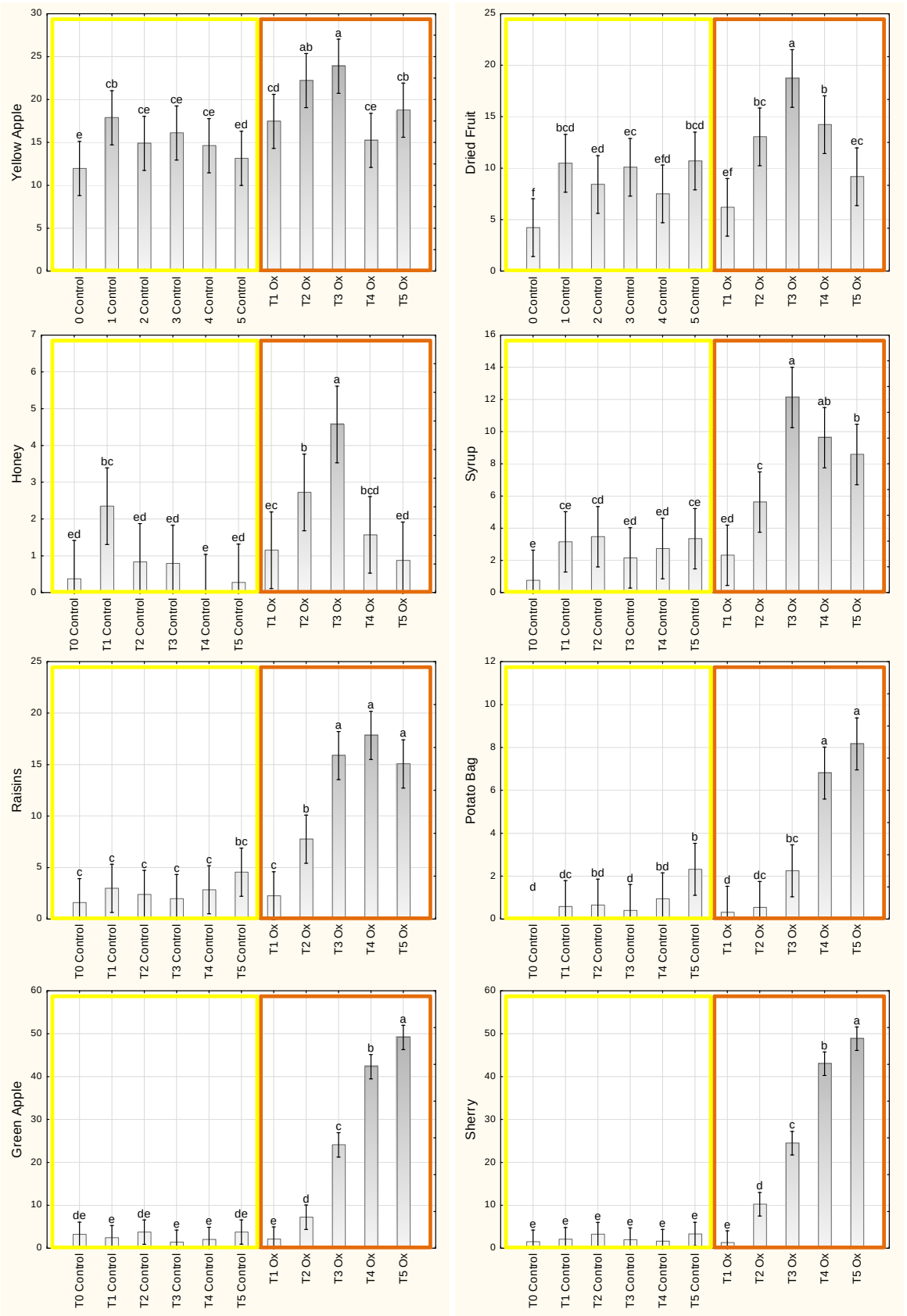


Figure 4.26 Mean values for each sensory attribute. Tests were done in triplicate; different letters indicate significant differences at $p<0.05$.

Attributes that scored very low in general included *lemon*, *banana*, *honey* and *potato bag*. For these attributes samples were never scored more than 10 intensity units. When compared to the Ox samples, Control samples were scored higher in attributes *fresh green*, *lemon*, *passion fruit*, *guava*, *pineapple* and *grapefruit*. However, in most cases this is not true for T1 Ox, which is mostly not significantly lower than the Control samples. T1 Ox was actually rated significantly higher than T1-T5 Control for the attributes *fresh green*, *passion fruit* and *grapefruit*. As seen in the PCA biplot (Figure 4.25), there was not much difference between the attributes for T1-T5 Control. T5 Control did in some cases show significant difference and would explain the separation observed in the PCA biplot.

The only attribute which seems to show some significant tendency or pattern in the Control samples is *cooked green*. Although this is not clear from the PCA biplot, the *cooked green* attribute increased in intensity from T0 Control to T5 Control with mostly significantly lower intensities in the Ox samples. The compound responsible for the perception of this attribute is not exactly known, however the development of 'reductive' odours which are often described to impart "cooked vegetables", "onions" and "cabbage" aromas are caused by sulphur-containing compounds such as H_2S, mercaptans, disulphides and dimethyl sulphides which occur due to low oxygen content in a wine (Rauhut, 1993; Brajkovich *et al.*, 2005). These compounds have low perception thresholds and are known to negatively influence young white wines, imparting unpleasant odours (Rauhut, 1993; Godden *et al.*, 2001).

As the Control samples did not receive any oxygen at the beginning of the study (maintaining low oxygen content), it is very likely that these compounds developed in the wine. Interestingly, even though T1 Ox seems to be better associated with the Control samples than the Ox samples, it scored significantly lower in the *cooked green* attribute when compared to T2-T5 Control. The prevention of mercaptan formation by the addition of oxygen at the beginning of the study could explain this observation, however such speculation certainly needs to be tested by appropriate experiments designed to investigate this hypothesis.

Fresh green also varied between the treatments. The persistence of IBMP concentrations in the samples would indicate that this attribute would remain at higher intensities throughout the treatment, however this is not the case. As mentioned previously, the "green" attributes in wines have sometimes shown a weak correlation to the methoxypyrazine concentrations, which could be the case in this study. The suppressive effect of other compounds (such as the 'reductive' odours or aldehydes in the oxidative treatments) on the *fresh green* attribute should also not be underestimated. *Banana*, *honey*, *yellow apple* and *dried fruit* were used more often for the first oxidation stages, while *syrup*, *raisins*, *potato bag*, *green apple* and *sherry* were used more often for the last oxidation stages. Attributes such as *green apple* and *sherry* became quite potent and

overpowering at the end of the aging period as the fresh and fruity attributes (with the exception of *yellow apple*) were in all cases scored below 5 intensity units for the last two sampling periods.

4.3.3.2 Sensory analysis according to wine colour

In wines, phenolic compounds undergo non-enzymatic oxidation in the presence of oxygen, yielding polymerized polyphenols that finally precipitate in the form of brown pigments (Singleton, 1987). In this study the wines were subjected to visual analysis and ranked from "least oxidised" to "most oxidised" according to their colour only (Figure 4.27).

Similar to the PCA, cluster analysis (CA) is used to classify objects into groups characterized by the values of a set of variables (in this case the observation of the colour of the wine). CA is therefore an alternative to PCA for examining classificatory structure of data (Massart *et al.*, 1997) and allows obtaining groups based on their similarities (Camiña *et al.*, 2008). Figure 4.28 shows the dendogram obtained from the samples subjected to the evaluation of their colour. The Ward's method which calculates the distances between objects of a cluster was used as amalgamation criterion, while Euclidean distance was used as association criterion.

Two main groups separated the Control samples from the Ox samples. It is thus evident that there were clear differences between these two groups which the panellist could easily distinguish. On the x-axis the samples are thus arranged from "most oxidised" (left) to "least oxidised" (right) according to their colour. The next grouping separates T1 and T2 Ox from T3, T4 and T5 Ox. T1 and T2 Ox were thus classified as being "less oxidised" when compared to the other three Ox samples. T1 Control seems to be somewhat separated from T0, T2, T3, T4 and T5 Control. It is difficult to explain this observation.

In order to see individual ranking according to the colour of the wine, the data was subjected to further analysis. Figure 4.29 shows the ranking position of each treatment.

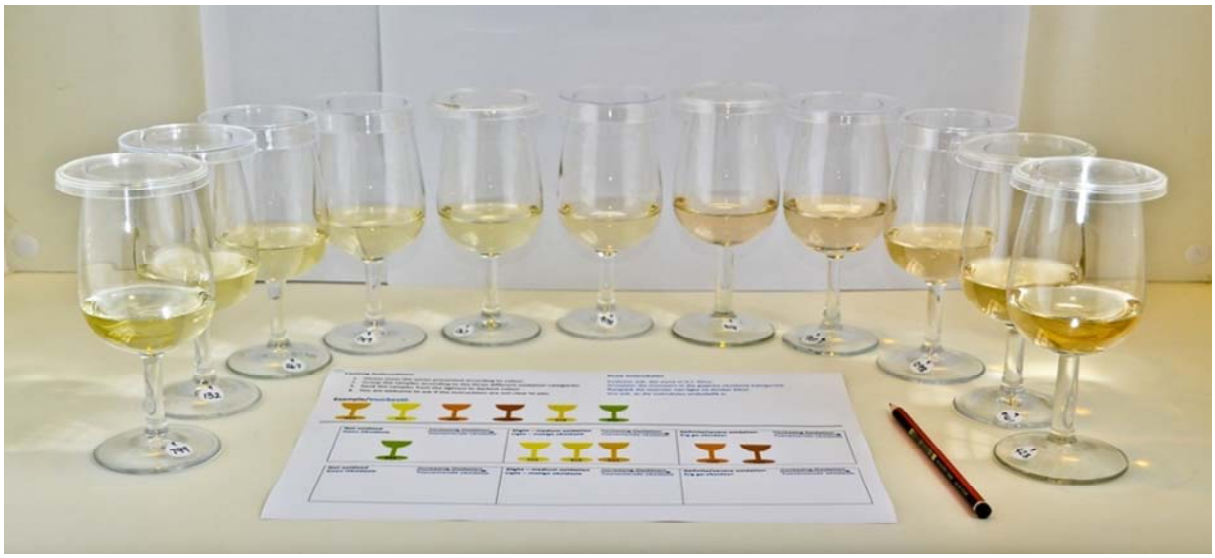


Figure 4.27 Sample set arranged from least oxidised to most oxidised according to colour only.

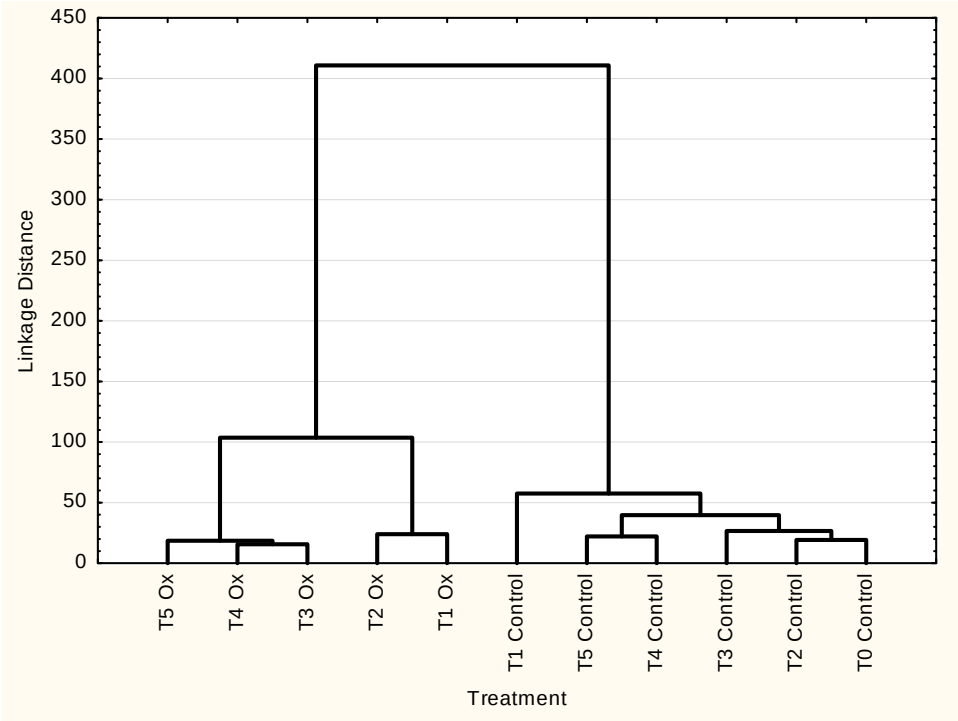


Figure 4.28 Dendogram of wine samples obtained by cluster analysis.

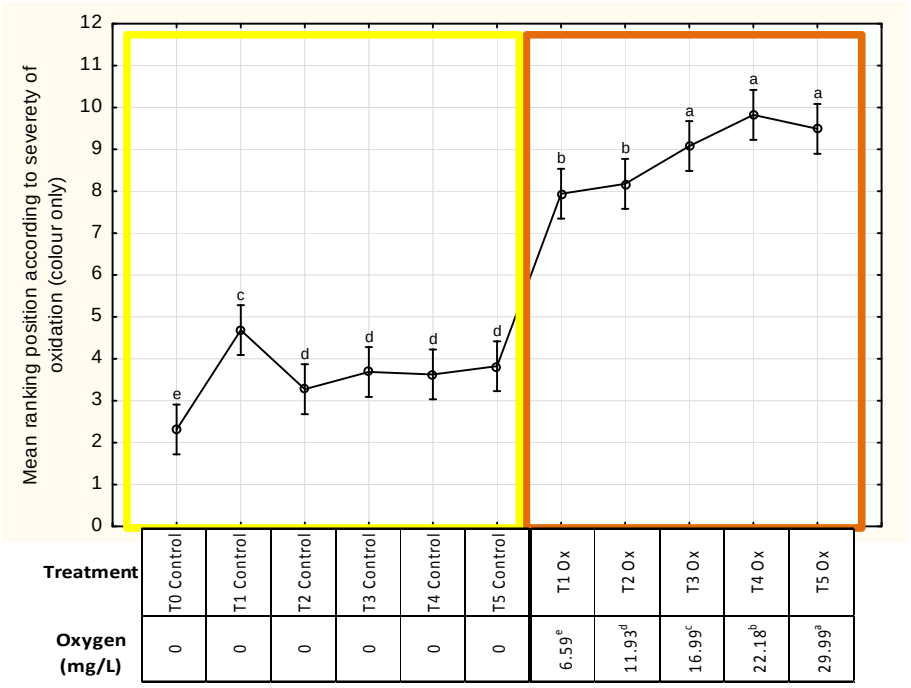


Figure 4.29 Average ranking position of wines subjected to sensory evaluation according to colour only. Panellists were asked to rank the wines from least oxidised (0) to most oxidised (11).

T0 Control was ranked as the "least oxidised" sample followed by T2, T3, T4 and T5 Control which did not differ significantly from each other. As mentioned previously, T1 Control was ranked somewhat more oxidised compared to the other Control samples. The mean ranking of the wines increased significantly when comparing the Control samples to the Ox counterparts. This confirms the ability of the panel to easily distinguish between these two groups. Of the Ox samples, T1 Ox and T2 Ox were classified as being least oxidised while there were no significant difference between T3, T4 and T5 Ox. As seen previously, T1 Ox was described as more fresh and fruity than most of the Control samples when considering the descriptive analysis applied to the samples.

The visual analysis did not coincide with the results from the olfactometric analysis and the T1 Ox sample was situated to the "oxidised" side of the spectrum. Thus, according to the visual analysis, the wine looked oxidised, however analysing the wines aromatically proved otherwise. This is contradictory to previous studies which report a decrease in certain aroma compounds during oxidation first which is then followed by a change in wine colour (Singleton & Kramling, 1976; Singleton *et al.*, 1979; Escudero *et al.*, 2000b). In the current study, the development of an oxidised colour preceded the disappearance of the pleasant aroma and could indicate the sequence of these occurances to not take place as described previously. The results from this study would suggest a change of colour after which a decrease in pleasant aroma occurs with an increase in oxidation

aroma after that. Differences in the chemical composition between wines could also perhaps account for these discrepancies.

4.3.4 Chemical and sensory data combined

Combining the data obtained from the chemical analaysis with the data from the sensory evaluation could reveal other findings not observed when looking at the data sets individually. It is also of interest to assess whether there may be predictive ability of the chemical data for the degree of "fresh and fruity" or "oxidative" character of the wine.

A partial least square regression (PLS) analysis was performed to explore the relationships between the different concentrations of chemical compounds (placed in the X-space) and the sensory descriptors (placed in the Y-space). The PLS constructed explained 55% of the variance of X and 70% of the variance in Y for dimensions 1 and 2 (Figure 4.30). There was a strong relationship between many of the chemical measurements and attributes. The attributes follow a similar pattern to the loadings when a PCA was performed using the sensory data alone (Figure 4.25).

Two groups appear on opposite sides of the PLS, showing a strong negative association. On the left, attributes *syrup*, *raisins*, *sherry*, *green apple* and *potato bag* correspond strongly and on the right, attributes *pineapple*, *grapefruit*, *passion fruit*, *guava*, *fresh green* and *lemon* correlated well. Descriptors *dried fruit*, *yellow apple and honey* had a weak correlation to the oxidation-related attributes (on the left). *Banana* correlated weakly with the fresh and fruity attributes located to the right of the PLS. *Cooked green* associated strongly with the latter group. Most of the scores were located between the two ellipses explaining between 50 and 100% of the variance.

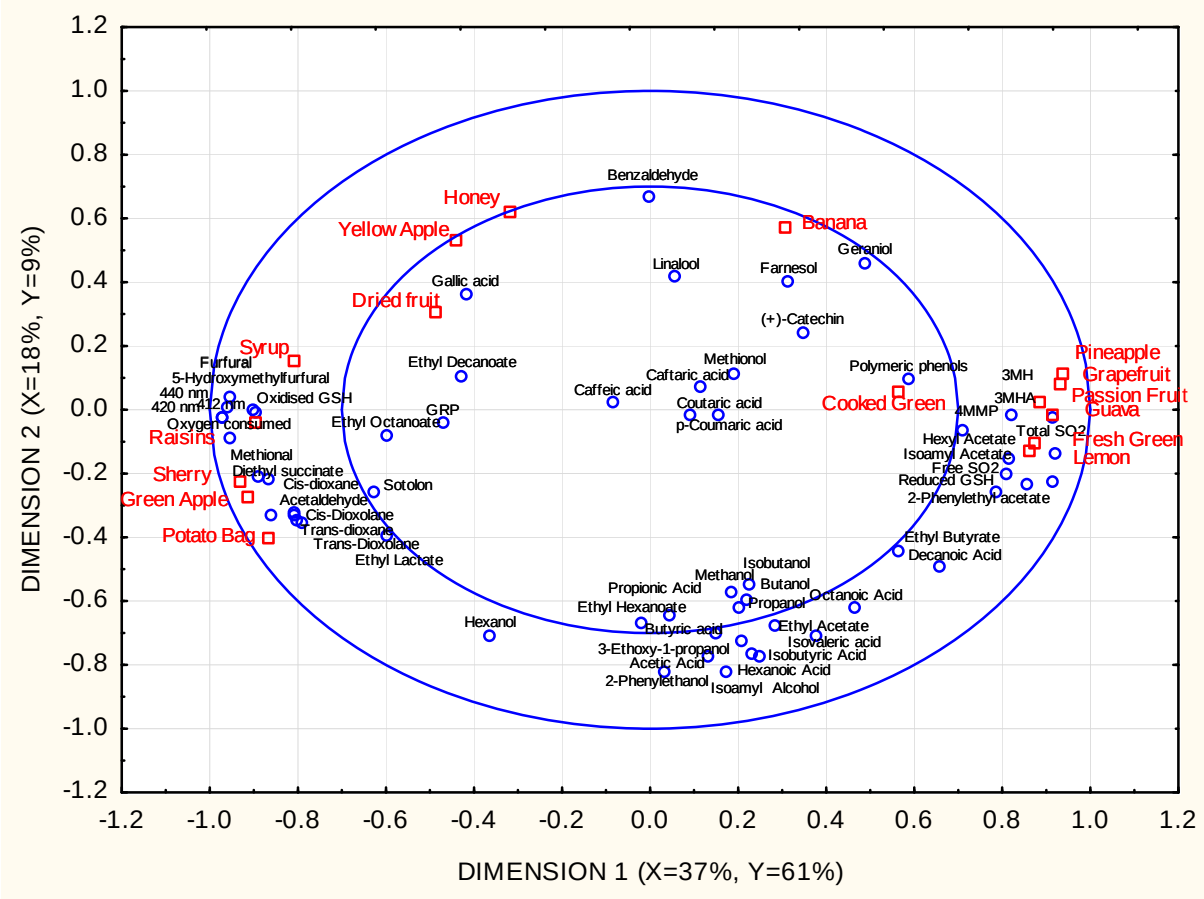


Figure 4.30 PLS plot of component 1 and component 2 containing chemical information in the X-space and the sensory descriptive data in the Y-space.

Three groupings of chemical compounds can be observed. On the left, compounds such as furfural, 5-HMF, oxidised GSH, methional, acetals, acetaldehyde and sotolon associated strongly with each other and the oxidation-related sensory attributes. On the right, correlating strongly with the fruity and fresh attributes, the compounds 3MH, 3MHA, 4MMP, hexyl acetate, isoamylacetate, 2-phenylethyl acetate and reduced GSH are grouped. The volatile thiols and a few acetate esters could be the factors driving the fresh and fruity flavours found in the wine. As the concentrations of these compounds decreased, the associated attributes also decreased. As seen previously, compounds grouping at the bottom centre of the PLS consisted of various acids including hexanoic acid and octanoic acid as well as various alcohols such as isoamyl alcohol and 2-phenylethanol. These compounds did not correlate to either of the sensory groups formed. However, the group in general was more closely located to the fresh and fruity side (right) of the PLS compared to the oxidative side (left) and could thus contribute to the overall fresh, fruity wine aroma without contributing to specific aroma attributes identified in this study.

Interestingly, various polyphenolic compounds including *trans*-caftaric acid, *p*-coutaric acid and (+)-catechin were located in the centre of the PLS and was not significantly influenced by the treatment. This phenomenon was also seen in a study done by Fracassetti *et al.* (2013). The monoterpenes (linalool, farnesol and geraniol) were also located inside the inner ellipses contributing to less than 50% of the variance.

Table 4.5 provides a summary of the correlation coefficients, with consumed oxygen concentration, free and total SO_2, A_{420} and reduced GSH being correlated with the sensory scores for all the aroma attributes. For consumed oxygen and A_{420}, positive correlations were observed with the attributes associated with aging and oxidation, while positive correlations were seen between the fresh and fruity attributes and free and total SO_2 as well as reduced GSH. The strongest correlations were seen for A_{420}, consumed oxygen and free SO_2 and would indicate a good ability to identify the development of oxidation aromas and a loss in fruity characters. Godden *et al*. (2001) also found good correlations between some of these parameters and sensory characteristics of white wines.

Table 4.5 Pearson correlation coefficients among the sensory variables with consumed oxygen, free and total SO_2, reduced GSH and A_{420}.

	Consumed O_2		Free SO_2		Total SO_2		Reduced GSH		A_{420}	
Fresh Green	-0.7995	***	0.8241	***	0.8182	***	0.8215	***	-0.8469	***
Lemon	-0.8222	***	0.8909	***	0.8555	***	0.8255	***	-0.8618	***
Passion Fruit	-0.8349	***	0.8211	***	0.8286	***	0.7922	***	-0.8743	***
Guava	-0.8997	***	0.8835	***	0.8963	***	0.7922	***	-0.9275	***
Pineapple	-0.9182	***	0.8422	***	0.8657	***	0.7613	***	-0.9396	***
Grapefruit	-0.9376	***	0.8443	***	0.8883	***	0.7241	***	-0.9476	***
Cooked Green	-0.7220	***	0.5874	***	0.7008	***	0.2722	ns	-0.6738	***
Banana	-0.2923	ns	0.0147	ns	0.0893	ns	0.0551	ns	-0.2405	ns
Honey	0.3398	ns	-0.5299	**	-0.5128	**	-0.3885	*	0.4193	*
Dried Fruit	0.4362	*	-0.6091	***	-0.5599	***	-0.6153	***	0.5089	**
Yellow Apple	0.4404	*	-0.6319	***	-0.5898	***	-0.5336	***	0.5144	**
Syrup	0.8000	***	-0.8235	***	-0.8350	***	-0.7291	***	0.8428	***
Raisins	0.9056	***	-0.8603	***	-0.8917	***	-0.7303	***	0.9301	***
Potato Bag	0.8739	***	-0.6978	***	-0.7219	***	-0.5922	***	0.8403	***
Green Apple	0.9519	***	-0.7677	***	-0.8357	***	-0.6153	***	0.9301	***
Sherry	0.9640	***	-0.8004	***	-0.8648	***	-0.6541	***	0.9485	***

ns = not significant at P=0.05; * = significant at P < 0.05; ** = significant at P<0.01; * = significant at P < 0.001**

The addition of low amounts of oxygen to the wine could indeed improve the wine sensory quality. An improved expression of wine fruity attributes was also observed when Sauvignon blanc wine was exposed to low (< 3mg/L) or moderate (about 6 mg/L) oxygen exposure in conjunction with a decrease in 'reductive' attributes (Lopes *et al.*, 2009). In the current study, the occurrence of *cooked green* could be an indication of the development of 'reductive' odours in the Control samples. As seen in the sensory results, T1 Ox was considered to be more fresh and fruity when compared to T1 Control. As T1 Ox associated better with the Control samples when looking at the chemical data, the question remains how does this sample score fresher and fruitier than the Control samples as seen in the sensory evaluation? The similar chemical composition would suggest that the wine be sensorially more similar to the other Control samples, however the addition of moderate amounts of oxygen to this wine could inhibit the formation of 'reductive' off-odours. This also raises the question of the ability of these 'reductive' odours to mask and suppress fresh and fruity odours considering the similar chemical composition otherwise. Follow-up studies should investigate the formation of the 'reductive' odours and what specific oxygen concentrations to administer to benefit the wine quality. Interactive effects between the 'reductive' sulphur-containing odours and other fresh associated odours such as the volatile thiols and the methoxypyrazines need to be investigated.

The role of exact oxygen measurements, free and total SO_2 as well as A_{420} measurements as a predictive tool was also investigated. Importantly, the study has allowed the compositional measurements of sulphur dioxide, degree of browning and oxygen to be directly related to the sensory properties of the Sauvignon blanc wine and gives reasonable ability for predicting the degree of "fruitiness" or "oxidation" during storage of white wines. The measurement procedures for these parameters are fairly simple providing the necessary equipment is available. Furthermore, the strong correlations observed between especially free SO_2 and total SO_2 (r=0.9651) and free SO_2 and A_{420} (r=-0.9234) indicate that only the measurement of free SO_2, a relatively simple test that is commonly performed by many wineries, needs to be performed in a commercial situation.

A certain "cut-off" free SO_2 concentration for fruity style white wines has been contemplated in a previous study (Godden *et al.*, 2001). The current study confirms the detrimental sensory effect as soon as a wine reach levels below 10 mg/L free SO_2 at which point insufficient SO_2 is available to bind newly-formed oxidised compounds, allowing the development of unpleasant wine flavour and aroma. This seems to be applicable to a certain wine style: fruity, no oak contact, relatively low pH, handled relatively anaerobically and also bottled with approximately 30 mg/L free SO_2. It would seem that the periodic measurement of SO_2 together with consumed oxygen and A_{420} could be used by wine companies, distributors and retailers to identify wines that are approaching a critical level. It

is also possible that by increasing the concentration of SO_2 in wines at bottling, winemakers could compensate for the losses over time identified in this study. This could potentially increase longevity of the bottled wines, however increases in SO_2 concentrations in wines are highly undesirable as the US FDA estimates that as many as 1% of the general population show an increased degree of sensitivity to sulphites (Papazian, 1996).

Considering the concentration of various fruit-driven aromatic compounds to be higher than the perception threshold (Table 4.3) even in the later oxidative stages it is evident that some aromatic compounds could have powerful odourous influences causing complete masking or suppressing effects leading to significant decrease in the perception of the fruity attributes. So, not only is the loss in fruity characters likely to be caused by oxidative damage to flavour compounds, but also by interactive effects of especially aldehydes being formed. On the other hand, some of the unpleasant oxidation-related compounds that developed remained below the perception threshold of that compound (such as sotolon). The effect of these compounds even when present at very low concentrations should also be investigated as enhancing effects could take place contributing to the overall oxidation flavour.

In this study, the first administration of oxygen resulted in a 6.59 mg/L dissolved oxygen concentration which was all consumed before the second oxygen dose was administered. This concentration in white wines terms would be considered to be quite high. Even being at the higher range of dissolved oxygen for white wines, the treatment still scored higher in fresh and fruity attributes when compared to samples where no oxygen was added. It would however be interesting to investigate the effect of smaller dosages of oxygen. This would give a better idea of at what exact concentration the intensity of the fruity attributes will reach its peak. The proposed study would probably result in the wines being very similar in sensorial perceptions, however the chemical content should again be monitored to identify the oxygen dose where the best sensory perceptions are matched with the ideal chemical situation. After the second oxygen dose (T2 Ox; 11.98 mg/L consumed oxygen) the fresh and fruity attribute intensities dropped significantly, however the wines will probably only be classified as an extreme case of oxidation between T3 and T4 Ox (16.99 and 22.18 mg/L respectively). This suggests that even though the wine quality decreased, the wine was able to consume more oxygen than expected without developing the overpowering oxidation character. Only in a very few studies has the actual degree of oxygen exposure been precisely determined or at least estimated (Godden *et al.*, 2001; Brajkovich *et al.*, 2005; Lopes *et al.*, 2009; Caillé *et al.*, 2010; Nygaard *et al.*, 2010), however in some of these studies the oxygen measurements fluctuated causing substantial variations between repeats.

Considering the amount of days aged, the Control samples seem to have developed the 'reductive' odour within 2 months of aging. There was no significant difference between the sensory profiles of the T1-T4 Control samples which would indicate the aging of 64 days vs. 204 days had little effect, however the role of the 'reductive' odours masking and suppressing abilities could play an important role and should be investigated before making any further conclusions regarding the aging period of the Control samples. Another topic worth investigating would be the effect of further aging on the T1 Ox sample. The aging process was effectively stopped by freezing the sample after 64 days of aging. However, would the sensory perception of the wine remain as desirable for a longer aging period and if so, for what length of time? In other words, the aging capacity of this type of wine (where oxygen exposure took place) should be investigated.

4.4 CONCLUSION

The results in this study agree with results reported in other studies. Herbst *et al*. (2011) also reported the decrease in the volatile thiols during aging as well as the initial increase in 3MH concentrations, while Silva Ferreira *et al.* (2002) reported the increase in oxidation compounds in white wines. In the latter publication, the results reported agree with the present study in that methional was formed during the aging period, however substantial phenylacetaldehyde and sotolon formation was also reported by Silva Ferreira *et al.* (2002) which was not observed in the present study. Aging conditions such as higher aging temperature could account for this observation. Results reported by Godden *et al.* (2001) coincide with the present study, especially considering the SO_2 levels and formation of yellow/brown colour. The sensory data reported in the present study is supported by previous publications (Godden *et al.*, 2001; Lopes *et al.*, 2009), however this is the first study where a comprehensive overview of both chemical (aromatic and non-aromatic) as well as sensory data has been obtained during repetitive oxidation of the wines. This provides insight into the advantages as well as the disadvantages of various levels of oxygen exposure as well as the capacity of a wine to consume oxygen.

It is still rather difficult to determine the ideal amount of oxygen that can be beneficial for a wine during bottle aging, especially considering the wide range of varieties and wine styles. Review articles have attempted to shed some light on the overall effect of oxygen on the composition of wines during aging (Coetzee & Du Toit, 2012; Ugliano, 2013), however the chemical mechanisms involved in the formation of many key aging compounds remain to be established and further research is needed to understand the various mechanisms. The results in this study will add to the data base of wine research regarding oxygen and could serve as an indication to the amount of

oxygen that could be beneficial for especially fruit-driven Sauvignon blanc wines. It can also give guidance as to whether enough oxygen exposure has taken place to adversely affect the wine at a later stage of storage. This information would be most valuable, not only for wineries but also for packaging developers.

Given the diversity that occur between individual wines, the great challenge that remains would be the ability to predict the tendency of a wine to lose desirable aroma compounds and develop oxidative odours and, in general, to define how much oxygen is needed to achieve the perfect balance in order to obtain a wine at its best potential and most balanced in terms of aromatic composition. The impact of oxygen on wine is context-specific due to factors such as must and wine composition, additives and timing of oxygen exposure which can all influence the outcome. The addition of oxygen to white wine in commercial wineries is often kept to a minimum, which should be re-assessed. Little data exists regarding an oxygen specification level that white wines require. However, such specifications would probably differ from wine to wine. Wine producers should thus conduct their own trials to confirm the influence of oxygen on their wines whenever possible.

Oxygen management in wine is generating considerable interest especially regarding topics such as methods to improve the wine quality, improving the winemaker's control and the formation of credible solutions on how to manage the oxygen during winemaking (Nygaard, 2010).

Through integration of flavour chemistry and sensory research such as the current study, wine producers are given guides that enable them to tailor make wines according to the target market. A better understanding of potential sensorial improvements based on oxygen management could lead to the development and implementation of novel winemaking practices.

4.5 ABBREVIATIONS USED

3-mercaptohexan-1-ol (3MH); 3-mercaptohexan-1-ol acetate (3MHA); 4-mercapto-4-methylpentan-2-one (4MMP); 3-isobutyl-2-methoxypyrazine (IBMP); 3-isopropyl-2-methoxypyrazine (IPMP); 3-*sec*-butyl-2-methoxypyrazine (SBMP); light-emitting diode (LED); p-hydroxymercurybenzoate (p-HMB); butylated hydroxyanisole (BHA); selective ion monitoring mode (SIM); limit of quantification (LOQ); limit of detection (LOD); gas chromatography-flame ionization detector (GC-FID); gas chromatograph (GC); solid phase extraction (SPE); gas chromatography–olfactometry (GC-O); ultra performance liquid chromatography-tandem mass spectrometric (UPLC-MS/MS); mass spectrometer (MS); glutathione (GSH); oxidised glutathione (GSSG); grape reaction product (GRP); multiple reaction monitoring mode (MRM); high performance liquid chromatography (HPLC); reverse phase-high performance liquid chromatography (RP-HPLC); aldehyde dehydrogenase (Al-DH); nicotinamide-

adenine dinucleotide (NAD); ultraviolet (UV); analysis of variance (ANOVA); partial least square regression (PLS); cluster analysis (CA); principle component analysis (PCA).

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Chapter 5

Research results

Acetaldehyde and its masking/enhancing abilities –

A sensory study in model wine

Chapter 5: Research results

Acetaldehyde and its masking/enhancing abilities – A sensory study in model wine

5.1 INTRODUCTION

Acetaldehyde is one of the most important carbonyl compounds in wine and constitutes up to 90 % of the total amount of aldehydes found in wine (Nykänen, 1986). The possible negative effect acetaldehyde can have on the organoleptic quality of wine and its ability to combine rapidly with sulphur dioxide (SO_2) makes the accumulation of this compound critical during winemaking (Burroughs & Sparks, 1973). Acetaldehyde is formed by the yeast during alcoholic fermentation (Margalit, 1997) and can also originate from the activity of other microbes such as lactic acid bacteria and acetic acid bacteria (Drysdale & Fleet, 1988; Liu & Pilone, 2000). The amount of acetaldehyde formed by the yeast will depend on many factors such as yeast strain, temperature, pH, sugar content, oxygen and SO_2 (Ough & Amerine, 1958; Rankine & Pocock, 1969; Herraiz *et al.*, 1989; Romano *et al.*, 1994; Cabranes *et al.*, 1998; Garde-Cerdán *et al.*, 2008). Other winemaking processes can also have a significant effect on acetaldehyde concentrations and moderate to important acetaldehyde increases usually occur post-fermentation (Jackowetz & De Orduña, 2013) of which the most important production is due to the oxidation of ethanol (Wilderandt & Singleton, 1974; Ribéreau-Gayon, 1998, 2004b).

The oxidation of ethanol is not direct, but rather via coupled auto-oxidation of certain phenolic compounds (Wilderandt & Singleton, 1974). Free SO_2 present in the wine will slow this oxidation by reacting with intermediate oxidation products as well as with acetaldehyde, thereby forming an odourless sulphite combination with acetaldehyde (hydroxysulphonate) which is stable in acid medium. It has been estimated that only 0.04% of acetaldehyde is in the free form in the presence of 30 mg/L free SO_2 (Blouin, 1965). Free acetaldehyde concentration will thus increase as soon as all the free SO_2 has been converted to the bound form. The free acetaldehyde can have a significant influence on the aroma and has been reported to contribute odours such as “green apple”, “overripe bruised apple”, “grassy”, “pungent”, “nutty” and “sherry” (Margalith, 1981; Henschke & Jiranek, 1993; Miyake & Shibamoto, 1993; Frivik & Ebeler, 2003).

The perception threshold of free acetaldehyde in 10% ethanol solution has been reported to be 0.5 mg/L (Guth, 1997), which can make this compound a potent contributor to the oxidation character in wine. In wines, this perception threshold is considerably higher (100-125 mg/L) (Zoecklein *et al.*, 1995), however the interactive effects between aromatic (and non-aromatic) compounds could influence this determination and might not be applicable to all wine types and styles. A survey done on 127 white wines reported the total acetaldehyde concentration (the sum of free and bound) to range between 7 and 240 mg/L averaging at 40 mg/L (Lopes *et al.*, 2009; Jackowetz & De Orduña, 2013). Other sources reported average levels in white wine to be around 80 mg/L (McCloskey &

Mahaney, 1981). Fortified wines had the highest acetaldehyde content (average 118 mg/L, range 12-800 mg/L) (Lachenmeier & Sohnuis, 2008). While high levels of acetaldehyde are generally undesirable in table wines, it is considered to be a unique feature of sherry-type wines (Sponholz, 1993; Cortes *et al.*, 1998).

Sauvignon blanc wines present a large diversity of wine styles and aroma. On the one hand, the volatile thiols (4-mercapto-4-methylpentan-2-one (4MMP), 3-mercaptohexan-1-ol (3MH) and 3-mercaptohexan-1-ol acetate (3MHA)) can lead to "tropical/fruity" style wines, while the methoxypyrazines (3-isobutyl-2-methoxypyrazine (IBMP), 3-isopropyl-2-methoxypyrazine (IPMP) and 3-*sec*-butyl-2-methoxypyrazine (SBMP)) can contribute to the "green pepper", "grassy" and "asparagus" odour (Lacey *et al.*, 1991; Tominaga *et al.*, 1998; Swiegers *et al.*, 2006; Coetzee & Du Toit, 2012). Both of these odour groups are often sought after for the production of quality Sauvignon blanc wines (Lund *et al.*, 2009b; King *et al.*, 2011).

The volatile thiols are oxidation sensitive and can also undergo acid hydrolysis which leads to a decrease in these compounds during aging or oxidation (Tominaga *et al.*, 2004; Herbst *et al.*, 2008; Nikolantonaki *et al.*, 2010). Correspondingly, a loss in the fruity odour of the wine will occur. The grape derived methoxypyrazines are not sensitive to oxidation and studies have shown methoxypyrazine concentrations to remain stable during oxidative handling of juice and wine (Marais, 1998; Coetzee *et al.*, 2013). Even though the concentrations of especially the volatile thiols decrease during aging, concentrations at the end of an oxidative aging period of eight months still proved to be well above the odour perception threshold (Chapter 4). The methoxypyrazine concentration remained the same (above the odour perception threshold) during the aging period. However, the odour of the wine was dominated by attributes such as *potato bag*, *green apple* and *sherry* at the end of the aging period. These sensory attributes are likely due to the formation of methional (*potato bag*) and acetaldehyde (*green apple* and *sherry*).

Interactive effects of aroma compounds (suppression or enhancement) have been reported before (Chapter 3) (Ribéreau-Gayon *et al.*, 1975; Ferreira *et al.*, 2002; Campo *et al.*, 2005; Escudero *et al.*, 2007; Pineau *et al.*, 2007; King *et al.*, 2011; Van Wyngaard, 2013) however, the effect of acetaldehyde has not been elucidated. In some studies the contribution of acetaldehyde to the oxidative aroma of wines oxidised during a short period of time has been reported as negligible and attributed the oxidised aroma to the formation of other compounds (Escudero *et al.*, 2002). However, in general, it is still believed that acetaldehyde is one of the main compounds causing the typical oxidative aroma especially in wines stored for a longer period of time (Baro & Quiros Carrasco, 1977; Ribéreau-Gayon *et al.*, 1977; Usseglio-Tomasset, 1985; Meirland & Pernot, 1992). Methional has proven to suppress the attributes that arise due to the presence of 3MH (Chapter 3)

and could possibly enhance the *cooked beans and cooked potato* odour when alone or in combination with IBMP. The goals of the research presented in this chapter are to define the aroma attributes caused by acetaldehyde at varying concentrations and to determine the role of acetaldehyde in the perception of pleasant aromas brought by typical Sauvignon blanc compounds such as 3MH and IBMP.

5.2. MATERIALS AND METHODS

5.2.1 Medium

Model wine consisted of distilled water, 5 g/L tartaric acid and 12% ethanol and pH adjusted to 3.5 (using sodium hydroxide). After preparing the model wine, the composition was confirmed using WineScan FT 120 instrument (FOSS Analytical, Denmark).

5.2.2 Chemicals and spiking

The compounds used in this study were 3MH, IBMP and acetaldehyde. Solutions of 733.34 mg/L 3MH (Interchim) and 250 mg/L IBMP (Sigma) were prepared in 99.5% ethanol (Merck Chemicals, South Africa). The exact concentration of 3MH was also confirmed by Ellman's reagent (Ellman, 1959). Acetaldehyde (Sigma) solutions were made fresh daily from the undiluted compound. The 3MH and IBMP solutions were stored in the dark at -80˚C, while acetaldehyde was stored at 4˚C. These solutions were used to spike the model wine to the desired concentrations one hour prior to tasting.

5.2.3 Experimental design

Initially each compound was spiked individually in order to assess the effect of concentration changes on the aromatic perception. The concentrations used throughout this study were kept constant and can be seen in Table 5.1. This was done to compare profiles of both trials (singular additions and multiple additions). Samples are numbered from 1 to 5 according to the level of each of the compounds. Using the level as the indicator of the concentration simplifies the interpretation of the various figures in this study. For specific concentrations of the compounds refer to Table 5.1.

Table 5.1 Concentrations tested

Compound	1	2	3	4	5
3-Mercaptohexan-1-ol (ng/L)	40.0	60.0	500.0	2000.0	6000.0
3-Isobutyl-2-methoxypyrazine (ng/L)	1.0	2.0	10.0	20.0	40.0
Acetaldehyde (mg/L)	0.5	30	60	100	200

A central composite design was used for the sensory analysis of the samples containing multiple compounds (to assess interactions). The suitability of the central composite design lies in the fact that it can be used to test interactions between different factors. Ideally a factorial design could be used to test all combinations, however this design would result in a very large number of combinations to be assessed. The central composite design only selects a certain amount of combinations specifically chosen by the design. The use of this method led to a design with six star points, eight cube points and one centre point which resulted in 15 samples in total to be tested (Table 5.2).

Table 5.2 Compositions (concentration levels) of various samples in the central composite design.

	Compound		
Sample	**3-Mercaptohexan-1-ol**	**3-Isobutyl-2-methoxypyrazine**	**Acetaldehyde**
Centre	3	3	3
Cube 1	2	2	2
Cube 2	2	2	4
Cube 3	2	4	2
Cube 4	2	4	4
Cube 5	4	2	2
Cube 6	4	2	4
Cube 7	4	4	2
Cube 8	4	4	4
Star 1	1	3	3
Star 2	5	3	3
Star 3	3	1	3
Star 4	3	5	3
Star 5	3	3	1
Star 6	3	3	5

5.2.4 Sensory analysis

The sensory panel consisted of 12 judges. Intensity rating was done using a 100 mm unstructured line scale (ranking intensity from “none” to “intense”). Testing was done in booths using standard ISO wine tasting glasses. The booths had standard artificial daylight lighting and temperature control at 20±2˚C. Sample glasses were marked with a random three-digit code (unique for each judge) and glasses were covered with a plastic lid prior to sensory assessment to prevent the aroma contaminating the laboratory environment. The order of the samples was random and balanced across the assessors. Along with the set of spiked samples, a blank glass containing the unspiked model wine only, was also provided for comparison. Panellists evaluated the samples orthonasally only and the data was collected on a paper ballot. For reasons of human ethics, a brief explanation of the addition of flavour to the samples was given prior to testing, although care was taken to exclude any information that could have caused bias.

Compounds were first analysed individually. Six training sessions (one hour each) were conducted in an attempt to reach consensus regarding different samples and terminology used. In this way, the panellists were exposed to each compound twice. At each training session, the panel received all five levels of a compound as well as a blank sample consisting only of model wine. A range of reference standards was also given. During the first discussion session, the panel generated attributes and a brief line scaling exercise was done. During the second training session the descriptors and the sample’s position on the line scale was finalised. Each individual compound was analysed during a formal test session, which contained 3 replicates.

The profiling of the samples containing multiple compounds was done after the evaluation of the singular compounds. At this stage the judges were already familiar with the compounds. During profiling, the samples were divided into two subsets as all samples could not be analysed in one session. The first subset was star points 1-6 with the second subset containing cube points 1-8. The centre sample always accompanied the sets during training and testing. The experimental layout and the combinations of compounds for each sample and subset can be seen in Table 5.2. Reference standards were available for the panellists during the trial.

Five training sessions consisting of 1 hour each were used per subset (5 hours per subset). During training, the panellists were not informed of the composition of each sample. Samples were scaled on a 100 mm unstructured line scale using the attributes generated during the profiling of the singular compounds. Analyses were performed as 2 different tests (each subset separately) with 3 replicates each. With the set of spiked samples (along with the centre sample), a blank glass

containing model wine only, was provided for comparison. Star points were tested first, followed by the cube points. The judges took regular breaks between samples to prevent fatigue.

5.2.5 Data Analysis

Assessor performance was evaluated using PanelCheck (Version V1.4.0, Nofima, Tromsø, Norway) according to the workflow as described by Tomic *et al.* (2010). Sensory descriptive data for each attribute was analysed using mixed model analysis of variance (ANOVA). Principle component analysis (PCA) biplots were created using the correlation matrix of the mean data. Post-hoc Fisher's LSD tests were used to test for significance of sensorial differences between compound combinations. In a custom built perl program, t-tests were used to test for the significance of the sensorial differences between compound combinations, and the significant statistical relationships were modelled as a network using Cytoscape 3.0.1. (Shannon *et al.*, 2003) in which coloured nodes were compound levels that yielded a statistically significant difference in a sensory attribute as compared to the control levels that were indicated by an uncoloured node located at the centre of the network. Response surface plots were constructed (Statistica 10, Statsoft Inc., Tulsa, USA) to investigate interactions. A p value threshold of 0.05 was used for the determination of statistical significance ($p<0.05$).

5.3 RESULTS AND DISCUSSION

5.3.1 Panel evaluation

3-Way ANOVA was used to determine significant attributes and Tucker plots were used to assess the consensus amongst judges (Figure 5.1) and the grouping indicated that the panellists were well trained and homogeneous in terms of the wine profiling. For most of the attributes this grouping was situated between the inner and outer ellipses. This shows good panel consensus with 50-100% of the variance explained by the judges.

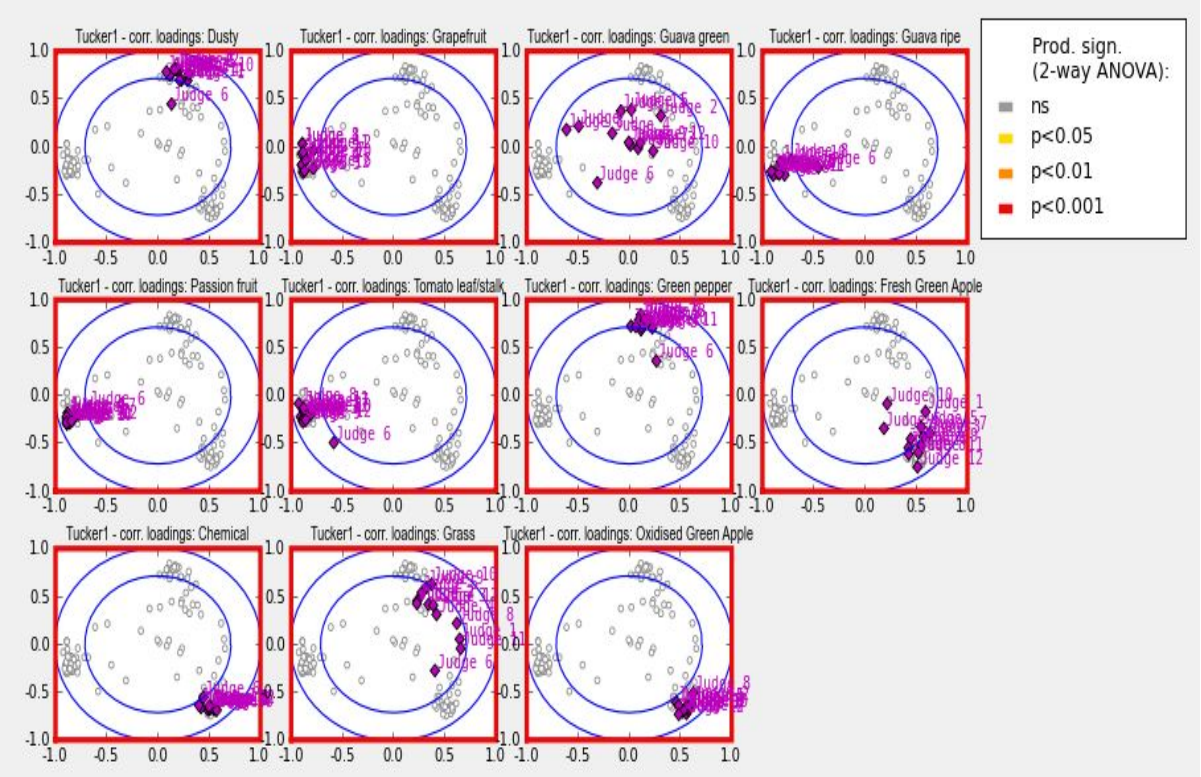

Figure 5.1 Tucker plot of panellist performance**.**

5.3.2 Individual compound evaluation

Model wine was the preferred choice of spiking medium as the complexity of real wine could interfere with the interaction study. Even a neutral or dearomatized wine has sensory properties due to certain aromatic compounds that remain in the wine. For the purpose of this study, the absence of any other aroma compounds or non-aromatic compounds (that could influence the perception of the aroma) was critical (Lund *et al.*, 2009a).

The concentrations tested (Table 5.1) were chosen based on concentrations of the aroma compounds found in white wines as reported in literature, as well as a preliminary sensory assessment of the individual aroma compounds and mixtures of the compounds. Pre-screenings by experienced wine tasters (all who completed a certificate course in wine evaluation) were done before finalising the concentrations to ensure that the levels chosen fulfil the requirements for the aim of this study. The concentrations for 3MH and IBMP in this study were chosen as follows: the first level was chosen to be below the perception threshold, as this would give an indication of enhancing or suppressive effects of these compounds even when they are not present in concentrations high enough to be individually perceived. The second level was at the perception

threshold as reported in literature (Buttery *et al.*, 1969; Dubourdieu *et al.*, 2006). The third level was at a low to medium concentration while the fourth level was at medium to high concentration found in wines according to literature. The fifth level was considered to be very high according to levels found in white wines in general (Dubourdieu *et al.*, 2006; Alberts *et al.*, 2009; Swiegers *et al.*, 2009). In this way, the effect of these compounds below, at and above their perception threshold, could be investigated. Acetaldehyde concentrations were chosen to be present at higher concentrations. The first level was chosen at the perception threshold as reported in literature (Guth, 1997). The second level was considered to be close to the average level found in non-oxidised young white wines (Jackowetz & De Orduña, 2013). Levels 3 and 4 had an increased acetaldehyde concentration to represent aging white wines, while level 5 had a very high acetaldehyde concentration according to literature (Jackowetz & De Orduña, 2013).

The attributes and definitions generated by the panel as well as the reference standards used during training are shown in Table 5.3. The effect of varying levels of 3MH and IBMP can be seen in Figures 3.3 and 3.4 in Chapter 3. These results have been discussed in Chapter 3 section 3.3.2 and will not be repeated here.

Attributes generated for the profiling of the samples containing acetaldehyde included *fresh green apple*, *grass*, *chemical* and *oxidised green apple.* These attributes have been mentioned in literature previously for the description of the aroma of acetaldehyde (Margalith, 1981; Henschke & Jiranek, 1993; Miyake & Shibamoto, 1993; Frivik & Ebeler, 2003). Interestingly, the attribute “sherry” was not used when profiling the model wines containing acetaldehyde. It is well known that acetaldehyde is responsible for the typical odour of sherry wines due to its presence at high concentrations (Sponholz, 1993; Cortes *et al.*, 1998). In this study, the attribute was not used to describe the odour even at 200 mg/L of acetaldehyde. This would suggest that the typical “sherry” odour (which can also develop in table wines as seen in Chapter 4) probably occurs due to the combined effect of acetaldehyde and other aroma compounds. As discussed in Chapter 3, various interactions can occur between wine aroma compounds creating the overall bouquet of the wine. The combination that delivers this attribute can be potentially hazardous for especially white wine quality and further investigations should be done to identify the origin of this attribute.

Table 5.3 Attributes and reference standards used for descriptive analysis of spiked model wines at various concentrations.

Descriptor	Definition	Reference standard
Dusty	Smell associated with a closed basement or cupboard	none
Grapefruit	Typical grapefuit	Grapefuit pieces in model wine
Guava green	Unripe guava (green skin)	Unripe green guava pieces in model wine
Guava ripe	Very ripe guava (pink skin)	Ripe guava pieces in model wine
Passion fruit	Typical passion fruit	Passion fruit pieces in model wine
Tomato leaf/stalk	Fresh tomato plant stalk	Fresh cherry tomato on the vine with long stalk
Cooked beans	Cooked green beans	Canned cooked beans in model wine
Green pepper	Typical green pepper	Fresh green pepper
Fresh green apple	Freshly sliced green apple	Freshly grated green apple (Granny Smith)
Chemical	Chemical acetone like smell	none
Grass	Freshly cut grass	Freshly cut grass
Oxidized green apple	Grated green apple kept at room temperature for 24 hours	Grated green apple (Granny Smith) kept at room temperature for 24 hours

As seen in Figure 5.2, no specific attribute was used to describe the odour at 0.5 mg/L acetaldehyde. This concentration has been reported as the aroma detection threshold of acetaldehyde in a model wine (Guth, 1997). However the panel struggled to detect any aroma at this concentration and a brief triangle test proved that the panel was not able to distinguish between the blank model wine sample and the sample containing 0.5 mg/L acetaldehyde (results not shown). The reason for this might be the use of 12% ethanol model wine solution instead of the 10% ethanol model wine solution as reported by Guth, (1997). Higher ethanol concentration will increase the solubility of the odorant causing a decrease in the amount of volatiles reaching the pituitary. Even more, ethanol has proven to mask or suppress the fruity notes of esters (Escudero *et al.*, 2007) and could potentially influence the detection of acetaldehyde as well.

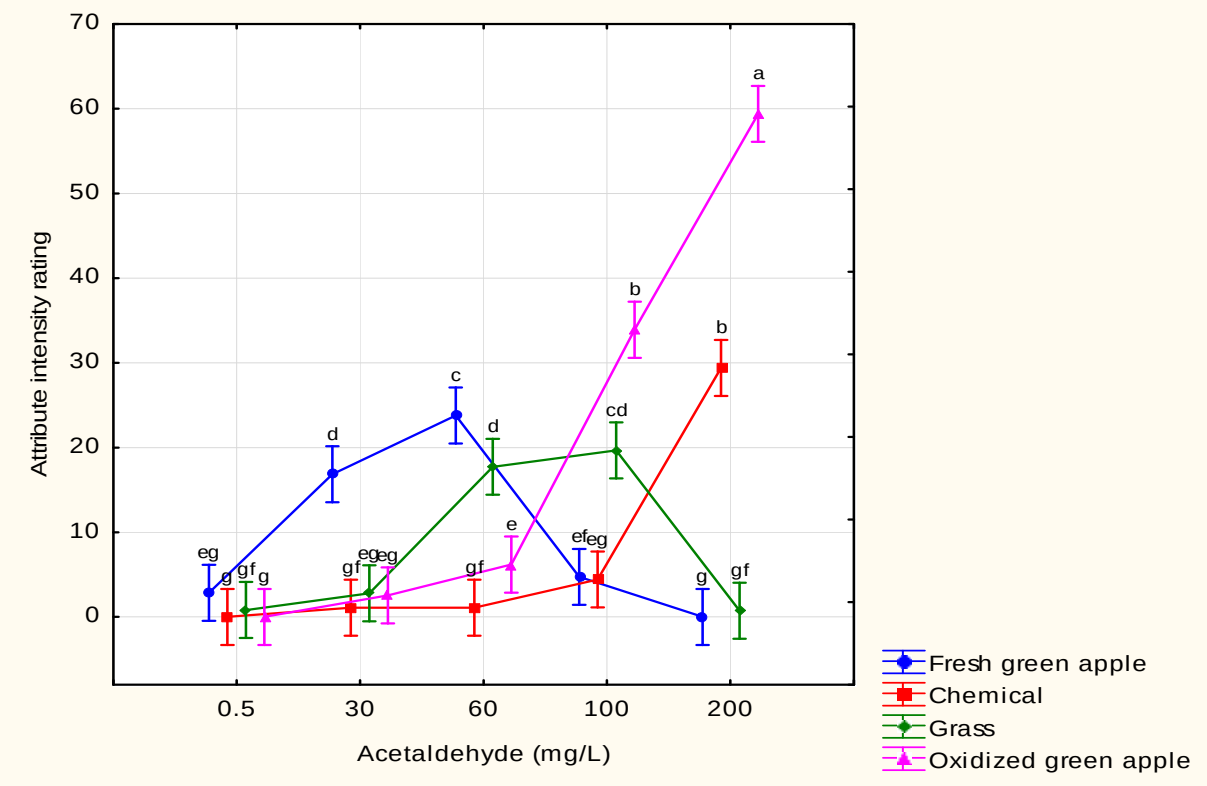

Figure 5.2 Effect of acetaldehyde concentration on the various attribute intensities. Different letters indicate significant differences at $p<0.05$.

At 30 mg/L, the panel was able to distinguish between the blank model wine sample and the sample containing acetaldehyde and described the odour of the sample as *fresh green apple* at an intensity of 16.8 units. It is thus evident that the detection threshold of free acetaldehyde in a 12% ethanol model wine solution should lie somewhere between 0.5 and 30 mg/L. Various other threshold studies done in water reported the threshold value to be between 0.7 µg/L and 2.5 mg/L (Nijssen *et al.*, 1996) and mention the difficulty of determining this compound's threshold value, probably due to its high volatility. In the present study, a full odour detection threshold was not done, however the exact detection threshold value of this compound should be tested in future.

There was an increase in the *fresh green apple* attribute from 16.8 to 23.8 intensity units when acetaldehyde concentrations were increased from 30 to 60 mg/L. This could explain the contribution of acetaldehyde to the fruity aroma of wines when present at lower concentrations. The intensity of this attribute decreased significantly at 100 and 200 mg/L. At 60 mg/L *grass* also contributed to the perceived aroma and was rated at an average of 17.2 intensity units. The intensity remained the same at 100 mg/L after which it decreased. Both *fresh green apple* and *grass* have a non-linear relationship with the compound concentration (as seen for 3MH and *guava green* in Chapter 3) overlapping at 60 mg/L.

At 100 and 200 mg/L, *oxidised green apple* was the attribute rated with the highest intensity. This odour is typically associated with oxidised wines. *Chemical* also started to develop at 100 mg/L reaching an intensity of 29.4 at 200 mg/L. At 200 mg/L the presence of *oxidised green apple* and *chemical* were dominant and would probably result in a wine being classified as very oxidised if perceived in a dry white wine. This is the first study that we know of that has sensorially profiled various concentrations of acetaldehyde (using model wine as medium). As mentioned earlier, in wines, the acetaldehyde is mostly in its bound form which decreases the aromatic potency substantially (Jackowetz *et al.*, 2011). The free form will only increase significantly in the absence of binding agents such as SO_2. During the production of free acetaldehyde, the reported attributes will start to significantly influence the wine aroma developing the typical oxidation character.

5.3.3 Multiple compound evaluation

Samples will be referred to as three digit codes. The codes are an indication of the level (concentration) of the specific compound as reported in Table 5.1. For example, code 1-3-5 would represent the sample containing level 1 of 3MH (40 ng/L), level 3 of IBMP (10 ng/L), level 5 of acetaldehyde (200 mg/L).

A PCA biplot was constructed to investigate possible interactions that occurred when multiple compounds were added in the same sample (Figure 5.3). Eighty five percent of the variation was explained by the first two PCs. Three groups of attributes can be seen: on the left the attributes *guava ripe*, *passion fruit*, *tomato leaf/stalk* and *grapefruit* are grouped together, while attributes *green pepper*, *dusty* and *grass* are located at the bottom of the PCA. The last group of attributes are *cooked beans*, *chemical*, *oxidised green apple* and *fresh green apple* grouped at the top of the PCA.

During the evaluation of the single compounds, *grass* was used to describe the odour of both IBMP and acetaldehyde. As seen in the PCA, *grass* does not correlate strongly with either of the groups and is located in-between, however it is slightly better correlated with the attributes normally associated with IBMP compared to those associated with acetaldehyde. *Cooked beans* correlated well with the attributes normally associated with acetaldehyde. This result is surprising as this attribute was not used to describe the odour of acetaldehyde during the single compound evaluation.

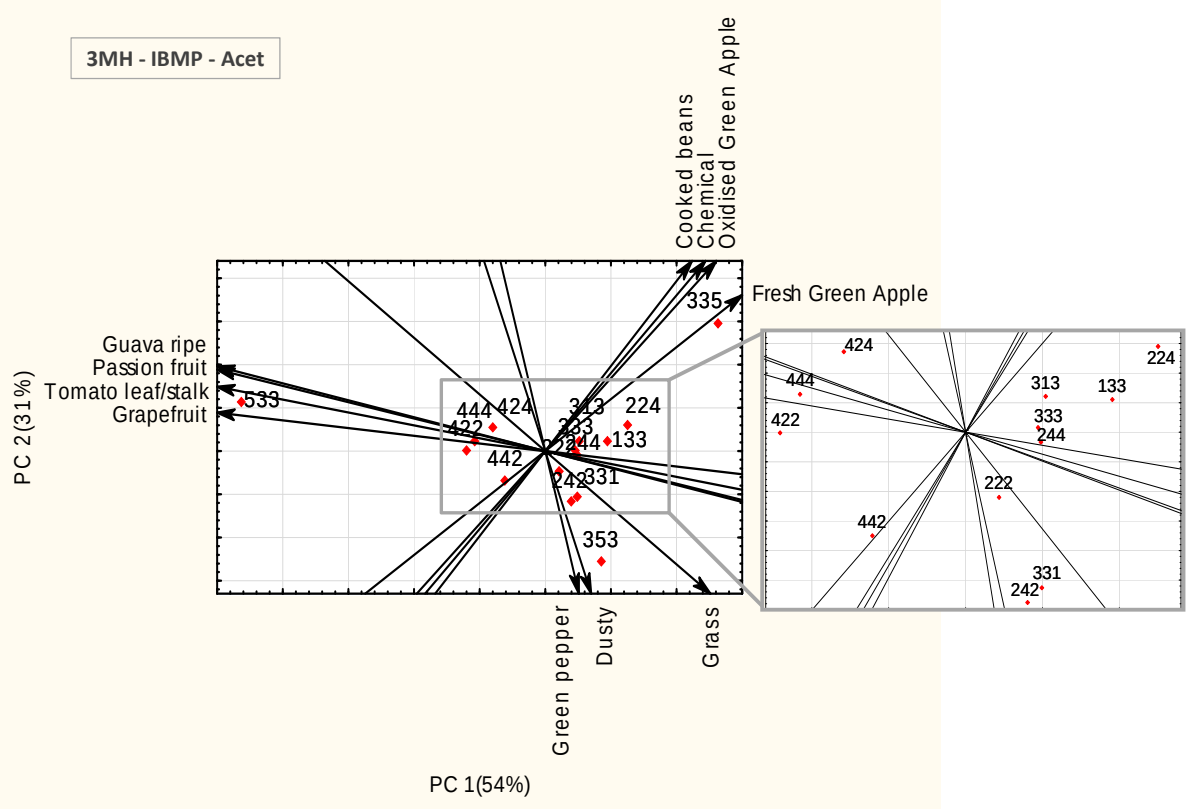

Figure 5.3 PCA biplot showing PC1 and PC2. The selected are is enlarged to better evaluate the clusters in the centre of the PCA.

As expected, all the samples containing a compound at level 5 correlated well with the associated attributes: sample 5-3-3 with 3MH, 3-5-3 with IBMP and 3-3-5 with acetaldehyde. The centre cluster was enlarged to investigate further correlations (Figure 5.3). As with the level 3 and level 5 combinations, the samples containing only one compound at level 4 with the other two compounds present at level 2 (4-2-2, 2-4-2 and 2-2-4), also had a strong correlation with the associated attributes.

Samples located to the higher end of the 3MH attribute intensities are 4-4-4, 4-2-4 and 4-4-2. All three of these samples contained 3MH at level 4. Interestingly, sample 4-4-4 was not situated in the middle of the PCA as in the case of sample 4-4-4-4 in Chapter 3 (Figure 3.7). The correlation of this sample is indeed interesting considering the location of sample 4-4-2 which had a weaker correlation with the fruity attributes when compared to 4-4-4. This would suggest that the presence of acetaldehyde could enhance the fruity odours in the presence of 3MH also al level 4. The same was seen for IBMP as sample 4-2-4 had a slightly weaker correlation to the fruity attributes when compared to 4-4-4. The combination of all three compounds at relatively high concentrations

enhanced the perceived fruity attributes. In Chapter 3 and in other literature, a slight suppression of 3MH by IBMP was observed (Van Wyngaard, 2013), however in Chapter 3 the samples were in combination with methional and phenylacetaldehyde which could change the interactive effect. Samples better associated with the IBMP attributes were 2-4-2, 3-3-1, 4-4-2 and 2-2-2. Except for sample 2-2-2, all these samples contained IBMP at a relatively higher concentration. Sample 3-3-1 was strongly correlated with the IBMP associated attributes and the presence of acetaldehyde could suppress these attributes considering the location of 3-3-3 at the lower intensities of the IBMP attributes. This observation is supported by the location of sample 4-4-2 compared to sample 4-4-4. In fact, all of the samples correlating well with the IBMP-associated attributes contained low concentrations of acetaldehyde.

Sample 2-4-4 was also much more strongly correlated to the acetaldehyde attributes even though IBMP was present at relatively high concentrations (with lower 3MH levels). Even 2-2-2 was better correlated to the IBMP attributes when compared to 2-4-4. This all supports the suppressive effect acetaldehyde seems to have on the methoxypyrazine attributes. The presence of 3MH also at level 3 in sample 3-3-1 does not seem to have a suppressive effect on the IBMP-related attributes, however, sample 4-4-2 was slightly weaker correlated to these attributes which could indicate a suppressive effect when 3MH is present at higher concentrations.

With the exception of sample 4-4-4, all other samples containing acetaldehyde at levels 3 or 4 were located to the higher end of the acetaldehyde attribute intensity. Samples 3-1-3 and 1-3-3 resembled the acetaldehyde descriptors even in the presence of either 3MH or IBMP at level 3. Furthermore, sample 3-3-3 was also correlated with acetaldehyde attributes. In the near absence (level 1) of either 3MH or IBMP in these samples, a slightly better correlation to the acetaldehyde attributes was observed and could indicate to suppression effects (3-1-3 and 1-3-3 compared to 3-3-3). This was also seen with samples 2-4-4 and 4-2-4 (compared to 2-2-4), however it should be kept in mind that even with the slight suppression these samples still had relatively strong sensory characteristics smelling of acetaldehyde.

As mentioned in Chapter 3, the PCA biplot does have a few restrictions when interpreting this data. The main concern is the grouping of the attributes which does not allow the observation of sample influences on individual attributes. Another restriction is the question regarding the extent of the increase or decrease. For example, *cooked beans* seems to be highly correlated with the acetaldehyde-related attributes when referring to Figure 5.3, the question still remains how often this attribute was used as a whole and what the increases in attribute intensities were when it was used. These questions could be answered by constructing statistical networks. Statistical networks investigate each attribute separately by using coloured nodes. Node colours were scaled to the

change in the mean intensity units in the corresponding sensory attribute. Red nodes indicate an increase, while blue nodes show a decrease. In other words, the networks provide visual indications of how the various samples influence the intensity of the attribute as compared to the centre sample only. The attributes that are affected by the samples and the samples that have a statistically significant difference from the centre sample are shown in the network.

Table 5.4 shows the average intensity rating of each attribute for the three samples used as centre samples in Figures 5.4-5.6 (3-3-3, 2-2-2 and 4-4-4). Using this Table as a reference, the intensity scoring and increases and/or decreases can be understood.

Table 5.4 Average intensity scoring of attributes for samples 3-3-3, 2-2-2 and 4-4-4.

Descriptor	3-3-3	2-2-2	4-4-4
Dusty	5.5	9.6	3.3
Grapefruit	15.8	13.9	29.9
Guava green	12.8	5.3	11.9
Guava ripe	1.9	2.4	16.0
Passion fruit	2.0	1.8	19.0
Tomato leaf/stalk	2.0	1.7	17.9
Cooked beans	0.0	0.0	0.0
Green pepper	3.7	5.0	8.0
Fresh green apple	11.4	5.1	17.7
Chemical	2.1	0.0	0.3
Grass	12.8	6.3	4.5
Oxidized green apple	14.3	4.4	6.6

Figure 5.4 shows the statistical network constructed using sample 3-3-3 as the centre sample. As expected, attributes *grapefruit*, *guava ripe*, *passion fruit* and *tomato leaf/stalk* all increased with 41.2 to 54.4 intensity units when the 3MH was increased to level 5. *Guava green* did not increase significantly in sample 5-3-3. The non linear relationship observed between *guava green* and 3MH concentration (Chapter 3, Figure 3.3) could explain this observation. The intensity of this attribute

did however increase (5.2 units) at low levels of IBMP. This is contradictory to what has been reported in Chapter 3 where an increase in both 3MH and IBMP led to an increase in *guava green*. The presence of acetaldehyde in the sample (compared to methional and phenylacetaldehyde), could have a different interactive effect.

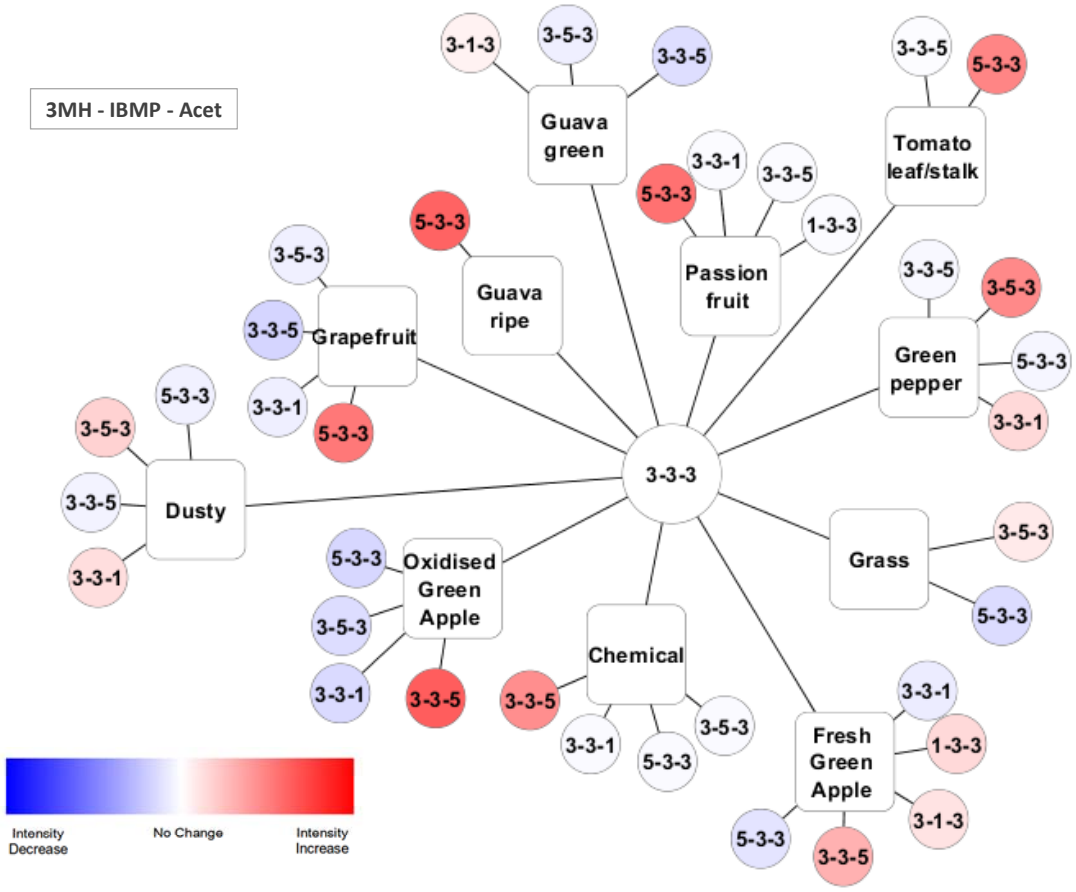

Figure 5.4 A statistical network (centre sample 3-3-3) showing the effect of sample combinations on attribute intensity. Edges between attributes and coloured nodes indicate a statistically significant difference from the centre sample. Red nodes indicate attribute intensity increase; blue nodes indicate attribute intensity decrease. Darker colour shows larger increase/decrease.

As seen with the PCA biplot, the decrease of acetaldehyde to level 1 (3-3-1) caused a decrease in intensity for attributes *grapefruit* and *passion fruit* (-6.1 and -2.0 units respectively). This confirms the contribution of acetaldehyde to these attributes at certain concentrations. However, an increase of acetaldehyde concentration to level 5 seems too severe and attributes *grapefruit*, *passion fruit* and *tomato leaf/stalk* decreased in intensity (-6.1, -2.0 and -1.6 units respectively). Acetaldehyde seems to have an enhancing effect on these 3MH-associated attributes when present at certain

concentrations, however if the concentration increases the interaction could change to a suppressive one.

Attributes associated with IBMP (*green pepper* and *grass*) also increased significantly with increased IBMP concentrations (5-3-3). This increase was however much larger for *green pepper* (41.2 units) when compared to *grass* (7.9 units). The non-linear relationship observed between *grass* and IBMP concentration during the single profiling of IBMP (Chapter 3, Figure 3.4) could explain the slight increase in this attribute. *Green pepper* also increased in the absence of acetaldehyde (13.6 units) in sample 3-3-1 and could indicate a suppressive influence of acetaldehyde regarding IBMP. In the presence of either 3MH or acetaldehyde at level 5, a decrease in *green pepper* and *grass* was observed. *Cooked beans* did not appear in any of the statistical networks as the changes in intensities were not significant (Table 5.4).

This is a good example of the usefullness of the statistical network when interpreting this type of data. Even though the PCA biplot gives a good overview of the sample interactions, some of the observations could be misleading. It would thus be advised to use the PCA biplot in conjunction with another statistical approach such as the statistical networks showed in this study.

Fresh green apple, *chemical* and *oxidised green apple* increased as acetaldehyde concentration increased to level 5 with 27.5, 39.7 and 56.6 intensity units respectively. The *fresh green apple* intensity also increased in samples where either 3MH or IBMP were at level 1 (13.8 and 10.4 units respectively) showing a possible suppressive effect of these two compounds on this attribute.

Figure 5.5 shows the statistical network with sample 2-2-2 as centre sample. As expected, most of the nodes are coloured in red indicating an increase in intensity of the attributes as the sample combinations changed. For the attributes associated with 3MH (*grapefruit*, *guava ripe*, *guava green passion fruit* and *tomato leaf/stalk*), all samples containing 3MH at level 4 increased in attribute intensity with the exception of *guava green* where surprisingly an increase in IBMP and acetaldehyde (2-4-4) led to an increase (6.9 units). This could again be due to the non linear relationship between the attribute's intensity and 3MH concentration. However, the presence of acetaldehyde in combination with IBMP could contribute to the perception of this attribute.

In the case of *grapefruit*, *guava green*, *passion fruit* and *tomato leaf/stalk*, the increase in both 3MH and IBMP (4-4-2) led to increased attribute intensity, however in all cases the increase in 3MH alone (4-2-2) was higher with between 5.0 and 8.4 units. In the samples containing both 3MH and acetaldehyde at level 4 (4-2-4), an increase in the intensities of *grapefruit*, *guava ripe*, *passion fruit* and *tomato leaf/stalk* was observed. Again these increases were still between 0.9 and 5.8 units lower when compared to increased 3MH alone (4-2-2). *Grass* was influenced by the increase in IBMP only, while *green pepper* intensity increased with IBMP alone at level 4 (16.2 units) or in combination

with either 3MH or acetaldehyde at level 4 (14.8 and 8.0 units respectively). Samples 4-4-2 and 2-4-4 did not cause any significant change in the *grass* aroma intensity. The presence of 3MH or acetaldehyde at level 4, even in the presence of IBMP also at level 4, seems to suppress this attribute, however in the case of 3MH the suppression was only characterised by 1.4 intensity units (comparing sample 2-4-2 to 4-4-2). The combination of IBMP and acetaldehyde, both at level 4, led to the smallest increase in intensity which again indicate the suppressive effect of acetaldehyde on *green pepper*.

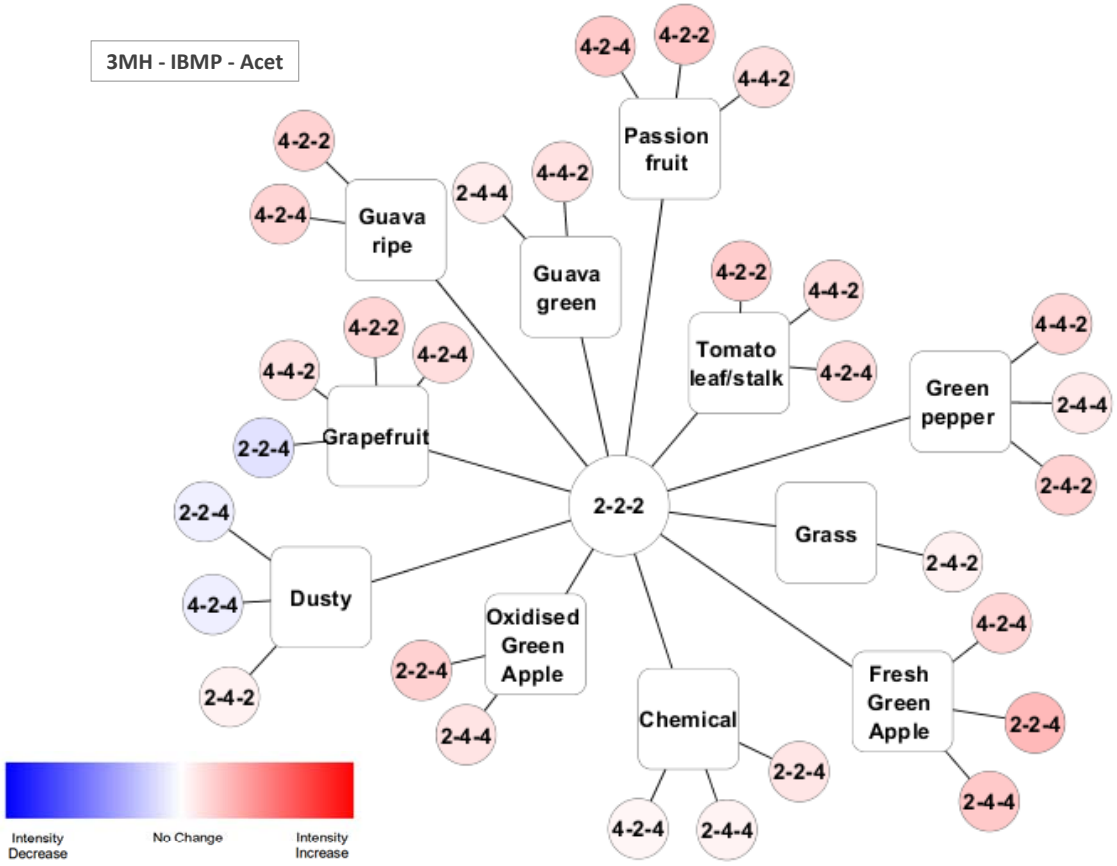

Figure 5.5 A statistical network (centre sample 2-2-2) showing the effect of sample combinations on attribute intensity. Edges between attributes and coloured nodes indicate a statistically significant difference from the centre sample. Red nodes indicate attribute intensity increase; blue nodes indicate attribute intensity decrease. Darker colour shows larger increase/decrease.

Of all the acetaldehyde associated attributes, *fresh green apple* increased the most in intensity when comparing sample 2-2-2 with sample 2-2-4. For sample 2-2-4, *fresh green apple*, *chemical* and *oxidised green apple* increased with 24.8, 9.4 and 16.3 intensity units respectively. *Fresh green apple* was not used as the main attribute when profiling the sample containing only acetaldehyde at level

4. However, with acetaldehyde in combination with 3MH and IBMP, *fresh green* apple increased signifianctly. When acetaldehyde was alone in the medium (level 4) the odour was predominantly described as *oxidised green apple* (Figure 5.2), but in combination with 3MH and IBMP (even at low levels), the attribute changed and the descriptor *fresh green apple* was predominantly used. It would thus seem as if the interaction could change the perception of the compound and subsequently another attribute is mainly used to describe the aroma. *Fresh green apple* also increased when the sample contained either 3MH or IBMP at level 4 (15.0 and 19.1 units respectively) in combination with acetaldehyde at level 4. This also applied to *chemical* where the increase of sample 4-2-4 (3.6 units) and 2-4-4 (4.7 units) was observed, however this increase was not as high as the increase in acetaldehyde alone (2-2-4; 9.4 units). *Oxidised green apple* only increased in samples 2-2-4 (16.3 units) and 2-4-4 (9.9 units). The addition of 3MH at level 4 in conjunction with acetaldehyde at level 4 (4-2-4) did not lead to any significant changes and would thus indicate a suppressive effect of 3MH on the *oxidised green apple* attribute.

The statistical network containing sample 4-4-4 as centre sample gave further information regarding interactions between the compounds (Figure 5.6). For the 3MH-related attributes, all intensities decreased when 3MH decreased to level 2. For attributes *grapefruit*, *passion fruit* and *tomato leaf/stalk*, the decrease was the smallest in the samples where both IBMP and acetaldehyde was still present at level 4 (i.e. sample 2-4-4 decreased by less when compared to 2-4-2 or 2-2-4).

Green pepper intensities increased in the absence of acetaldehyde (11.8 and 13.2 units for samples 4-4-2 and 2-4-2 respectively). This again confirms the suppressive effect of acetaldehyde on this attribute. *Grass* increased in the absence of both 3MH and acetaldehyde (2-4-2) and also increased in the absence of 3MH and IBMP (2-2-4). *Grass* was used as a descriptor for the profiling of both IBMP and acetaldehyde and could thus explain this increase. *Fresh green apple*, *chemical* and *oxidised green apple* increased as the concentrations of 3MH and IBMP decreased and the attribute intensity decreased as acetaldehyde concentrations decreased.

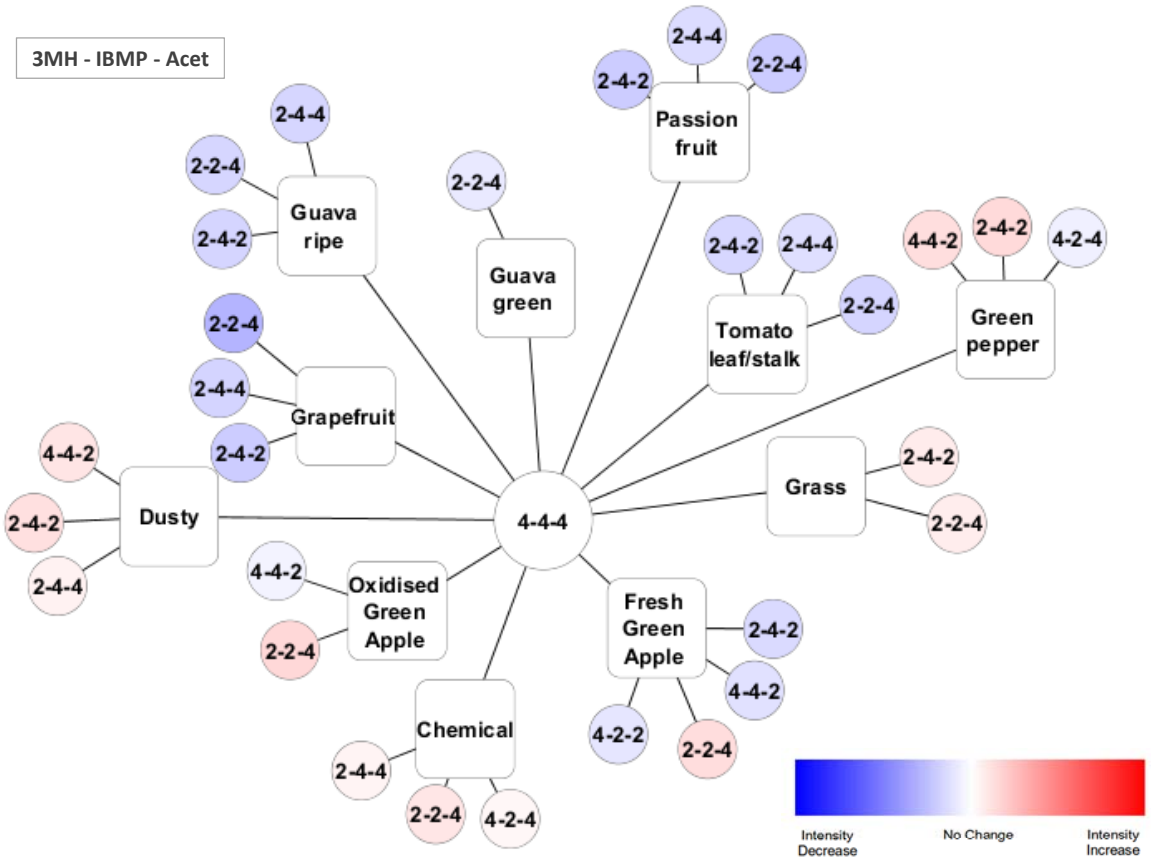

Figure 5.6 A statistical network (centre sample 4-4-4) showing the effect of sample combinations on attribute intensity. Edges between attributes and coloured nodes indicate a statistically significant difference from the centre sample. Red nodes indicate attribute intensity increase; blue nodes indicate attribute intensity decrease. Darker colour shows larger increase/decrease.

Surface plots were constructed to investigate supportive information regarding the tendencies observed in the statistical networks. The suppression of acetaldehyde on the perception of *green pepper* is shown in Figure 5.7.

The suppression of acetaldehyde on *green pepper* can have important oenological implications. As mentioned previously, white wines and especially Sauvignon blanc wines can lose flavour and aroma during aging, however the persistence of the methoxypyrazines (which are not as sensitive to acid hydrolysis or oxidation as some volatile thiols) can, to some extent, guarantee the typical *green pepper* or *grassy* aroma to remain in the wine. However, it would seem that the *green pepper* attribute can decrease significantly if acetaldehyde were to develop in the wine. The suppressive effect could thus alter the overall wine bouquet, especially if the wine style is driven to a more

"green" flavour. 3MH also exerted a suppressive effect on the *oxidised green apple* flavour which was also observed in the biplot (Figure 5.8).

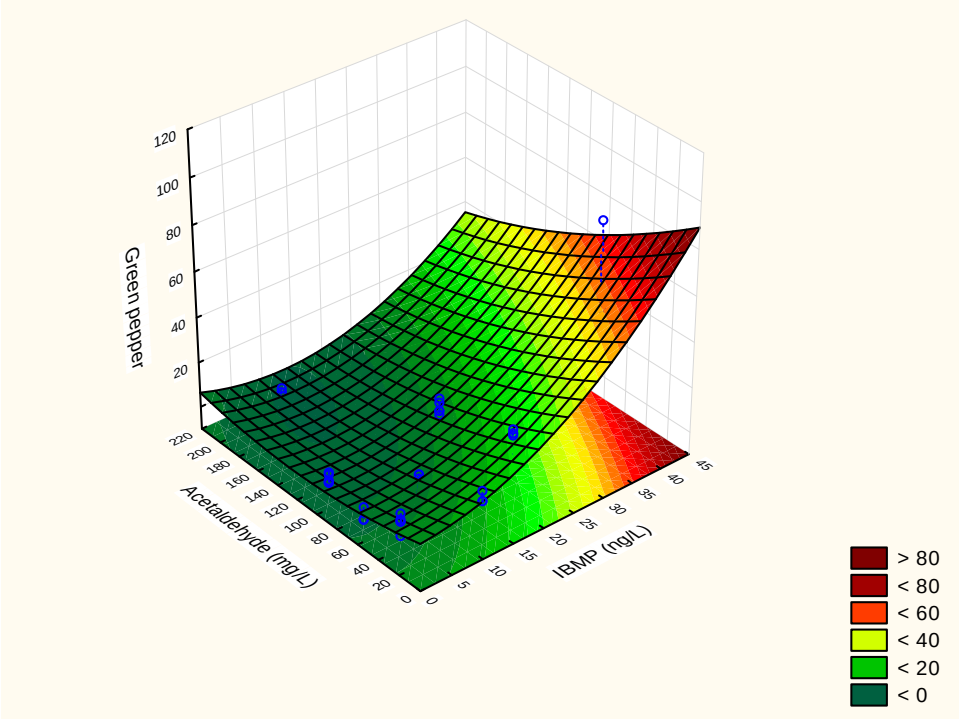

Figure 5.7 Response surface plot of the *green pepper* attribute as influenced by IBMP and acetaldehyde.

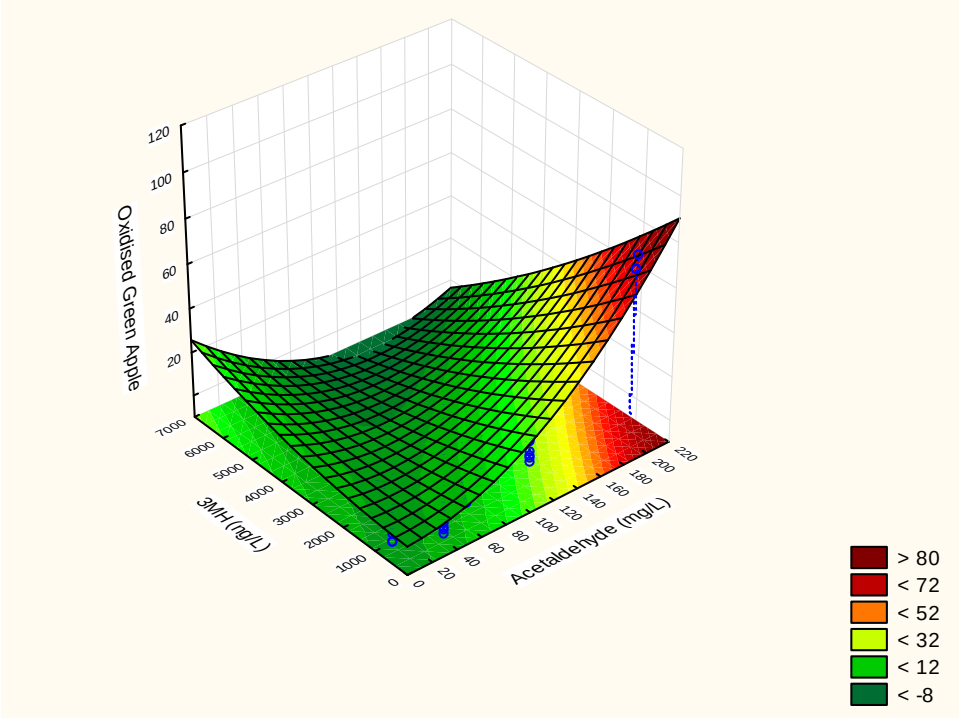

Figure 5.8 Response surface plot of the *oxidised green apple* attribute as influenced by acetaldehyde and 3MH.

Sauvignon blanc winemaking techniques are often designed to produce as much volatile thiols as possible and also to protect the thiols against degradation during aging (Coetzee & Du Toit, 2012). Practices to increase or protect 3MH levels in wine include machine harvesting, protection against oxidation during pressing (Mattivi *et al.*, 2012), SO_2 additions to must (Coetzee *et al.*, 2013), yeast choice (Swiegers & Pretorius, 2007) and storing wine at a lower temperature (Makhotkina *et al.*, 2012). This is done to preserve the pleasant fruity flavours exerted by these compounds. The present study also highlights the importance of protecting the volatile thiols due to the suppressive effect 3MH has on the *oxidised green apple* flavour. In Chapter 4, the presence of 3MH at the end of the oxidative storage could perhaps delay the occurrence of the *oxidised green apple* flavour during storage. However, as the acetaldehyde concentration increases, the suppressive effect of 3MH on *oxidised green apple* will become less prominent.

In the presence of medium to high concentrations of 3MH, acetaldehyde could enhance the fruity attributes. This was especially prominent when observing the PCA biplot and some of the statistical networks where the samples containing both 3MH and acetaldehyde simultaneously in the medium in relatively high concentrations, were reported to have an increased intensity of the fruity attributes when compared to samples where acetaldehyde was very low. In the presence of lower concentrations of 3MH, increasing acetaldehyde concentrations suppressed the remaining 3MH character. Excessive levels of acetaldehyde should thus be avoided, due to its suppression effect on positive aroma attributes of Sauvignon blanc and its binding capacity of SO_2 (Jackowetz & De Orduña, 2013). This can be performed by preventing excessive oxygen pick up during the vinification process and using SO_2 judiciously (Du Toit *et al.*, 2006). The change in attribute when acetaldehyde was added alone or in combination is also important as it was observed that the *oxidised green apple* attribute was replaced by *fresh green apple* when acetaldehyde was added in combination with 3MH and IBMP.

Acetaldehyde readily binds to glutathione or individual amino acids (Liu & Pilone, 2000) such as cysteine whose addition to beer has proven to reduce acetaldehyde concentration (Suovaniemi *et al.*, 2006). The sensory experience during the suppression of 3MH (also being a sulphur-containing compound) could be due to the orthonasal reactions or possibly reactions that occur in the wine model medium. This needs further investigation.

In a real wine situation, acetaldehyde would be one of many compounds formed during aging or oxidation. Other aldehydes such as methional and phenylacetaldehyde are also likely to form (Silva Ferreira *et al.*, 2003) which could contribute to the masking or suppression of the pleasant wine aroma. In a study where oxidation-related branched aliphatic aldehydes were added in a synthetic wine medium, a synergistic relationship was observed contributing to the “sweet orange”, “dried

fruit" and "fusel" character (Culleré *et al.*, 2007). The possible synergism between aldehydes could explain the sensory experience during the profiling of the Ox samples in Chapter 4. In these wines, the samples T4 and T5 Ox theoretically contained 52.10 and 70.34 mg/L free acetaldehyde respectively and had high intensities of the "oxidised green apple" and "sherry" odours. In a model wine medium, the addition of free acetaldehyde at concentrations similar to this did not lead to the same oxidative intensity. The enhancing or additive effects of other aroma compounds in the real wine medium could play a very important role in the overall wine aroma.

5.4 CONCLUSION

The contribution of acetaldehyde is not always detrimental to the wine aroma even when present at relatively high concentrations, provided that compounds such as 3MH are also present at medium to high concentrations. Acetaldehyde does, however, have strong suppressive effects on attributes linked to IBMP and should be considered when aging wines of which the aroma composition is driven by methoxypyrazines.

Moderate acetaldehyde concentrations could enhance the fruity aroma, however together with the formation of acetaldehyde during oxidation, a decrease in pleasant volatile compounds (such as 3MH) will occur; thus the positive contribution of acetaldehyde could be lost if 3MH concentrations are too low. The aromatic contribution of the bound acetaldehyde should also be investigated further. Many studies report acetaldehyde concentrations in wine without specifying whether the compound is in the free or bound form (Liu & Pilone, 2000; Lachenmeier & Sohnuis, 2008). In the presence of sufficient free SO_2, the acetaldehyde reported should be in the bound form and should thus not have a sensory impact, however, Lund *et al*. (2009a) has reported the sensory impact of non-volatile compounds on aromatic compounds, thus the sensory ability of the hydroxysulphonate should not be underestimated unless proven otherwise.

5.5 ABBREVIATIONS USED

3-mercaptohexan-1-ol (3MH); 3-isobutyl-2-methoxypyrazine (IBMP); principle component analysis (PCA)

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Chapter 6

General discussion and conclusions

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6.1 GENERAL DISCUSSION AND CONCLUSIONS

The olfactory sensation linked to the act of drinking a glass of wine is undoubtedly associated with the presence of different chemical compounds, most of these being aromatic. Understanding the role played by these odourants and their interactions on wine flavour is not easy. The correct use of analytical instruments such as gas chromatography has led to the development of rigorous protocols for the isolation, identification and quantification of aroma compounds, however the interpretation of the sensory roles of these compounds and the overall sensory perception of a complex product such as wine is a challenging task.

This study clearly showed the complexity of the interactions that occurs between various chemical compounds in a wine medium. Masking, suppressing and enhancing effects complicate the interpretation of a wine's sensory composition when observing from a chemical viewpoint alone. In a complex medium such as wine, these effects should always be considered.

By investigating individual compounds in a simplified medium such as a synthetic wine, it is possible to observe direct sensory interactions. The possible complex interactions in these types of studies neccessitates a simple testing medium such as a model wine. The disadvantage of the model wine medium is the investigation of direct sensory interactions only. The challenge still remains to investigate the interactions that occur in a real wine. The interactions observed in this study might not be replicated in a real wine medium and the complexity and diversity of different wines might cause different interactive relationships between the compounds.

In the present study, the suppressive effect of methional on the perception of fruity attributes brought by 3MH was evident, while the enhancing or additive effects of methional and IBMP on each other in the same medium could have an important sensory influence on the wine. 3MH has shown to suppress the odours caused about by acetaldehyde, which highlights the importance of preserving this volatile thiol in the wine during aging. Acetaldehyde could also contribute to the fruity characters of a wine when present at certain concentrations.

The relationships observed in this study and in future studies should all be considered as relative, meaning that as conditions change (presence of specific compounds as well as the concentration) the interactive effects of the aroma compounds could also change. This complicates the dynamic of wine sensory composition as winemaking practices that encourage a change in wine composition can lead to a change in the interactive effects between compounds. The analytical measurement of aroma compounds to predict the sensory composition of a wine at this stage should thus be used as an approximate tool as this study clearly showed the complex interactions between various compounds influencing the perception of the overall aroma. The present study also illustrates the

importance of investigating a range of concentrations occurring in wine when interaction studies between various aroma compounds are investigated. Such studies could give wine producers an idea on the sensory response elicited by a certain compound if present at specific concentrations.

The evolution of aromatic and non-aromatic compounds during repetitive oxidation of a Sauvignon blanc wine was also investigated. Most previous studies investigated a single oxygen dose to the wine and conditions (such as high temperature) were often adjusted to facilitate accelerated oxidation (Ferreira *et al.*, 1997; Escudero *et al.*, 2002; Silva Ferreira *et al.*, 2003; Loscos *et al.*, 2010; Cejudo-Bastante *et al.*, 2013). The present study aimed to assess the effect of multiple oxygen doses on the chemical and sensory composition of a fruity style Sauvignon blanc wine. This wine was then aged for a relatively long period of time at a temperature which normally occurs in a cellar. Other than that, this is the first study that administered oxygen to a South African Sauvignon blanc wine in a controlled manner by specifically measuring the amount of oxygen exposure after which a large range of chemical analyses as well as extensive sensory descriptive analysis were done. This could help the global understanding of the evolution of various compounds during white wine oxidation.

Some groups of compounds decreased in concentration, while other increased, contributing to the overall change in aroma composition. This raises more questions about the interactions occurring in these circumstances. A significant decrease in the volatile thiol concentration was observed in the oxidised samples while the methoxypyrazines maintained a steady concentration. This happened in concurrence with an increase in chemical compounds associated with oxidation such as certain aldehydes.

The formation of a ‘reductive’ odour was observed during the sensory evaluation and the compounds responsible could have had a significant influence on the perception of the wine aroma. One of the shortcomings of the study would be the inability to measure the reductive compounds, which should be included in future studies. The questions regarding interactive effects between reductive sulphur compounds and aroma compounds responsible for the fresh and fruity aroma also needs further investigation. An optimal degree of oxygen exposure should thus be identified in order to prevent the formation of ‘reductive’ off-odours without incurring an excessive loss of fruitiness due to oxidation and the formation of oxidation-related compounds.

The sequence of occurrence of various compounds is also quite complex. Some compounds are more oxidation sensitive than others, while certain odourants are produced at an early stage while others occur at a later stage. The development and sequence of such groups of compounds are also influenced by antioxidants.

The colour measurement of the wine throughout the process also delivered interesting results, indicating that certain white wines could be perceived as not oxidised when analysed orthonasally, however profiling the wine visually might indicate otherwise.

Determining the complete chemical content of the wine is a daunting task and will most certainly not include all the compounds at this stage. However, studying the main groups known to significantly influence the sensory perception of a wine might give some insight to the reactions occurring during oxidation. Combining the chemical with the sensory analysis confirmed the difficulty in matching the two data sets precisely, as predictions according to chemical data alone did not always coincide with the sensory profiling of the wine. The presence of compounds not measured in the study could play an important role in this observation.

Due to large chemical variation between wines, it could be argued that the results found in this study are to some extent only applicable to the specific wine investigated. This is in fact something to consider as each individual wine will have a unique chemical fingerprint and wines would thus not react in the precise same manner, however similarities will probably be evident. Future studies should attempt confirm trends observed in this study using other Sauvignon blanc wines. A better understanding of potential improvements to wine sensory quality associated with oxygen exposure could also lead to the development of novel winemaking practices based on improved oxygen management. This study, although performed on only one wine or a synthetic wine, could serve as a valuable basis for future studies on this topic.

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