Volatiles playing an important role in South African Sauvignon blanc wines

by

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Thesis presented in partial fulfilment of the requirements for the degree of
Master of Agricultural Science

at
Stellenbosch University
Department of Viticulture and Oenology, Faculty of AgriSciences

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March 2013

Declaration

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Date: 26 February 2013

Summary

Sauvignon blanc wines have become progressively more important in the commercial market. Extensive research is being done in various countries to gain more understanding about the aroma compounds found in Sauvignon blanc wines and the interactions between them. Sauvignon blanc wines often have either have a green or tropical style. The green style is caused by the methoxypyrazines while the volatile thiols are important contributing compounds to the tropical style. Various international studies have focussed on measuring the chemical composition of Sauvignon blanc wines. However, more research is required on South African Sauvignon blanc wines. Little is known of the volatile thiols content of South African Sauvignon blanc wines, although the methoxypyrazine content has been extensively reported on. Although methoxypyrazines and volatile thiols are seen as the most important aroma compounds contributing to Sauvignon blanc character, other compounds contribute as well. Esters, monoterpenes and phenols have been found to influence Sauvignon blanc aroma and interact with the methoxypyrazines and volatile thiols. The complex interaction between the compounds responsible for the aroma of Sauvignon blanc wines are still not fully understood and further research is thus needed. The first part of the current study investigated the interaction between a specific methoxypyrazine and volatile thiol. Five different concentrations of 2-isobutyl-3-methoxypyrazine (ibMP) and 3-mercaptohexan-1-ol (3MH) were spiked in dearomatize, neutral Sauvignon blanc wine. The single compounds as well as every possible combination of the range of concentrations were evaluated using sensory descriptive analysis. It was found using various statistical approaches that ibMP suppressed the tropical attributes associated with 3MH and that 3MH suppressed the green attributes that correlated with ibMP. The concentrations at which the suppression occurred and the degree of suppression was different for each attribute. The second part of the current study focussed on commercial South African Sauvignon blanc wines. Sensory descriptive analysis and chemical analysis were used to assess the wines and measure the volatile thiol and methoxypyrazine concentrations. The concentrations of volatile thiols and methoxypyrazines were found to be in line with international Sauvignon blanc wines. It was also shown for the first time that the mutually suppressive trend between the volatile thiols and methoxypyrazines can be seen in commercial Sauvignon blanc wines as well. Future work is needed to fully understand the complex interaction between the various compounds in Sauvignon blanc wines. Further research could focus on investigating the mechanism of interaction between the volatile thiols and methoxypyrazines as well as other aroma compounds.

Opsomming

Sauvignon blanc-wyne word toenemend belangriker in die kommersiële mark. Omvattende navorsing word in etlike lande gedoen om meer begrip te ontwikkel van die aromaverbindings wat in Sauvignon blanc-wyne teenwoordig is, asook van die interaksies tussen hulle. Sauvignon blanc-wyne het in baie gevalle óf ’n groen óf ’n tropiese styl. Die groen styl word veroorsaak deur metoksipirasiene, terwyl die vlugtige tiole belangrike bydraende verbindings is wat aanleiding gee tot die tropiese style. Verskeie internasionale studies het reeds gefokus op die meet van die chemiese samestelling van Sauvignon blanc-wyne, maar meer navorsing is nodig oor Suid-Afrikaanse Sauvignon blanc-wyne. Min is bekend oor die inhoud van vlugtige tiole in Suid-Afrikaanse Sauvignon blanc-wyne, hoewel daar reeds op groot skaal oor die metoksipirasieninhoud verslag gedoen is. Hoewel metoksipirasiene en vlugtige tiole die belangrikste aromaverbindings is wat tot Sauvignon blanc-karakter bydra, is daar ook ander verbindings wat ’n bydrae maak. Esters, monoterpene en fenole het almal ’n invloed op Sauvignon blanc-aroma en reageer op die metoksipirasiene en vlugtige tiole. Die komplekse interaksie tussen die verbindings wat vir die aroma van Sauvignon blanc-wyne verantwoordelik is, word nog nie volledig begryp nie en verdere navorsing is nodig. Die eerste deel van die huidige studie het die interaksie tussen ’n spesifieke metoksipirasien en vlugtige tiol ondersoek. Vyf verskillende konsentrasies van 2-isobutiel-3-metoksipirasien (ibMP) en 3-merkaptoheksaan-1-ool (3MH) is by ontgeurde, neutrale Sauvignon blanc-wyn gevoeg. Die enkel verbindings, asook elke moontlike kombinasie van die reeks konsentrasies, is deur middel van beskrywende sensoriese analise geëvalueer. Daar is met behulp van verskillende statistiese benaderings gevind dat ibMP die tropiese eienskappe wat verband hou met 3MH onderdruk het, terwyl 3MH die groen eienskappe wat verband hou met ibMP onderdruk het. Die konsentrasies waarteen die onderdrukking plaasgevind het en die vlak van onderdrukking het vir elke eienskap verskil. Die tweede deel van die studie het gefokus op kommersiële Suid-Afrikaanse Sauvignon blanc-wyne. Beskrywende sensoriese analise en chemiese analise is gebruik om die wyne te assesseer en die konsentrasies van vlugtige tiole en metoksipirasiene te meet. Die konsentrasies vlugtige tiole en metoksipirasiene was in lyn met dié van internasionale Sauvignon blanc-wyne. Daar is ook vir die eerste keer gewys dat die wedersyds onderdrukkende tendens tussen die vlugtige tiole en metoksipirasiene ook in kommersiële Sauvignon blanc-wyne gevind word. Toekomstige werk sou kon fokus op ’n begrip van die komplekse interaksie tussen die verskillende verbindings in Sauvignon blanc-wyne. Verdere navorsing sou ook kon fokus op ’n ondersoek van die meganisme van interaksie tussen die vlugtige tiole en metoksipirasiene, sowel as ander aromaverbindings.

This thesis is dedicated to my friends, family and the enigma that is wine

Biographical sketch

Elizma van Wyngaard was born in Caledon on 17 February 1988. She grew up in Oudtshoorn and matriculated in 2006 from Oudtshoorn High School. She obtained her BScAgric-degree in Oenology specialized in 2010 and went on to enrol for an MScAgric-degree in Oenology.

Acknowledgements

I wish to express my sincere gratitude and appreciation to the following persons and institutions:

- Dr WJ du Toit (Department of Viticulture and Oenology, Stellenbosch University), who acted as my supervisor, for the opportunities he provided me with and his guidance, motivation and contributions to this study.
- Jeanne Brand (Department of Viticulture and Oenology, Stellenbosch University) who acted as my co-supervisor, for her invaluable input with sensory and statistical analysis as well as reviewing of this manuscript
- Dr A Buica (Department of Viticulture and Oenology, Stellenbosch University) for her continuous guidance and assistance
- Dan Jacobson (Institute for Wine Biotechnology, Stellenbosch University) for his input with the statistical analysis of the data
- Prof Martin Kidd (Centre for Statistical Consultation, Stellenbosch University) for his assistance with statistical analysis and interpretation
- Carien Coetzee, for teaching me invaluable skills in the lab and showing me what can be achieved with hard work
- Katja Suklje, for her valuable assistance and hospitality, and everyone at the Agricultural Institute in Slovenia
- The South African Wine Industry (Winetech), THRIP and the NRF for financial support
- The academic and technical staff at the Department of Viticulture and Oenology for their assistance
- The various commercial cellars for their generosity and participation in this study
- My friends and family for their support and motivation

Preface

This thesis is presented as a compilation of 5 chapters.

Chapter 1	**General Introduction and project aims**
Chapter 2	**Literature review** Chemical composition and sensory analysis of Sauvignon blanc wines
Chapter 3	**Research results** Assessment of interaction between 3-mercaptohexan-1-ol and 2-isobutyl-3-methoxypyrazine in dearomatized Sauvignon blanc wine
Chapter 4	**Research note** Preliminary results on interaction between volatile thiols and 2-methoxy-3-isobutylpyrazine in commercial South African Sauvignon blanc wines
Chapter 5	**General discussion and conclusions**

Contents

Chapter 1

Introduction and project aims

1. Introduction and project aims

1.1 Introduction

Sauvignon blanc has become one of the most important cultivars in the South African wine industry. The consumption of Sauvignon blanc wines in South Africa has increased with over 4 million litres from 2005 to 2011. In 2011 it was the most sold and most exported natural white grape variety in South Africa (SAWIS, 2012). South African Sauvignon blanc wines need to be better characterized in order for South African winemakers to keep on producing wines that are equal in quality to their Australian, New Zealand and French counterparts.

Sauvignon blanc wines have been found to express either a green or tropical style (Swiegers *et al.,* 2006). The volatile thiols have been identified as the group largely responsible for the tropical aromas of Sauvignon blanc wines. The first discovery of 4-mercapto-4-methylpentan-2-one (4MMP) in wine was in 1995 (Darriet *et al.,* 1995), with 3-mercapto-hexylacetate (3MHA) and 3-mercaptohexan-1-ol (3MH) following soon thereafter (Tominaga *et al*., 1996; Tominaga *et al*., 1998). It was seen that 4MMP could contribute to box tree, blackcurrant bud and passion fruit aromas (Swiegers *et al.,* 2006; Roland *et al.,* 2011), while 3MH was perceived as grapefruit, guava and passion fruit aromas. It was found that 3MHA had passion fruit and box tree aromas (Swiegers *et al.,* 2005; Coetzee & Du Toit, 2012). Methoxypyrazines were identified as important contributing compounds to the green style of Sauvignon blanc wines. The dominant methoxypyrazine, 2-methoxy-3-isobutylpyrazine (ibMP), was described as green pepper and herbaceous aromas (Allen *et al.,* 1991; Lacey *et al.,* 1991; Marais, 1994), with 2-methoxy-3-isopropylpyrazine (ipMP) and 2-methoxy-3-*sec*-butylpyrazine (sbMP) contributing to asparagus and pea or bell pepper aromas respectively (Ebeler & Thorngate, 2009; Coetzee & Du Toit, 2012).

The aromatic character of Sauvignon blanc wines is thus largely influenced by the volatile thiols and methoxypyrazines. However, limited research has been done on the sensory interaction between the methoxypyrazines and volatile thiols. The studies that have been done mainly focussed on the group of compounds and not on the interaction between individual chemical compounds. These studies did not evaluate a range of concentrations, but focussed only on medium and high concentrations (Campo *et al.,* 2005; King *et al.,* 2011). A number of international studies have compared the chemical composition and sensory character of Sauvignon blanc wines (Parr *et al.,* 2007; Lund *et al.,* 2009; Lopes *et al.,* 2009; Parr *et al.,* 2010; Pineau *et al.,* 2011; Benkwitz *et al.,* 2012a; Benkwitz *et al.,* 2012b), but no such research has been done on South African Sauvignon blanc wines. Very little is thus known about the volatile thiol content of South African Sauvignon blanc wines, with only two studies reporting levels in a few South African Sauvignon blanc wines (Lund *et al.,* 2009; Benkwitz *et al.,* 2012b). Research is thus needed to fully understand the sensory and chemical composition of commercial South African Sauvignon blanc wines and how thiols and methoxypyrazines interact in these wines. Such characterizations could aid South African wine producers to better understand the composition and aromatic expression of their Sauvignon blanc wines.

1.2 Project aims

This work consisted of two main sections. The main part of this study focussed on the interaction between two key aroma compounds in terms of sensory properties in Sauvignon blanc wines. The specific aims of this part were thus:

(i) To determine if sensory interaction occur between ibMP and 3MH;

(ii) To investigate whether the nature of the interaction is suppressive or synergistic;

(iii) To determine the concentrations at which interaction occurs.

The second part focussed on the sensory and chemical profiling of South African Sauvignon blanc wines in terms of volatile thiols and ibMP. The specific aims of this part were:

(i) To characterize the aroma profile of commercial South African Sauvignon blanc wines using sensory analysis

(ii) To determine volatile thiol and methoxypyrazine levels in these wines and investigate their possible sensory interactions

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Chapter 2

Literature review

Chemical composition and sensory analysis of Sauvignon blanc wines

2. Chemical composition and sensory analysis of Sauvignon blanc wines

2.1 Chemical composition of Sauvignon blanc wines

2.1.1 Introduction

Sauvignon blanc grapes are known across the world for the dry, aromatic white wines they produce (Swiegers *et al.,* 2009). First made popular in Sancerre and Pouilly in France, it has spread to all other wine producing countries (Swiegers *et al.,* 2006). South African Sauvignon blanc wines can generally be divided into two distinctive styles, green and tropical. The green aromas, such as green pepper, grassy, asparagus and tomato leaf, are usually derived from methoxypyrazines while volatile thiols are largely responsible for the tropical, grapefruit, passion fruit, gooseberry and guava aromas (Marais, 1994; Swiegers *et al.,* 2006; Swiegers *et al.,* 2009; Coetzee & Du Toit, 2012). Although methoxypyrazines and volatile thiols are seen as impact compounds of Sauvignon blanc wines, many other volatile compounds influence its flavour.

Together with the methoxypyrazines and volatile thiols this review will assess the influence of acetate and ethyl esters, monoterpenes, C_{13}-norisoprenoids, higher alcohols, phenols and acids on Sauvignon blanc aroma. Although data on the chemical composition of wine can give valuable insights, sensory analysis is imperative in order to assess the aromatic expression of the chemical compounds. The complex and largely unknown sensory interaction between the aroma compounds of Sauvignon blanc wines will be discussed in this chapter as well (King *et al.,* 2011; Benkwitz *et al.,* 2012a).

2.1.2 Methoxypyrazines

First identified by Augustyn *et al*., in 1982, in Sauvignon blanc wines, methoxypyrazines are known as one of the main contributors to the typical aroma of certain Sauvignon blanc wines (Augustyn *et al.,* 1982; Lacey *et al.,* 1991). The biosynthesis of methoxypyrazines has not yet been fully explained, (Ebeler & Thorngate, 2009) but since they are nitrogen-containing ring structures, it is accepted that methoxypyrazines are formed as secondary products during the metabolism of amino acids (Marais, 1994; Swiegers *et al.,* 2006; Ebeler & Thorngate, 2009). It has been proposed that the amino acids leucine, isoleucine and valine might act as precursors during this reaction (Gunter, 2007).

The three methoxypyrazines generally present in Sauvignon blanc wines are 2-methoxy-3-isobutylpyrazine (ibMP), 2-methoxy-3-isopropylpyrazine (ipMP) and 2-methoxy-3-*sec*-butylpyrazine (sbMP) (Lacey *et al.,* 1991; Marais, 1994). IbMP is regarded as the main methoxypyrazine, normally being present in the highest concentrations in Sauvignon blanc wines. It contributes to herbaceous and green pepper aromas in wine and has a very low detection threshold of 2 ng/L in water (Lacey *et al.,* 1991; Marais, 1994). While the other two methoxypyrazines are usually present at much lower concentrations than ibMP, (Ebeler & Thorngate, 2009) ipMP and sbMP can still contribute to the aroma and complexity of the wine if present above their sensory thresholds (Marais, 1994). IpMP is associated with aromas of asparagus and green bean aromas and sbMP with pea and bell pepper aromas (Ebeler & Thorngate, 2009).

Methoxypyrazines were found to be present in the grapes and were less affected by vinification than viticultural practices (Marais, 1994; De Boubee *et al.,* 2002; Hunter *et al.,* 2004; Maggu *et al.,* 2007). It was found that the major methoxypyrazine, ibMP, is mainly located in the skins, stems and seeds. The percentage of ibMP located in the seeds and stems decrease during ripening while it increases in the skins. Although extensive skin contact and hard pressing is mostly unwanted in Sauvignon blanc wines, the effects of these practices on the extraction of methoxypyrazines were researched. It was established that most of the ibMP is extracted during crushing because of its high solubility in aqueous solutions. Although pressing and skin contact do have an effect on ibMP levels in the must, it is not as significant as expected when considering the high levels located in the skins (De Boubee *et al.,* 2002; Maggu *et al.,* 2007). Methoxypyrazine levels are thus mostly affected by viticultural practices such as canopy management and climate. Light exposure has a major effect on the formation of methoxypyrazines during ripening. It was found that light exposure before veraison can increase methoxypyrazine levels, while light exposure during ripening and after harvest is known to decreases methoxypyrazine levels (Hashizume & Samuta, 1999; Hunter *et al.,* 2004). Methoxypyrazine levels are thus much higher in unripe grapes than in fully ripe grapes (Hashizume & Samuta, 1999; Hunter *et al.,* 2004; Suklje *et al.,* 2012*)*, with higher levels also occurring in grapes grown in cooler areas (Coetzee & Du Toit, 2012). Berry diameter, even at the same total soluble solids levels, influences ibMP levels in Sauvignon blanc during ripening (Suklje *et al.,* 2012). In countries where it is legal, grapes can be harvested earlier and supplemented with sugar to obtain maximum methoxypyrazine levels (Pineau *et al.,* 2011). The main methoxypyrazine, ibMP, was found to be relatively resistant to oxidation in wine (Marais, 1998), but all methoxypyrazines were found to be relatively sensitive towards light exposure after bottling (Marais, 1994).

The methoxypyrazine aromas, detection thresholds and concentrations found in international and South African Sauvignon blanc wines are summarised in table 2.1. South African methoxypyrazine concentrations seem to correspond to international levels found in Sauvignon blanc wines.

Table 2.1 Aroma descriptors, odour thresholds and concentrations of methoxypyrazines found in international and South African Sauvignon blanc wines (source given in parentheses).

Methoxy-pyrazines	Aroma	Odour threshold (OT)	OT determined in	Concentration in Sauvignon blanc	Concentration in South African Sauvignon blanc
2-isobutyl-3-methoxypyrazine	earth, spice, green pepper [1]	2 ng/L [2]	water	2.2- 47.2 ng/L [3,5]	0.48- 44 ng/L [4,5]
2-isopropyl-3-methoxypyrazine	asparagus, earthy [6]	2 ng/L [4]	water	6- 13.7 ng/L [3,5]	6.3- 9.1 ng/L [3]
2-*sec*-butyl-3-methoxypyrazine	green pepper [7]	2 ng/L [4]	water	0.1- 1.3 ng/L [2]	0.03 – 3.2 ng/L [4]

1. Francis & Newton, 2005; 2. Lacey *et al.,* 1991; 3. Lund *et al.,* 2009b; 4. Alberts *et al.,* 2009; 5. Benkwitz *et al.,* 2012b. 6. Coetzee & Du Toit, 2012; 7. Ribereau-Gayon *et al.,* 2006.

2.1.3 Volatile Thiols

Volatile thiols contribute to the tropical characteristics of Sauvignon blanc wine. Volatile thiols are usually present as non-volatile precursors in the grapes and are released during fermentation by the yeast (Roland *et al.,* 2011b). The yeast strain chosen during fermentation can have a notable influence on the final thiol concentration of the wine. It has been proposed that the yeast uses an enzyme called β-lyase for the release of volatile thiols from the corresponding precursor (Swiegers *et al.,* 2006; Roland *et al.,* 2011a) by the cleavage of the S-C (sulpur-carbon) bonds (Swiegers & Pretorius 2007; Thibon *et al.,* 2009).

The three main volatile thiols present in Sauvignon blanc wines are 4-mercapto-4-methylpentan-2-one (4MMP) (Darriet *et al.,* 1995), 3-mercaptohexan-1-ol (3MH) and 3-mercaptohexyl acetate (3MHA) (Tominaga *et al.,* 1996; Tominaga *et al.,* 1998a). Potential precursors have been suggested for both 4MMP and 3MH, but these still only account for a fraction of the volatile thiols formed. 4MMP is released from S-4-(4-methylpentan-2-on)-L-cysteine and S-4-(4-methylpantan-2-one)-glutathione during fermentation and contribute to aromas of box tree, passion fruit and blackcurrant bud (Swiegers *et al.,* 2006; Roland *et al.,* 2011a). Schneider *et al.,* (2006), proposed that mesityl oxide may also be a possible precursor of 4MMP by direct or indirect formation (Schneider *et al.,* 2006). 4MMP has an extremely low perception threshold of only 0.8 ng/L, making it a potent and prominent aroma compound in Sauvignon blanc wines (Swiegers *et al.,* 2006).

S-3-(hexan-1-ol)-L-cysteine and S-3-(hexan-1-ol)-glutathione have been identified as precursors of 3MH (Tominaga *et al.,* 1998c; Peyrot des Gachons *et al.,* 2002) but thus explains only a small percentage of 3MH formation during fermentation (Subileau *et al.,* 2008). *E*-2-Hexenal was also proposed as a potential precursor of 3MH either through direct addition with H_2S or indirectly by C-S lyase activity of the yeast (Schneider *et al.,* 2006). 3MH contributes to aromas of passion fruit, guava and grapefruit and has a perception threshold of 60 ng/L. 3MHA is produced during fermentation from 3MH by yeast ester-forming alcohol acetyltranferase. 3MHA has a perception threshold of 4 ng/L and different aromas for each enantiomer exist. (R)-3MHA has more passion fruit aromas while (S)-3MHA gives more herbaceous and box tree aromas (Darriet *et al.,* 1995; Peyrot des Gachons *et al.,* 2002; Swiegers *et al.,* 2006; Swiegers & Pretorius, 2007; Fedrizzi *et al.,* 2009; Swiegers *et al.,* 2009; Roland *et al.,* 2010; Roland *et al.,* 2011a).

Table 2.2 is a summary of the aroma descriptors and odour thresholds for the volatile thiols. The table also includes the concentrations found in local and international Sauvignon blanc wines. Very little research has been done on the volatile thiol content of South African Sauvignon blanc wines and the two studies mentioned only measured six Sauvignon blanc wines (Lund *et al.,* 2009b; Benkwitz *et al.,* 2012b).

Table 2.2 The aromas and odour thresholds of volatile thiols and the concentrations found in local and international Sauvignon blanc wines (literature sources given in parentheses).

Volatile thiols	Aroma	Odour threshold (OT)	OT determined in	Concentration in Sauvignon blanc	Concentration in South African Sauvignon blanc
4-mercapto-4-methylpentan-2-one (4MMP)	cat urine, box tree, blackcurrent, broom [1]	0.8 ng/L [2]	aqueous alcohol solution (12 v/v %)	5.5 - 24 ng/L [3]	6.6 ng/L [3]
3-mercapto-hexylacetate (3MHA)	passion fruit, boxtree [1]	4 ng/L [2]	aqueous alcohol solution (12 v/v %)	0- 2507 ng/L [4]	10.1-119.2 ng/L [3,4]
3-mercapto-hexan-1-ol (3MH)	passion fruit, grapefruit, guava [1,5]	60 ng/L [2]	aqueous alcohol solution (12 v/v %)	687.7 - 18681.3 ng/L [4]	1013- 2955 ng/L [3,4]

1. Swiegers *et al.,* 2005; 2. Tominaga *et al.,* 1998b; 3. Benkwitz *et al.,* 2012b; 4. Lund *et al.,* 2009b; 5. Coetzee & Du Toit, 2012.

The precursors of 3MH were found to be mainly located in the skins while those of 4MMP were mostly found in the skin and pulp (Roland *et al.,* 2011b). Although skin contact and harder pressing can increase precursor concentrations in the must, lower thiols levels were found in the wine. This is probably due to higher levels of oxidation in the skin contact and harder pressed juice (Maggu *et al.,* 2007; Patel *et al.,* 2010). It has been established that fermentation temperature has an effect on volatile thiol levels in the wine. Higher levels of 4MMP, 3MH and 3MHA were found in fermentations performed at 20°C compared to those at 13°C. Thus fermentation at 18-20°C can be used to increase volatile thiol levels (Masneuf-Pomarede *et al.,* 2006).

Recent studies have shown interesting results concerning the harvesting methods used for Sauvignon blanc grapes. In New Zealand it was found that machine harvested grapes had higher 3MH and 3MHA concentrations than handpicked grapes. It was speculated that the increased maceration and enzymatic activity occurring during machine harvesting contributed to the increased thiol content and led to more tropical style Sauvignon blanc wines (Allen *et al.,* 2011). Grapes infected with *Botrytis cinerea* have also been found to have higher concentrations of volatile thiols, especially 3MH (Thibon *et al.,* 2009). Pineau *et al.,* (2011), reported that 3MH concentrations of the finished wine increased as the grape ripeness increased, but that juice chaptalisation can lead to a decrease in 3MH and 3MHA concentration.

Oxidation in the absence of sufficient sulphur dioxide (SO_2) leads to lower thiol levels in model and real wine. This is probably due to the association of the thiol to quinones, the product of phenolic oxidation (Coetzee & Du Toit, 2012). Changes in the volatile thiol concentrations were observed during the aging of Sauvignon blanc wines. It was found that the 3MHA concentration decreased rapidly during aging with an estimated loss of 40% after 3 months of bottling and complete disappearance after one year in the bottle. The 3MH concentration stayed constant with increases seen after seven months of bottle aging. It was proposed that the increase in 3MH levels can be attributed to 3MHA being hydrolysed to 3MH (Herbst-Johnstone *et al.,* 2011).

2.1.4 Monoterpenes

Monoterpenes are renowned for their floral and citrus aromas in Muscat cultivars and other white wines like Gewürztraminer, Riesling and Sauvignon blanc (Marais, 1994; Carrau *et al.,* 2005; Ebeler & Thorngate, 2009). Monoterpenes are 10-carbon compounds and are all produced from the same precursor, geranyl pyrophosphate, by plants, algae, fungi and yeasts. *Vitis vinifera* is one of the plant species that can produce monoterpenes (Swiegers *et al.,* 2005). Monoterpenes mainly exist as non-odorous precursors bound to glucose and other sugars or in some cases in their free form in grapes. These compounds are released from their sugars during fermentation either by acid or enzymatic hydrolysis. Acid hydrolysis can cause rearrangement of odourless monoterpenes to more aromatic monoterpenes and thus increase the total monoterpene concentration (Marais, 1983; Swiegers *et al.,* 2005; Ebeler & Thorngate, 2009). Monoterpenes can also be produced by *Saccharomyces cerevisiae* through *de novo* synthesis (Carrau *et al.,* 2005).

Monoterpenes that are generally present in wines above their perception thresholds are linalool, geraniol, nerol, citronellol and α-terpineol. These are normally the monoterpenes found at the highest concentration in wines (Marais, 1983; Carrau *et al.,* 2005). Monoterpenes have a broad scope of aromas ranging from floral, citrus, rose-like (geraniol, nerol, rose oxide), coriander (linalool) and herbaceous (Marais, 1983; Swiegers *et al.,* 2005; Ebeler & Thorngate, 2009). Although monoterpenes may occur below the detection threshold in Sauvignon blanc wine, they may also contribute synergistically to its overall aroma (Ribereau-Gayon *et al.,* 1975; Ribereau-Gayon *et al.,* 2006).

Monoterpenes are synthesised in the berries and are predominatly located in the grape skins (Ebeler & Thorngate, 2009). Monoterpenes are mainly absent in unripe grapes and increases during ripening. However, a decrease may be seen in very warm areas and in overripe grapes (Marais, 1983). Solar radiation and temperature are the two main factors affecting monoterpene concentration in the vineyard. Generally higher concentrations were seen in cooler climatic areas and cooler seasons compared to warmer areas and seasons. Solar radiation plays a vital role since it was found by Marais *et al.,* (1999), that canopies with higher light intensity yielded higher monoterpene levels (Marais *et al.,* 1999). Monoterpene concentrations can thus already be manipulated in the vineyard.

Pressing and skin contact can affect monoterpene concentrations in Sauvignon blanc wines significantly. Higher concentrations are generally found in press juice compared to free-run juice and in macerated musts. Heat-treatment or thermovinification also increases the monoterpene concentration significantly, but too high temperatures can cause rearrangement to less aromatic monoterpenes. Higher fermentation temperatures, such as 20°C, can enhance acid hydrolysis to increase the release of monoterpenes (Marais, 1983; Swiegers *et al.,* 2005). Monoterpene concentrations found in international and South African Sauvignon blanc wines are given in table 2.3 as well as their aroma descriptions and odour thresholds.

Table 2.3 Monoterpene aromas, odour thresholds and concentrations found in South African and international Sauvignon blanc wines (literature cited in parentheses).

Mono-terpenes	Aroma	Odour threshold (OT)	OT determined in	Concentration in Sauvignon blanc	Concentration in South African Sauvignon blanc
Linalool oxide	Sweet, woody, floral [1]	Not found	Not found	Not found	9.2-8.9 µg/L [2]
± Linalool	Floral, citrus, lavender [3,4]	15 µg/L [5]	water/ethanol (90 + 10, w/w)	7- 15 µg/L [3,6,8]	Not found
		25.2 µg/L [7]	synthetic wine		
Geraniol	Freshly cut grass, rose, geranium [3,4]	30 µg/L [5]	water/ethanol (90 + 10, w/w)	4-12 µg/L [3,6]	Not found
Β-Farnesol	Lemon, anise, floral, peach, honey, pollen, raspberry [9]	100 µg/L [9]	12% ethanol/water mixture	Not found	127.4 – 143.4 µg/L [2]

1.Veverka *et al.,* 2012; 2. Coetzee *et al.,* 2012; 3. Kozina *et al.,* 2008; 4. Francis & Newton, 2005; 5. Guth, 1997; 6. Sefton *et al.,* 1994; 7. Ferreira *et al.,* 2000; 8. Benkwitz *et al.,* 2012b; 9. Li *et al.,* 2008.

2.1.5 Ethyl and Acetate Esters

Esters have been found to be the largest group of aromatic compounds present in wine. Esters are volatile compounds contributing to fresh and fruity aromas, but a single ester is very rarely linked to a specific aroma property. Esters usually act in a group to cause a synergistic effect referred to as fermentation bouquet. Esters are formed during fermentation by yeast as secondary metabolites of glycolysis. Esters can be divided into two groups, acetate esters and ethyl esters. The first step in ester formation is the activation step when a fatty acid combines with coenzyme A to form acetyl-CoA. Acetyl-CoA can then combine with either ethanol or higher alcohols to form ethyl esters or acetate esters respectively (Lambrechts & Pretorius, 2000; Swiegers *et al.,* 2005).

Ethyl acetate is quantitatively the most significant ester present in white wine with concentrations ranging from 50 to 150 mg/L. At lower concentrations it can have a pleasant sweet and fruity aroma but at high concentrations it imparts nail polish and solvent-like aromas. Other prominant esters are isoamyl acetate, reminiscent of banana and pear, 2-phenylethyl acetate with honey, fruity flowery aromas, and ethyl hexanoate reminiscent of apple and violet aromas (Lambrechts & Pretorius, 2000; Swiegers *et al.,* 2005; Ebeler & Thorngate, 2009). In white wines it was also found that ethyl esters contribute more to tree fruit aromas and acetate esters more towards tropical fruit aromas (Lambrechts & Pretorius*,* 2000).

Ester concentrations can be dependent on many factors such as grape maturity, sugar concentrations, yeast strain, fermentation temperature, cultivar and sulphur dioxide levels. All these factors affect the yeast's production of esters and therefore the yeast strain used is one of the most important parameters affecting ester concentrations (Lambrechts & Pretorius*,* 2000). It was found by Marais, (1998), that Sauvignon blanc grapes stored at overnight temperatures of 0°C had higher ester concentrations than grapes stored at 20°C. Contradicting evidence was available on the effect of SO_2 addition on the ester concentration. Some studies found no consistent difference between ester concentrations of wines produced from reductive and oxidised juice (Marais, 1998; Coetzee *et al.,* 2012). High storage temperatures can drastically decrease ester concentrations during storage (Lambrechts & Pretorius*,* 2000). It was found that storage temperatures of 5 to 10°C led to higher concentrations of acetate esters and ethyl esters of fatty acids in Sauvignon blanc wines after one year of storage. An increase in the ethyl esters of branched acids, such as ethyl lactate, diethyl malate and diethyl succinate, was found at uncontrolled room temperature and 18°C for Sauvignon blanc wines (Makhotkina *et al.,* 2012).

Esters become more important during the aging of Sauvignon blanc wines, especially the first year, when 3MHA concentrations declined. Esters as a group have been found to impact not just the attributes normally associated with volatile thiols but other attributes contributing to the general character of Sauvignon blanc wines. Benkwitz *et al.,* (2012a), found esters were associated with cats pee, sweet-sweaty-passion fruit and passion fruit-skin-stalk as well as apple lolly, stone fruit, apple and tropical aromas. This suggests a greater impact of esters on Sauvignon blanc varietal characteristics than previously thought (Benkwitz *et al.,* 2012a). Table 2.4 is a summary of ethyl and acetate ester aromas, odour thresholds and concentrations found in both international and South African Sauvignon blanc wines.

Table 2.4. Ethyl and acetate ester aroma descriptions, odour thresholds and concentrations found in international and South African Sauvignon blanc wines (literature cited in parentheses).

Esters	Aroma	Odour threshold (OT)	OT determined in	Concentration in Sauvignon blanc	Concentration in South African Sauvignon blanc
Ethyl Acetate	pineapple, varnish, balsamic [1,2]	7500 µg/L [3]	water/ethanol (90 + 10, w/w)	37 µg/L [4]	30.22- 233.58 mg/L [5,6]
Ethyl Butyrate	sour fruit, strawberry, fruity, apple [1,7]	20 µg/L [3]	water/ethanol (90 + 10, w/w)	246- 680 µg/L [4,8]	0.05- 0.8 mg/L [5,6]
Isoamyl Acetate	banana, fruity, sweet [2]	30 µg/L [9]	synthetic wine	999-3690 µg/L [4,8]	1.09- 18.45mg/L [5,6]
Ethyl Hexanoate	green apple, fruity, strawberry, anise [7]	5 µg/L [3]	water/ethanol (90 + 10, w/w)	614-1330 µg/l [4,8]	0.22- 2.11 mg/L [5,6]
		14 µg/L [9]	synthetic wine		
Hexyl Acetate	sweet, perfume [10]	0.7 mg/L [10]	Wine	43-523 µg/L [4,8]	0.07-2.48 mg/L [5,6]
Ethyl Lactate	lactic, raspberry, buttery, cream, sweet, fruity [7,2]	150 mg/l [2]	10%(v/v) ethanol-water solution, ph 3.5 with tartaric acid	2023-3699 µg/L [11]	1.56-29.52 mg/L [5,6]
Ethyl Caprylate	pineapple, pear, floral, fruit, fat [1,7]	2 µg/L [3]	water/ethanol (90 + 10, w/w)	790-2505 µg/l [4,8]	0.27-1.77 mg/L [5,6]
		5 µg/L [9]	synthetic wine		
Ethyl Caprate	fruity, fatty, pleasant, grape [1,7]	200 µg/L [9]	synthetic wine	78-608 µg/l [4,8]	0.23-2.58 mg/L [5,6]
Diethyl Succinate	fruity, wine [2]	1200 mg/l [2]	10%(v/v) ethanol-water solution, ph 3.5 with tartaric acid	230 µg/L [4]	0.09-4.89 mg/L [5,6]
2-Phenyl-ethyl Acetate	Fruity [2]	250 µg/L [3]	water/ethanol (90 + 10, w/w)	354 µg/L [4]	0.04-1.47 mg/L [5,6]
		1.8 mg/L [2]	10%(v/v) ethanol-water solution, ph 3.5 with tartaric acid		

1. Francis & Newton, 2005; 2. Peinado *et al.,* 2004; 3. Guth, 1997; 4. Kozina *et al.,* 2008; 5. Malherbe, 2011; 6. Louw *et al.,* 2010; 7. Li *et al.,* 2008; 8. Benkwitz *et al.,* 2012b; 9. Ferreira *et al.,* 2000; 10. Swiegers *et al.,* 2005; 11. Makhotkina *et al.,* 2012.

2.1.6 Higher Alcohols

Higher alcohols constitute a large part of the aroma compounds found in alcoholic beverages. They can impart strong, pungent aromas if present at too high concentrations (above 400 mg/L). However, at concentrations below 300 mg/L higher alcohols can have a desirable effect on the complexity of the wine. Higher alcohols can be formed by anabolic or catabolic pathways by the yeast. The anabolic pathway involves the *de novo* synthesis from sugars and the catabolic pathway, or Ehrlich pathway, uses amino acids as substrate. A few well known higher alcohols in wine are propanol, isoamyl alcohol, 2-phenylethanol and hexanol (Lambrechts & Pretorius*,* 2000; Swiegers *et al.,* 2005; Bell & Henschke, 2005).

The major factor affecting higher alcohol concentration is the nitrogen composition of the must. Contradicting results were found when looking at the effect of increasing nitrogen on higher alcohol concentration, with some higher alcohol increasing while others decreased. It was then found that the catabolic synthesis of higher alcohols increased with increasing amino acid concentration, but that the anabolic pathway was suppressed (Lambrechts & Pretorius*,* 2000; Swiegers *et al.,* 2005; Bell & Henschke*,* 2005). The addition of SO_2 and oxygen to Sauvignon blanc must did not seem to have a significant effect on the higher alcohol concentration found in the corresponding wines (Coetzee *et al.,* 2012).

South African Sauvignon blanc wines were found to have significantly higher concentrations of isoamyl alcohol, 2-phenylethanol and isobutanol than South African Chardonnay wines. These higher alcohols were all present above their respective odour thresholds thus contributing to the character of South African Sauvignon blanc wines (Louw *et al.,* 2010). The higher alcohols are summarised in table 2.5 together with aromas, odour threshold and concentrations found in international and South African Sauvignon blanc wines.

Table 2.5 Higher alcohol concentrations found in local and international Sauvignon blanc wines and aromas and odour thresholds (literature cited in parentheses).

Higher alcohols	**Aroma**	**Odour threshold (OT)**	**OT determined in**	**Concentration in Sauvignon blanc**	**Concentration in South African Sauvignon blanc**
Methanol	Alcohol [1]	500 mg/L [1]	10%(v/v) ethanol-water solution, ph 3.5 with tartaric acid	Not found	16.02-180.47 mg/L [2,3]
Propanol	ripe fruit, alcohol [1]	50000 µg/L [4] 306 mg/l [1]	water/ethanol (90 + 10, w/w) 10%(v/v) ethanol-water solution, ph 3.5 with tartaric acid	17.5 mg/L [5]	10.34-82.65 mg/L [2,3]
Isobutanol	wine, solvent, bitter [6]	40000 µg/L [7]	synthetic wine	15-44.5 mg/L [5,8]	2.26- 66.32 mg/L [2,3]
Butanol	alcohol, solvent [1]	50 mg/l [1]	10%(v/v) ethanol-water solution, ph 3.5 with tartaric acid	Not found	0.2-2.54 mg/L [2,3]
Isoamyl alcohol	whiskey, malt, burnt [6]	30000 µg/L [7]	synthetic wine	201-259 mg/L [5,8]	115.4- 394.35 mg/L [2,3]
Hexanol	resin, flower, green, cut grass [6]	8000 µg/L [4] 1.1 mg/L [1]	water/ethanol (90 + 10, w/w) 10%(v/v) ethanol-water solution, ph 3.5 with tartaric acid	0.5- 3.1 mg/L [5,8]	0.13- 4.11 mg/L [2,3]
3-ethoxy-1-propanol	Fruity [1]	0.1 mg/L [1]	10%(v/v) ethanol-water solution, ph 3.5 with tartaric acid	Not found	4 mg/L [2]
2-Phenyl Ethanol	flowery, pollen, perfume [9]	10000 µg/L [4]	water/ethanol (90+10, w/w)	17- 46 mg/L 5,[8]	6.89 - 32.78 mg/L [2,3]

1. Peinado *et al.,* 2004; 2. Malherbe, 2011; 3. Louw *et al.,* 2010; 4. Guth, 1997; 5. Kozina *et al.,* 2008; 6. Francis & Newton, 2005; 7. Ferreira *et al.,* 2000; 8. Benkwitz *et al.,* 2012b; 9. Li *et al.,* 2008.

2.1.7. C_{13}-Norisoprenoids

C_{13}-Norisoprenoids are a large group of compounds generally present at trace amounts in wines. However, many authors agree that their extremely low sensory thresholds still make them important aroma contributors in Sauvignon blanc and other wines. C_{13}-Norisoprenoids are secondary metabolites formed from grape carotenoids in the berry. C_{13}-Norisoprenoids are usually present in the odourless form bound to glucose in grapes, but can also exist in the free form.. The bound norisoprenoids are released mostly by acid hydrolysis during fermentation and storage (Swiegers *et al.,* 2005; Lee *et al.,* 2007; Ebeler & Thorngate, 2009).

Two common C_{13}-Norisoprenoids normally occurring in wine are β-ionone and β-damascenone. β-Ionone is more often found in young red wines and has a perception threshold of 700 ng/L. β-damascenone has an even lower perception threshold of 9-200 ng/L and is more likely to be found in white wines contributing to aromas such as tropical fruit, flowers and stewed apple (Swiegers *et al.,* 2005; Lee *et al.,* 2007; Ebeler & Thorngate, 2009). Other C_{13}-Norisoprenoids can also impart honey, lime and tea-like aromas in white and red wines. Recently a new C_{13}-Norisoprenoid, (E)-1-(2,3,6-trimethylohenyl)buta-1,3-diene (TPB), was identified that can be responsible for the green or cut-grass aroma in white wines (Marais, 1994; Swiegers *et al.,* 2005; Lee *et al.,* 2007; Ebeler & Thorngate, 2009).

Certain C_{13}-Norisoprenoids are thought to arise in berries because of photochemical degradation and their concentrations generally increase during ripening. It was found in most cultivars that increased sunlight exposure also increases the norisoprenoid concentration, but β-damascenone showed the opposite tendency. C_{13}-Norisoprenoids concentrations can thus be influenced by climate, grape maturity and sunlight exposure (Lee *et al.,* 2007; Ebeler & Thorngate, 2009).

In a recent study *β*-damascenone contributed to the fruity aromas of certain esters but suppressed the green aromas of ibMP (Benkwitz *et al.,* 2012a). The volatile thiols were found to have a synergistic effect with *β*-damascenone. This effect was seen for 3MH and 3MHA although it was different for each thiol. The combination of 3MH and *β*-damascenone seems to lead to increased passion fruit-skin-stalk, tropical and stone fruit aroma expression. The 3MHA and *β*-damascenone led to more cats pee and sweet-sweaty-passion fruit aromas as well as more tropical and stone fruit character (Benkwitz *et al.,* 2012a)

2.1.8. Acids

Organic acids are known to influence the sensory qualities of white wines in particular (Swiegers *et al.,* 2005; Ribereau-Gayon *et al.,* 2006). The effect on the aroma and flavour of the wine can be positive or negative, depending on the acid (Swiegers *et al.,* 2005). The most predominant organic acids found in grape juice are tartaric acid (2-3 g/L), malic acid (1-

2 g/L) and citric acid (0.5-1 g/L). These acids affect the acidity of grape juice and wine and thus influence the sensory quality. Tartaric acid is not affected during fermentation but malic acid can be formed or degraded by yeasts and bacteria. Succinic acid can also be present in trace amounts in the grapes, but higher concentrations are found in the wine. Succinic acid can be produced by the yeast at up to 2 g/L. Little is known about the specific effect of the acids on sensory properties of wine. Succinic acid has been found to impart an intensely bitter and salty taste in wine (Swiegers *et al.,* 2005; Ribereau-Gayon *et al.,* 2006). In one study malic acid and tartatic acid was negatively correlated with wine body and mouthfeel, but further research is needed for conclusive data (Skogerson *et al.,* 2009).

Volatile acids have been found to contribute to wine aroma and are mainly formed by fatty acid metabolism during alcoholic fermentation by yeast and bacteria. Acetic acid is the volatile acid present in the highest concentrations, but other volatile acids such as propionic, hexanoic and butanoic acid are also found in small amounts in wine (Lambrecht & Pretorius, 2000; Du Toit & Pretorius, 2002; Swiegers *et al.,* 2005). In a study done on South African wines Louw *et al.,* 2010, found that Sauvignon blanc wines had significantly higher concentrations of acetic acid, decanoic acid, octanoic acid and hexanoic acid than Chardonnay. Odour thresholds, aromas and concentrations of fatty acids found in international as well as South African Sauvignon blanc wines are shown in Table 2.6.

Table 2.6 Volatile acid aroma descriptions, detection thresholds and concentrations found in local and international Sauvignon blanc wines (references given in parentheses).

Acids and fatty acids	Aroma	Odour threshold (OT)	OT determined in	Concentration in Sauvignon blanc	Concentration in South African Sauvignon blanc
Acetic Acid	sour, pungent, vinegar [1]	200000 µg/L [2]	water/ethanol (90 + 10, w/w)	Not found	80.91-1191.02 mg/L [3,4]
Propionic Acid	pungent, rancid, soy [1]	8100 µg/L [1]		Not found	1.12- 43.01 mg/L [3,4]
		20 mg/L [5]	9.4 % grain spirit solution		
Isobutyric acid	rancid, butter, cheese [1]	200000 µg/L [2]	water/ethanol (90 + 10, w/w)	Not found	0.2-2.74 mg/L [3,4]
		2300 µg/L [6]	synthetic wine		
Butyric Acid	rancid, cheese, sweat [1]	10000 µg/L [2]	water/ethanol (90 + 10, w/w)	Not found	0.78-3.81 mg/L [3,4]
		2.2 mg/L [7]	10%(v/v) ethanol-water solution, ph 3.5 with tartaric acid		
		173 µg/L [6]	synthetic wine		
Iso-Valeric Acid	sweat, acid, rancid [1]	33.4 µg/L [6]	synthetic wine	708 µg/L [1]	0.15-2.52 mg/L [3,4]
Hexanoic Acid	sweat, rancid, cheese, fatty [1,7]	3000 µg/L [2]	water/ethanol (90 + 10, w/w)	2480 µg/L [8]	2.85-13.7 mg/L [3,4]
		420 µg/L [6]	synthetic wine		
Octanoic Acid	sweat, cheese [1]	500 µg/L [6]	synthetic wine	4475 µg/L [8]	1.73- 12.24 mg/L [3,4]
Decanoic Acid	rancid, fat [1]	15000 µg/L [3]	water/ethanol (90 + 10, w/w)	8653 µg/L [8]	0.43- 5.79 mg/L [3,4]
		1000 µg/L [6]	synthetic wine		

1. Francis & Newton, 2005; 2. Guth, 1997; 3. Malherbe, 2011; 4. Louw *et al.,* 2010; 5. Lambrecht & Pretorius, 2000; 6. Ferreira *et al.,* 2000; 7. Peinado *et al.,* 2004; 8. Kozina *et al.,* 2008.

2.1.9 Phenols

Phenols levels in white wines are normally quantitatively only about 10% of that found in red wine, but can still play an important role influencing the aroma of Sauvignon blanc wines. Various phenols have been found in white wine and include benzoic acids, cinnamic acids, catechins, procyanidins and flavonols (Goldberg *et al.,* 1999; Ribereau-Gayon *et al.,* 2006; Lund *et al.,* 2009a). Benzoic and cinnamic acid concentrations of 10 to 20 mg/L have been measured in white wines. The two main cinnamic acids, *p*-coumaric acid and caffeic acid, are mainly bound to tartaric acid through esterification. The cinnamic acids, together with other phenols, can cause browning of must. Phenolic acids are known to be colourless in alcoholic solutions but oxidation can cause yellowing of these compounds. White wines may also contain quercitin derivatives, catechins and procyanidins (Ribereau-Gayon *et al.,* 2006). In a study by Marais, (1998), the concentration of total polyphenols and total flavanoids in Sauvignon blanc wines were found to higher in wines produced with skin contact.

The addition of SO_2 and higher temperatures (20°C) during skin contact prior to fermentation also led to higher polyphenol and flavanoid concentrations. Techniques such as skin contact at high temperatures are however not recommended for Sauvignon blanc wines, as it can lead to oxidation of must and wines with high phenolic content (Marais, 1998). Maggu *et al.,* (2007) further investigated the effect of skin contact and pressure on polyphenol compounds in Sauvignon blanc must. It was seen that certain phenolic compounds increased with increasing pressure and skin contact, leading to the juice being more susceptible to oxidation (Maggu *et al.,* 2007). Most phenols found in white wine do not have a significant flavour or odour (Ribereau-Gayon *et al.,* 2006), but some have been linked to bitterness and astringency (Gawel, 1998). The flavan-3-ols, (+)-catechin and (-)-epicatechin, were found to contribute to bitterness and astringency in their monomeric forms in especially young wines (Noble, 1994; Gawel, 1998). An experiment done by Noble, (1994), showed that bitterness and astringency increase in white wine with increasing catechin concentration (Noble, 1994).

It has also been shown that phenolic compounds can influence the perception of key aroma compounds such as volatile thiols and methoxypyrazines in Sauvignon blanc wines, which will be discussed in more detail in the following section (Lund *et al.,* 2009a).

2.1.10 Interaction between aroma compounds

Researchers have been trying to explain the complex interactions between aroma compounds in wines for decades. Ribéreau-Gayon *et al.,* (1975) found evidence of the synergistic effect between mixtures of monoterpenes (Ribereau-Gayon *et al.,* 1975). Since then research has focussed on the interaction between aroma compounds of a variety of wines. Some of the most important aroma compounds in Sauvignon blanc wines, the methoxypyrazines and volatile thiols, have also been found to have a complex and still largely unexplained interaction (Marais & Swart, 1999; Swiegers *et al.,* 2006; Swiegers *et al.,* 2009).

In studies done in 2005 by Campo *et al.,* it was found that 3MHA positively correlated with tropical fruit notes. However the methoxypyrazine, sbMP, only had an effect on the perception of 3MHA at levels far above those normally found in Sauvignon blanc wines. In further experiments a combination of methoxypyrazines (sbMP, ibMP, ipMP) were also added to dearomatized wine spiked with 50 and 500 ng/L of 3MHA and ranked by sensory analysis according to the tropical fruit notes. It was clearly seen that the tropical fruit notes induced by 3MHA decreased with increasing methoxypyrazine concentration. This was seen at high and low 3MHA concentrations ($p<0.01$) (Campo *et al.,* 2005). In this study sbMP might not have been the ideal methoxypyrazine to use in the initial experiment, due to t not contributing to the vegetative/ green pepper aroma in Sauvignon blanc wines (Marais, 1994). A study by Lacey *et al.,* (1991), showed that sbMP was present at very low levels in only a few of the Sauvignon blanc wines tested. The results obtained by Campo *et al.,* 2005, using the mixture of methoxypyrazines, can be contributed to ibMP's dominant nature (Lacey *et al.,* 1991; Allen *et al.,* 1991; Marais 1994) or to the synergistic effect of all three methoxypyrazines. Since no experiments were done with the other methoxypyrazines their individual contribution is not clear.

Other volatile thiols and methoxypyrazines have also been shown to be impact aroma compounds in Sauvignon blanc wine. Marais & Swart, (1999), researched the effect of adding only one thiol and one methoxypyrazine in different concentration to deionised water, neutral non-Sauvignon blanc white wine and neutral Sauvignon blanc wine. This study focused on 3-isobutyl-2-methoxypyrazine and 4-mercapto-4-methylpentan-2-one and described their aroma profiles. It was found that both these compounds contribute to the complexity of Sauvignon blanc wines and a synergistic action was suggested. It was also stated that further research is needed on the relationship between these compounds pertaining to Sauvignon blanc aroma (Marais & Swart*,* 1999). This study did not evaluate the wines using quantitative descriptive analysis like other studies in this field (King *et al.,* 2011, Campo *et al.,* 2005). An expert panel, of fairly experienced people, were asked to evaluate the wines in only one session with no prior training. This sensory analysis did not provide quantitative results, only aroma descriptions, and thus intensity and interaction of the compounds could not be determined.

Another study by King *et al.,* (2011), added a combination of thiols (3MH, 3MHA and 4MMP) to a single methoxypyrazines (ibMP) at moderate and high concentrations. In the samples where ibMP concentrations were kept constant and the thiols increased, an increase in tropical and overall fruit aroma and a decrease in fresh green aroma were detected. This shows that the thiols in combination can overcome the 'green' characteristic of ibMP. Where thiols' concentrations were kept constant and the ibMP concentration was doubled the fresh green, green flavour and cooked green vegetal attributes increased significantly. Significant decreases in overall fruit aroma, tropical and cat urine/sweaty attributes were also found. It was concluded that ibMP and the thiols have a mutual suppression on one another when present in combination (King *et al.,* 2011). The thiols, however, were not investigated separately in combination with ibMP. It is not yet determined if the effect that the combined thiols exhibit on ibMP is also true for an individual thiol or if one of the thiols has a greater effect on ibMP than the others. This study also only focussed on moderate and high concentrations of the compounds investigated and varying concentrations of thiols and methoxypyrazines and their interactions thus needs further attention.

Research also found evidence that thiols can influence the aroma expression of esters (Campo *et al.,* 2005; King *et al.,* 2011; Benkwitz *et al.,* 2012a). The volatile thiol, 3MHA, suppressed the sweet, floral and muscat aromas associated with the monoterpene, linalool, and the ester, 2-phenylethyl acetate (Campo *et al.,* 2005). A thiol mixture at high concentrations was found to decrease the confectionary attribute correlated with a mixture of esters. The combination of esters also showed an increase in other attributes associated with thiols, such as tropical, overall fruit aroma and cooked green vegetal (King *et al.,* 2011). Similar results were found where the omission of a group of esters led to decreases in attributes associated with volatile thiols such as cats pee, sweet-sweaty-passion fruit, passion fruit-skin-stalk, tropical, apple and stone fruit (Benkwitz *et al.,* 2012a).

Most sensory research regarding phenols has focussed on their effect on bitterness and astringency in wine (Noble, 1994; Gawel, 1998; Lund *et al.,* 2009a). More recent findings have shown that polyphenols can indirectly affect sensory expression of wines by interacting with the aroma compounds. A study by Lund *et al.,* (2009a), researched the effect three polyphenols (catechin, caffeic acid and quercitin) had on the perception of aroma compounds of Sauvignon blanc wines. It was found that the aromas associated with ibMP were suppressed by catechin, caffeic acid and to a lesser extent by quercetin. Catechin and quercetin suppressed the perception of 3MH but caffeic acid had the opposite effect. The study speculated that caffeic acid could be suppressing other aroma compounds and thus indirectly enhancing 3MH expression. The three polyphenols had an insignificant effect on 3MHA expression and all three was found to suppress the ester, ethyl decanoate (Lund *et al.,* 2009a). This study highlights the importance of the polyphenols as well as the matrix effect on complex sensory interactions occurring in Sauvignon blanc wines.

2.2 Sensory analysis of Sauvignon blanc wines

2.1.1 Introduction

Sensory analysis, especially descriptive analysis (DA), is an important tool for the evaluation of wine aroma (Stone & Sidel, 1993; Lawless & Heymann, 1998). Although chemical analysis can identify the type and amounts of aroma compounds present only sensory analysis can describe how they are expressed in the wine. Extensive knowledge can be gained from performing DA on wines. Various studies have tried to identify the sensory characteristics of Sauvignon blanc wines (Parr *et al.,* 2007; Lund *et al.,* 2009b; Benkwitz *et al.,* 2012b). It was found that the sensory profile of Sauvignon blanc wines are very complex and diverse. Many studies have focussed on describing the typicality of a Sauvignon blanc wine from a certain terroir, especially from France and New Zealand (Parr *et al.,* 2007; Pardon *et al.,* 2008; Cadot *et al.,* 2010). However, few studies have been done on the aroma profile of South African Sauvignon blanc wines. Further research is thus needed to characterise the distinct aroma and flavour of South African Sauvignon blanc wines.

2.2.2 Panel Selection

In order to perform any sensory analysis a panel must be used that has some familiarity with the product being tested. In some sensory analyses, like descriptive analysis, training of the panel is crucial while in others, like discrimination testing, it is less important. Panel members must be trained to distinguish and correctly identify certain aroma compounds or off-odours in wine, depending on the study. By giving the panellists references and examples of the wine that will be tested they will learn to characterize the wine using the correct terminology, also called descriptors or attributes. In the end the panellists must be able to detect the compounds of interest, define these compounds using the right descriptors and rate the intensity of the compounds (Meilgaard *et al.,* 1999; Jackson, 2002).

In order to ensure that the panel is sufficiently trained for the specific study, panel members have to be monitored. A few methods have been established to evaluate panel performance, such as Tucker plots and ANOVA models, but the best results are seen if these methods are used together. Tucker 1 plots can be used to assess each panel member’s performance in relation to a specific attribute or the whole panel. If a panellist struggles to identify a specific attribute or rates it differently to the rest of the panel, it will be illustrated by the Tucker 1 plot. Tucker 1 plots will also show if the panellists struggle to differentiate between different wine samples. ANOVA models can be used to test the importance of a certain attribute and the significance of it in the wines analysed (Tomic *et al.,* 2007, Tomic *et al.,* 2010).

2.2.3 Descriptive Analysis

Descriptive analysis is the most accurate and extensive type of sensory evaluation available to fully describe a certain product. This is useful with a product as complex as wine since training can teach the panellist to focus on the attributes of interest. Training is important in this type of test, especially to teach the panellist to use the right terminology to describe the product. Choosing the right descriptors can be difficult in wine since different people may perceive aromas in different manners in wine. The most accurate option is to refer back to the chemical compound causing the specific aroma or taint if known. 2-methoxy-3-isobutylpyrazine, for instance, can be used as a reference for the attribute green pepper since it is known that this volatile compound contributes to this aroma in Sauvignon blanc (Stone & Sidel, 1993; Lawless & Heymann*,* 1998; Meilgaard *et al.,* 1999).

Descriptive analysis generally uses an unstructured line scale to rate the attributes. This can be used to rate the intensities of different attributes in the wine. Wine can be chemically similar but some aroma compounds might be more prominent in different wines and descriptive analysis will define that (Stone & Sidel, 1993; Lawless & Heymann*,* 1998; Meilgaard *et al.,* 1999). Lund *et al.*, (2009b), used DA to identify 16 key aroma attributes for the description of Sauvignon blanc wines. By rating the intensity of these attributes they were able to obtain differences between vintage and country of origin (Lund *et al.,* 2009b). Similar results were found by Green *et al.,* (2011), who showed that sensory analysis could be used to distinguish between wines from France, New Zealand and Austria. The New Zealand Sauvignon blanc wines exhibited more green, boxwood, and passion fruit notes where the French wines had more flinty, smoky and mineral characteristics. The Austrian Sauvignon blanc wines were in between and strong ripe, fruity, stone fruit and tropical aromas were perceived (Green *et al.,* 2011).

DA can help to define a problem if one (Lawless & Heymann*,* 1998) is detected. For example, identifying and rating the intensity of a certain off-odour in wine. White wines spoiled by oxidation were analysed to determine the key odorants responsible for this spoilage (Silva Ferreira *et al*., 2003). DA have further been used to assess wines spiked with specific compounds, like methoxypyrazines, volatile thiols and esters, and to determine interaction between these compounds (Campo *et al.,* 2005; King *et al.,* 2011; Benkwitz *et al.,* 2012a).

2.3 Conclusion

This chapter focussed on the chemical composition and descriptive analysis of Sauvignon blanc wines. The compounds contributing to Sauvignon blanc aroma are a vast and complex group of chemical substances. The concentrations and interactions of these compounds influence the style of Sauvignon blanc wines. Methoxypyrazines and volatile thiols are major compounds impacting the aroma of Sauvignon blanc wines. Many other aroma compounds also contribute to the overall aroma including esters, monoterpenes, C_{13}-Norisoprenoids and higher alcohols. Simply assessing the chemical composition of a Sauvignon blanc wine is at this stage probably not adequate to predict the aroma and quality profile of the wine. Complex interactions between the methoxypyrazines, volatile thiols, esters and other chemical compounds may influence the aromas perceived in Sauvignon blanc wines. These interactions are still mostly unknown and unexplained and need further research. Sensory analysis, such as descriptive analysis, is an important tool and can be used to acquire more knowledge about the aroma profile of wines. By combining the chemical and sensory data a more comprehensive representation of the aroma profile of Sauvignon blanc wines can be obtained. Advances in analytical chemistry, sensory analyses and the relevant software in future could help to unravel the complexity of Sauvignon blanc wine.

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Chapter 3

Research results

Assessment of interaction between 3-mercaptohexan-1-ol and 2-isobutyl-3-methoxypyrazine in dearomatized sauvignon blanc wine

3. Assessment of interaction between 3-mercaptohexan-1-ol and 2-isobutyl-3-methoxypyrazine in dearomatized Sauvignon blanc wine.

3.1 Introduction

A number of studies have been performed on the major aroma compounds impacting Sauvignon blanc wines. The two main groups of impact compounds were found to be methoxypyrazines and volatile thiols (Allen *et al.,* 1991; Tominaga *et al.,* 2000; Swiegers *et al.,* 2006).

The three methoxypyrazines often found in Sauvignon blanc wine are 2-isobutyl-3-methoxypyrazine (ibMP), 2-isopropyl-3-methoxypyrazine (ipMP) and 2-*sec*-butyl-3-methoxypyrazine (sbMP) (Buttery *et al.,* 1969; Allen *et al.,* 1991; Lacey *et al.,* 1991; Marais, 1994). 2-Isobutyl-3-methoxypyrazine is quantitatively the most important methoxypyrazine in Sauvignon blanc wines. It is normally present in higher concentrations than ipMP and sbMP and has a detection threshold of 2 ng/L in water (Buttery *et al.,* 1969; Allen *et al.,* 1991; Lacey *et al.,* 1991; Marais, 1994; Marais *et al.,* 2004). 2-Isobutyl-3-methoxypyrazine can contribute to green pepper, asparagus, vegetative and herbaceous aromas in Sauvignon blanc wines (Marais, 1994; Marais *et al.,* 2004; Swiegers *et al.,* 2006; Alberts *et al.,* 2009). In South African Sauvignon blanc wines IbMP concentrations ranging from 0.4 ng/L to 44 ng/L were found (Alberts *et al.,* 2009).

The volatile thiols have been found to play a very important role in Sauvignon blanc aroma contributing to the tropical nuances (Tominaga *et al.,* 2000; Swiegers *et al.,* 2006). The three main volatile thiols found in Sauvignon blanc wine are 4-mercapto-4-methylpentan-2-one (4MMP) (Darriet *et al.,* 1995), 3-mercaptohexan-1-ol (3MH) and 3-mercaptohexyl acetate (3MHA) (Tominaga *et al.,* 1996; Tominaga *et al.,* 1998). The detection threshold of 3MH is 60 ng/L and concentrations ranging from 733 ng/L to 12 822 ng/L have been measured in French wines (Dubourdieu *et al.,* 2006; Swiegers *et al.,* 2009). Limited research has been done on the 3MH concentrations found in South African Sauvignon blanc wines. One study showed levels of 1013 ng/L to 2955 ng/L measured in six South African Sauvignon blanc wines (Lund *et al.,* 2009b). Another study found an average concentration of 1526 ng/L 3MH but again only six South African Sauvignon blanc wines were measured (Benkwitz, *et al.,* 2012b). 3MH has been found to contribute to grapefruit and passion fruit aroma in Sauvignon blanc wines (Dubourdieu *et al.,* 2006). In a study done by Mateo-Vivaracho *et al.,* (2010) various concentrations of thiols, including 3MH, were added to dearomatized neutral white wine. The samples were then evaluated by a sensory panel and the aroma differences between the concentrations were described. At 3MH concentrations close to the detection threshold (21 or 81 ng/L) the aroma was negligible but at 148 ng/L it contributed to fruitiness. Above 246 ng/L of 3MH sulphur and burnt-like aromas were detected and at 1497 ng/L 3MH started to contribute to the tropical fruit aromas (Mateo-Vivaracho *et al.,* 2010).

Wine is an extremely complex medium with many chemical compounds that interact and contribute to the aroma. (Marais, 1983) The aroma composition of Sauvignon blanc wines are still being researched (Lund *et al.,* 2009b; Marais & Swart, 1999). Evidence has been found of the masking effect of ibMP on fruity characteristics (King *et al.,* 2011) and the suppression of the floral-sweet aromas due to linalool and 2-phenylethyl acetate by 3MHA (Campo *et al.,* 2005). The interaction, either masking or synergistic, between the methoxypyrazines and volatile thiols, in combination and individually, are however still not clear and should be further researched.

Since the overpowering nature of methoxypyrazines at high concentrations is known (Alberts *et al.,* 2009; King *et al.,* 2011) there have been speculations about their synergistic or masking effect on other aroma compounds found in Sauvignon blanc wines. Marais and Swart (1999), added a single thiol, 4MMP, and a single methoxypyrazine, ibMP, in various concentrations to water and neutral wine. The aroma descriptors were generated by an expert panel but no interaction was determined. 4MMP and ibMP were found to contribute to the complexity of Sauvignon blanc wines and a synergistic effect was proposed (Marais & Swart 1999). Later research found that by adding a methoxypyrazine mixture (ibMP, ipMP and sbMP) to dearomatized wine spiked with 3MHA, changes in aroma could be observed. By increasing the concentration of the methoxypyrazine mixture added to the 3MHA samples, tropical fruit notes decreased significantly (Campo *et al.,* 2005). Since these results were found with a mixture of methoxypyrazines the effect of an individual methoxypyrazine on thiols were still unclear. King *et al*., (2011), researched the effect of moderate and high concentrations of ibMP and thiols (4MMP, 3MH and 3MHA) added to a base wine. It was found that not only did the ibMP suppress the overall fruit aroma and tropical attributes associated with the thiols, but the opposite was true as well. When the thiol concentrations were increased and the ibMP was kept constant the fresh green attribute associated with ibMP showed a decrease. This showed that the methoxypyrazines and thiols can mutually suppress each other (King *et al.,* 2011). Since this study only determined the combined effect of the thiols on ibMP their individual effect still needs to be researched. This study also looked at only moderate and high concentrations and further research is required to evaluate various concentrations to determine at which concentrations interaction occur.

Limited research has focussed on the interaction between different concentrations of the methoxypyrazines and volatile thiols. The concentrations at which interaction occur needs further attention. No research to date has focused on the interaction effects between 3MH and ibMP in terms of the intensity of aroma attributes of Sauvignon blanc wines. In this study the methoxypyrazine, 2-isobutyl-3-methoxypyrazine (ibMP), and the volatile thiol, 3-mercaptohexan-1-ol (3MH) were added to dearomatized, neutral Sauvignon blanc wine at five different concentrations. The combinations were evaluated by a trained panel using descriptive analysis. The concentrations chosen varied from close to the detection limit to higher levels found in Sauvignon blanc wines.

3.2 Materials and methods

3.2.1 Dearomatization of Sauvignon blanc wine

A 2011 Sauvignon blanc wine, deemed neutral by the winemaker of the commercial South African cellar where it was produced, was dearomatized according to a method described in Mateo-Vivaracho *et al.,* (2010). Approximately 80L of wine was dearomatized using Amberlite XAD-2 (Sigma –Aldrich) resin for the purpose of spiking it with aroma compounds and sensorially evaluating the effect. The Amberlite was first activated using methanol (Sigma-Aldrich) followed by repeated washing with distilled water as specified by the manufacturers. The Amberlite was added at a concentration of 4 g/L to the wine and left on a shaker for 24 hours of gentle stirring (Mateo-Vivaracho *et al.,* 2010). After the 24 hours stirring the wine was decanted to remove the majority of the Amberlite. Activated charcoal was then added at 0.5 g/L for further removal of aroma compounds. The wine was left on the activated charcoal overnight and filtered the following day with a sheet filter.

3.2.2 Determination of spiking concentrations

The two compounds used in this study were 3-isobutyl-2-methoxypyrazine (Interchim) and 3-mercapto-1-hexanol (Interchim). It was decided to use five concentrations, or levels, of each compound. The concentrations of 2-isobutyl-3-methoxypyrazine (ibMP) and 3-mercapto-1-hexanol (3MH) used were initially determined by using the sensory feedback of an expert panel of 8 people. All panel members were either staff or post graduate students at the Department of Viticulture and Oenology, Stellenbosch University. Eight different concentrations of ibMP and 3MH respectively, selected from levels occurring in Sauvignon blanc wine (Alberts *et al.,* 2009; Lund *et al.,* 2009b; Mateo-Vivaracho *et al.,* 2010; King *et al.,* 2011) were initially separately evaluated by the expert panel. The ibMP concentrations initially assessed were 0.5, 1, 2, 5, 10, 20, 30 and 40 ng/L, while 3MH was initially assessed at 20, 70, 150, 250, 1000, 1500, 2500 and 3000 ng/L. Five levels of each compound were then selected for further use in the sensory evaluation. The lowest concentration was chosen below or at the detection threshold and the highest at maximum concentrations found in Sauvignon blanc wines. Certain concentrations were discarded mostly on the basis that they were deemed sensorially too similar to others by the expert panel. The final concentrations that were used for ibMP were thus 0.5 ng/L (level 1), 1 ng/L (level 2), 2 ng/L (level 3), 15 ng/L (level 4) and 40 ng/L (level 5). The final 3MH concentrations used were 70 ng/L (level 1), 250 ng/L (level 2), 1500 ng/L (level 3), 3000 ng/L (level 4) and 6000 ng/L (level 5).

3.2.3 Spiking procedure

All dilutions were made with absolute ethanol (Sigma-Aldrich) and all pure and diluted solutions stored at -80°C. To test the purity of the pure 3MH standard the Ellman method was used. Two dilutions (S1 and S2) were made from the pure compound (Ellman, 1959). The concentration of the S2 solution was determined using Ellman's reagent (Ellman, 1959) and calculated as 811.8 mg/L. The S2 solution was prepared monthly and used as the stock solution. Further dilutions were made daily from the stock solution for spiking and the stock solution was stored at -80°C. An ibMP dilution was made from the pure compound and kept at -80°C throughout the sensory analysis. This was used as the stock solution daily to prepare a dilution for spiking.

Spiking was done daily not more than an hour before the sensorial testing in 250ml volumetric flasks. All the diluting and spiking was performed in a fume hood. Absolute ethanol corrections were made to ensure that each treatment (including the control wine with no spiking) received the same amount of ethanol to rule out any sensory impact of the ethanol concentration (King *et al.,* 2011). The five levels of ibMP were spiked in combination with the five levels of 3MH in all possible combinations. The five levels of each compound were individually presented to the judges as well. A control with no addition was also used. In total 36 samples were thus evaluated (Table 3.1).

Table 3.1 Sample codes, levels and concentrations of ibMP and 3MH used for the spiking of the 36 samples evaluated during sensory analysis.

Sample Name/ Code	ibMP Level	IbMP Concentration (ng/L)	3MH Level	3MH Concentration (ng/L)
0-0	0	0	0	0
0-1	0	0	1	70
0-2	0	0	2	250
0-3	0	0	3	1500
0-4	0	0	4	3000
0-5	0	0	5	6000
1-0	1	0.5	0	0
1-1	1	0.5	1	70
1-2	1	0.5	2	250
1-3	1	0.5	3	1500
1-4	1	0.5	4	3000
1-5	1	0.5	5	6000
2-0	2	1	0	0
2-1	2	1	1	70
2-2	2	1	2	250
2-3	2	1	3	1500
2-4	2	1	4	3000
2-5	2	1	5	6000
3-0	3	2	0	0
3-1	3	2	1	70
3-2	3	2	2	250
3-3	3	2	3	1500
3-4	3	2	4	3000
3-5	3	2	5	6000
4-0	4	15	0	0
4-1	4	15	1	70
4-2	4	15	2	250
4-3	4	15	3	1500
4-4	4	15	4	3000
4-5	4	15	5	6000
5-0	5	40	0	0
5-1	5	40	1	70
5-2	5	40	2	250
5-3	5	40	3	1500
5-4	5	40	4	3000
5-5	5	40	5	6000

3.2.4 Sensory descriptive analysis

The sensory panel consisted of seven females and one male between the ages of 31 and 58. Training was done twice a week in six sessions that lasted two hours each. In the first session of training attributes were generated by the panel using the actual samples to be used for testing. Panellists completed 12 hours of training in total. Panellists were trained for nine specific attributes, which included overall green, green pepper, asparagus, green grass, dusty, overall tropical, passion fruit, grapefruit and guava. For most of the attributes two reference standards were presented, one fresh reference and one soaked in dearomatized Sauvignon blanc wine (Table 3.2). Lund *et al.,* (2009a), found natural product reference standards better represented the complexity of the attributes than using a single chemical compound (Lund *et al.,* 2009b).

Table 3.2 Reference standards used during training of descriptive analysis panel.

Attribute , Aroma	Reference Standards
Green pepper	Fresh green pepper [ab]
Green grass	Freshly cut green grass [a]
Asparagus	Canned asparagus (Koo) [ab]
Overall Tropical	Tropical fruit juice (Ceres) [a]
Passion fruit	Fresh passion fruit [ab]
Guava	Fresh guava [ab]
Grapefruit	Fresh grapefruit [ab]

[a] fresh fruit reference

[b] fresh fruit soaked in dearomatized Sauvignon blanc

Testing was done in a well ventilated sensory laboratory at a temperature of 20 ± 2°C with individual booths. ISO glasses were used with a sample volume of ± 30 ml. Lids were placed on each glass just after pouring to prevent aroma loss or contamination of the sensory laboratory. Pouring was never done more than 30 minutes before testing because of the volatile nature of the compounds used for spiking. A 120 mm unstructured line scale was used to assess the intensity of each attribute from “none” to “intense”. Glasses were marked with three digit codes and samples randomized within each repetition. Purified water and unsalted crackers were used as palate cleansers. Samples were only evaluated based on aroma, not taste.

A total of 36 samples (table 3.1) were evaluated using descriptive analysis by the trained panel in triplicate. Testing was done in five two hour sessions. Six to eight samples were tested per session in triplicate with a 15 minute break between repetitions to minimize sensory fatigue. A complete block design was used with randomization within repetitions;

therefore all the samples were tested by all the judges. The judges rated the nine attributes previously described per sample.

3.2.5 Statistical analysis of data

Panel performance was assessed using PanelCheck® software (Version 1.4.0, Nofima, Ås, Norway). Principle component analysis (PCA) was conducted using the mean data after weighing and centring was applied (Unscrambler X, version 10.1, CAMO Inc., Oslo, Norway). Analysis of variance (ANOVA) was used to investigate significant differences and interaction between the two chemical compounds and response surface graphs were obtained with (Statistica, version 10, Statsoft Inc., Tulsa, USA). Further exploration of the data was conducted using Cytoscape 2.8.2 (Shannon *et al.,* 2003). A significance level of 95% was chosen ($p<0.05$).

3.3 Results and discussion

A mixed model ANOVA was used to assess the significance of the attributes. The ANOVA showed the attribute dusty to be non-significant. All the other attributes analyzed by the ANOVA showed significant differences between samples with $p<0.001$ (Figure 3.1). Tucker plots were used to assess the judges' performance in terms of the attributes. All judges were grouped together between the inner and outer ellipses for almost all the attributes. This shows good panel consensus, since 50-100% of the variance was explained by all judges (Figure 3.2).

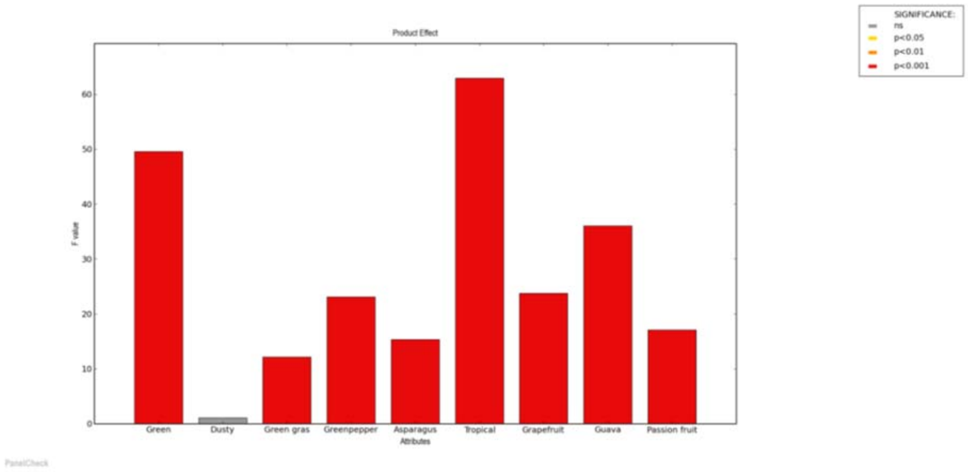

Figure 3.1 The product effect graph of the two-way ANOVA used to assess the panels' ability to discriminate between samples.

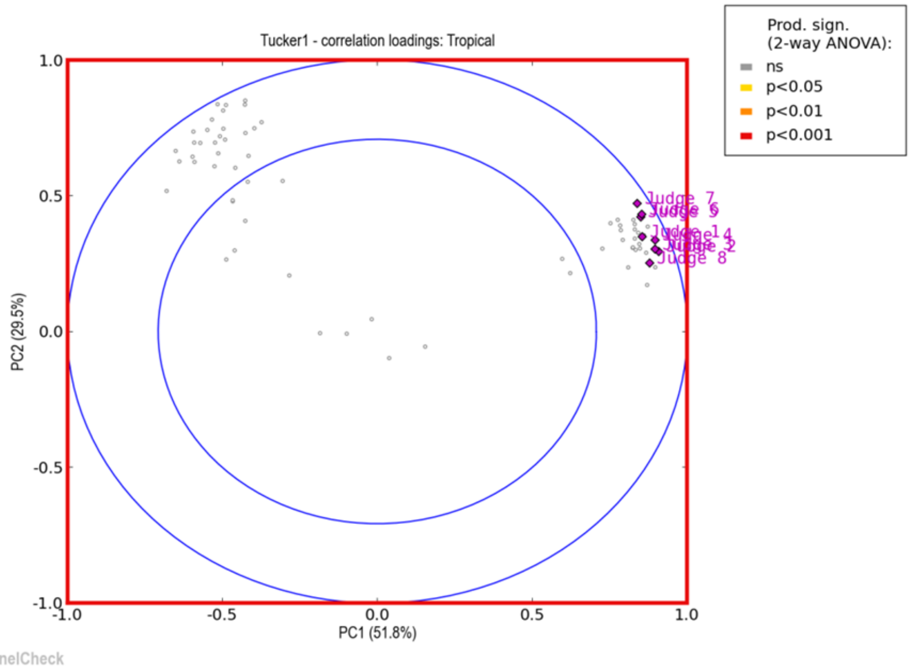

Figure 3.2 The Tucker plot for the tropical attribute to assess panel consensus.

Principle component analysis (PCA) was used to assess the variation between samples. In the PCA bi-plot of all the sensory data (Figure 3.3) 90% of the variance is explained by PC1 (57%) and PC2 (33%). The separation of the samples seen along PC1 is mainly based on the overall green, green pepper, asparagus, green grass and dusty on the right of the PCA plot, compared with the overall tropical, passion fruit, grapefruit and guava attributes on the left side.

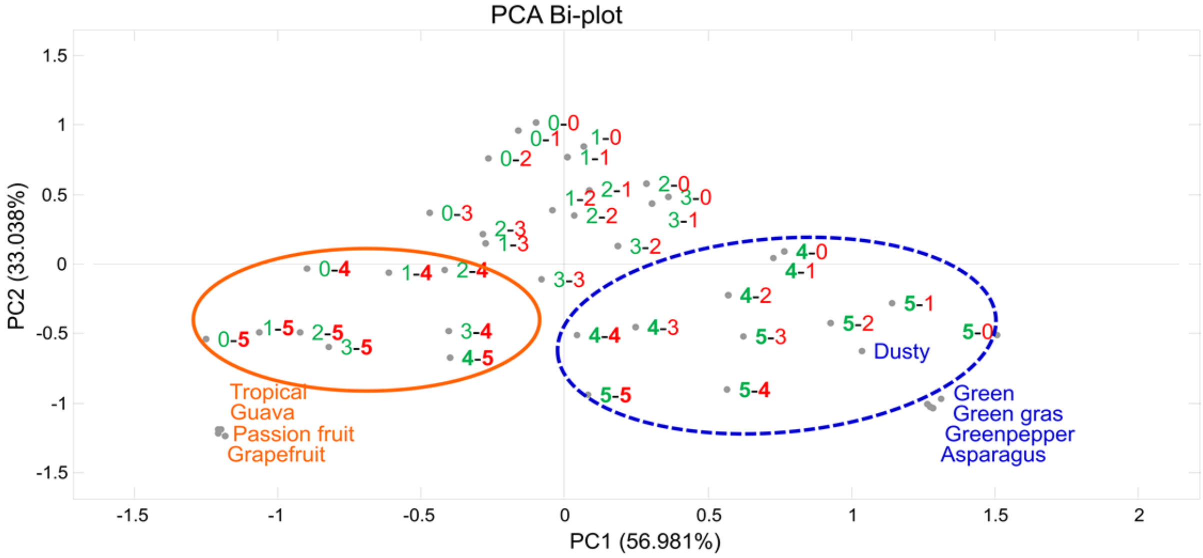

Figure 3.3 PCA Bi-plot of the combined sensory results of all the different combinations of ibMP and 3MH.

King *et al.,* (2011), showed similar results with a separation observed between fresh green, green flavour and cooked green vegetal attributes and overall fruit aroma, tropical and confectionary attributes. Attributes differed slightly from our study because only moderate and high concentrations were assessed in the study of King *et al.,* (2011), and esters were also considered. A positive correlation was seen in our study between the overall green, green pepper, asparagus and green grass attributes and the samples with higher levels of ibMP. The samples with higher levels of 3MH showed a positive correlation with the overall tropical, passion fruit, grapefruit and guava attributes, which grouped together. A previous study also showed that ibMP correlated with green attributes while 3MH enhanced tropical and citrus flavour (King *et al.,* 2011). Most of the intermediate levels (levels 1 to 3 of ibMP and 3MH) were situated in the centre of the PCA bi-plot. This showed that the intermediate samples had the same intensity for both the green and tropical attributes. The separation seen along PC2, which explained 33% of the variance, can be ascribed to the intensity of the perceived attributes. The levels of both the compounds increased from the bottom to the top of the PCA bi-plot.

Analysis of variance graphs were also used to assess the data. The data from the samples with no added 3MH and with level 0 to 5 of ibMP was plotted for all the attributes. It can be seen from the corresponding graph (Figure 3.4) that the overall green, green grass, green pepper and asparagus attributes significantly increased as the concentration of ibMP increased. The overall green and green pepper attributes also seemed to be the dominant attributes contributing to ibMP's aroma. These attributes showed the largest increase in intensity as the ibMP concentrations increased. The attributes overall tropical, grapefruit, guava and passion fruit showed no correlation with an increase in ibMP concentration. Similar results were found by Parr *et al.,* 2007, where ibMP correlated positively with grassy, green capsicum and herbaceous attributes. Other studies found samples containing ibMP to have green attributes (King *et al.,* 2011) or grassy, green pepper and herbaceous nuances (Marais & Swart, 1999).

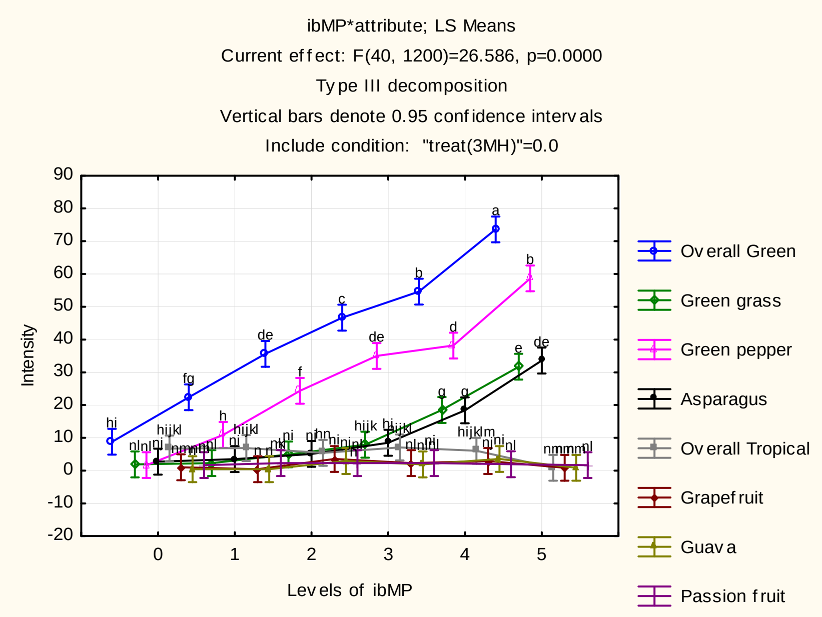

Figure 3.4 Intensities of attributes at different ibMP levels and containing no 3MH.

The samples containing no ibMP, but increasing levels of 3MH (Figure 3.5.) led to sharp increases of overall tropical, grapefruit, guava and passion fruit attributes, while having no effect on the overall green, green grass, green pepper and asparagus attributes. This showed that 3MH strongly correlated with the overall tropical, grapefruit, guava and passion fruit attributes as supported by literature (Duboubieu *et al.*, 2006; Swiegers *et al.*, 2009; King *et al.,* 2011). The overall tropical and guava attributes showed the largest increase in intensity as the 3MH concentrations increased. This showed that the overall tropical and guava attributes are the leading attributes contributing to 3MH's aroma. Few studies have included guava as an attribute for 3MH during sensory descriptive analysis. Although Swiegers *et al.,* (2006), proposed that 3MH has guava type aromas no statistical data is shown. In the current study the guava attribute was clearly correlated with increasing 3MH concentrations and showed to be one of the most dominant attributes of 3MH. This correlation can be due to the fact that a South African panel may be more familiar with the aroma of guava than for instance a French panel (Valentin, 2012).

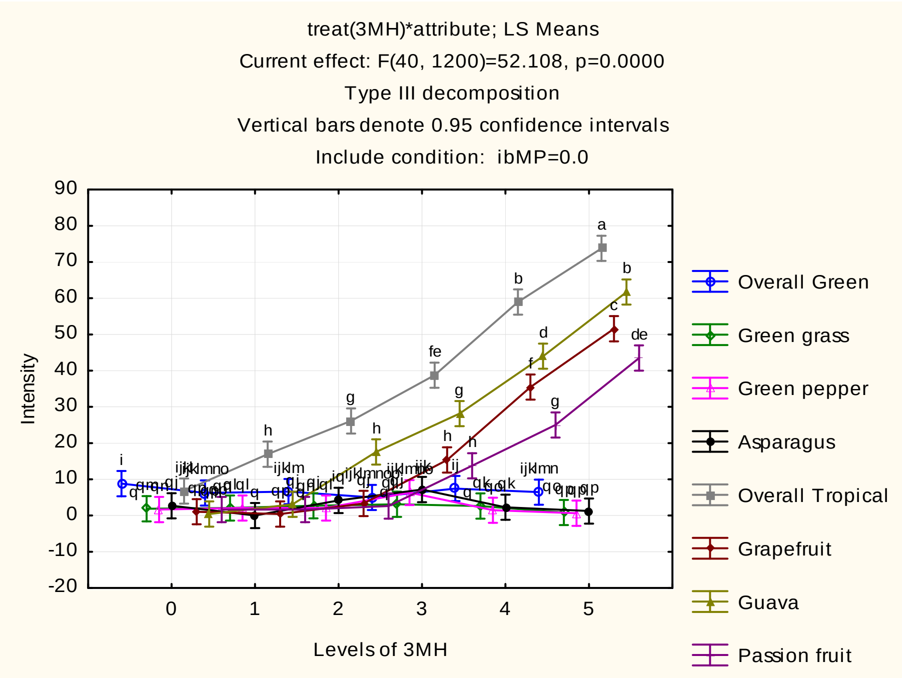

Figure 3.5 Intensities of attributes at different 3MH levels and containing no ibMP.

The PCA analysis showed the separation of all the data according to the attributes but no interaction could be seen with this statistical analysis. Various other statistical approaches were thus further used to analyse and visualise the interaction between ibMP and 3MH. Response surface graphs, ANOVA graphs and Cytoscape networks were used to represent the data.

The response surface graphs (Figure 3.6-3.13) are three dimensional representations of the data. The response surface graphs for the overall green attribute (Figure 3.6) and the grapefruit attribute (Figure 3.12) are shown as examples. The remaining graphs are given in appendix A. The three variables showed in the graphs are the 3MH levels, the ibMP levels and their effect on a specific attribute. The graphs indicated how the intensity of the attributes changed with changing concentrations of both 3MH and ibMP. The intensity of the green attributes (overall green, green grass, green pepper and asparagus (Figure 3.6-3.9) that correlated with ibMP showed a decrease as the concentration of 3MH increased. The amount that the intensity decreased for each attribute is different. The same trend is seen for the overall tropical, guava, grapefruit and passion fruit attributes (Figure 3.10-3.13) that correlated with 3MH. Decreases in the intensity of these attributes are seen as the ibMP concentration increased. The degree of decrease, as was the case with the green attributes, also differed per attribute. These decreases are also more clearly seen at the higher concentrations of 3MH and ibMP respectively. The response surface graphs do not show significant suppression but only show a trend that mutual suppression between 3MH and ibMP is possible. Similar results have been found by King *et al*., (2011), between ibMP and a combination of volatile thiols. These findings are more clearly explained by further statistical analysis using ANOVA and Cytoscape.

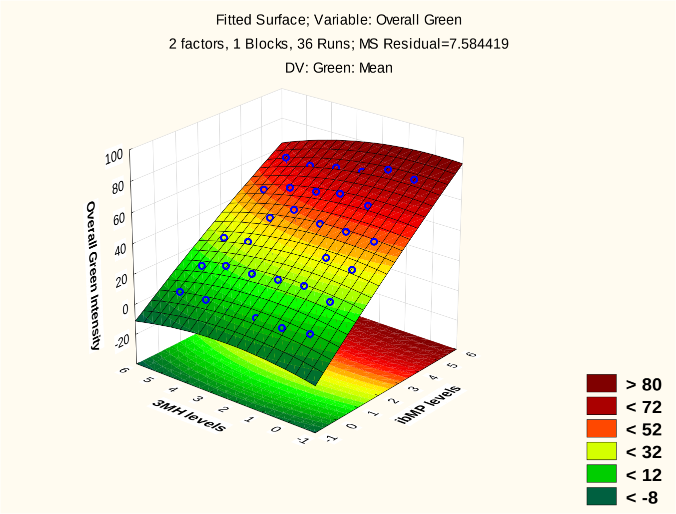

Figure 3.6 Response surface graph of the overall green attribute obtained from samples spiked with 3MH and ibMP at various levels.

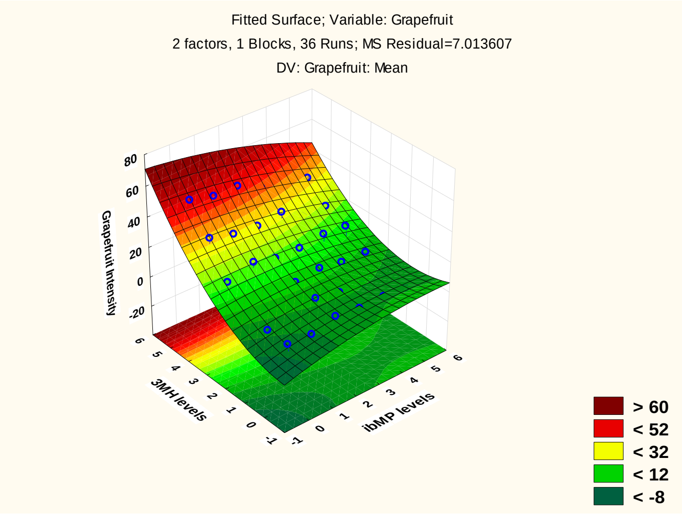

Figure 3.12 Response surface graph of the grapefruit attribute obtained from samples spiked with 3MH and ibMP at various levels.

Figure 3.14 to 3.21 showed two way ANOVA plots of the interaction between 3MH and ibMP for the different attributes. The attributes overall tropical, guava, grapefruit and passion fruit showed increasing intensity as the 3MH concentration increased (Figure 3.14-3.17). The same result was seen in the ANOVA graphs compiled of the samples containing only 3MH. Decreases in the intensity of the overall tropical, guava, grapefruit and passion fruit attributes were seen when comparing level 0 and 5 of ibMP at specific 3MH levels. However the level of 3MH where these decreases were significant was different for each attribute. The overall tropical and passion fruit attributes showed significant decreases at level 3, 4 and 5 of 3MH. However, decreases in the guava and grapefruit attributes were only seen at level 4 and 5 of 3MH. This confirmed that ibMP suppressed the attributes associated with 3MH and that some attributes were more affected than others. The overall green, green pepper, green grass and asparagus attributes correlated with ibMP and increases in their intensity was seen with increasing ibMP concentration. Decreases in the intensity of the attributes associated with ibMP were seen when comparing level 0 and 5 of 3MH at certain ibMP levels (Figure 3.18-3.21). The levels of ibMP at which a decrease induced by level 5 of 3MH differed between green associated attribute. The overall green attribute showed significant decreases at level 2, 3, 4 and 5 of ibMP and the green pepper attribute at level 3 to 5 of ibMP. The green grass and asparagus attributes only showed decreases at level 4 and 5 of ibMP when levels 0 and 5 of 3MH were compared. This showed that the intensity of the attributes associated with ibMP was suppressed by 3MH, but this normally occurred at medium higher levels of ibMP (3 to 5).

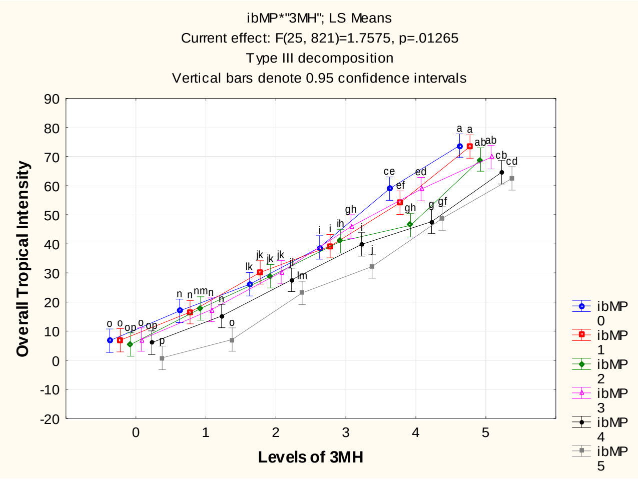

Figure 3.14 Effect of different 3MH and ibMP levels on the intensity of the overall tropical attribute.

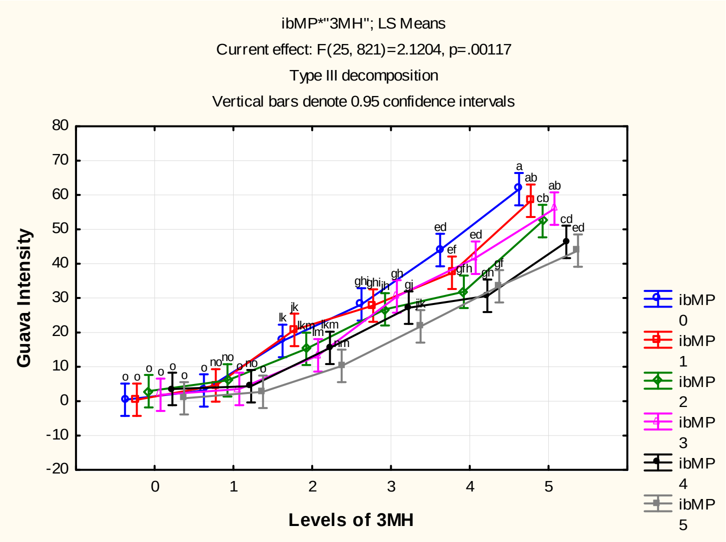

Figure 3.15 Effect of different 3MH and ibMP levels on the intensity of the guava attribute.

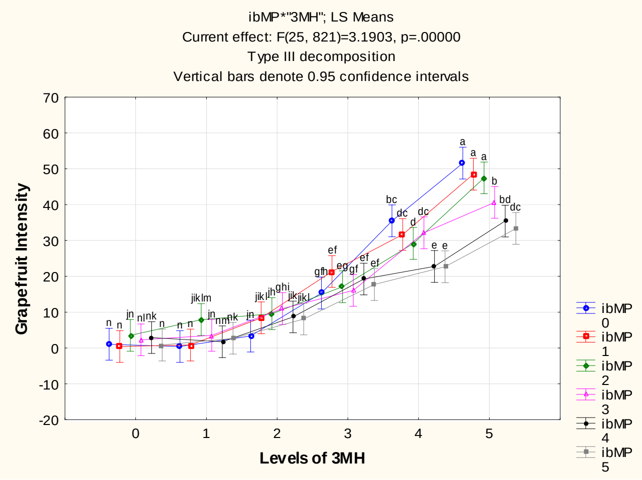

Figure 3.16 Effect of different 3MH and ibMP levels on the intensity of the grapefruit attribute.

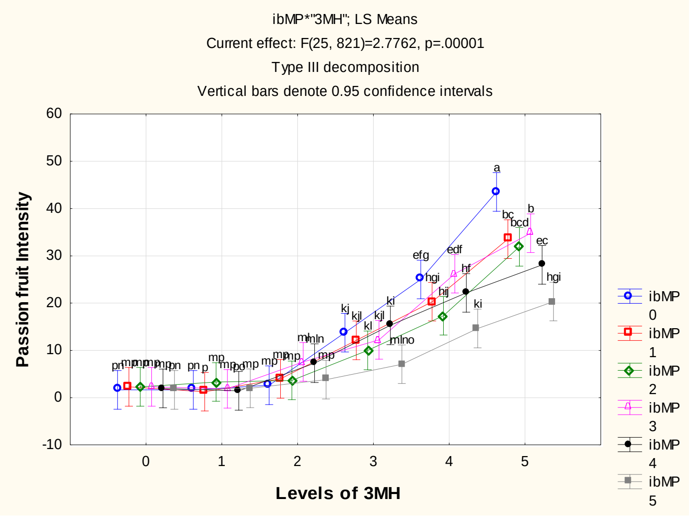

Figure 3.17 Effect of different 3MH and ibMP levels on the intensity of the passion fruit attribute.

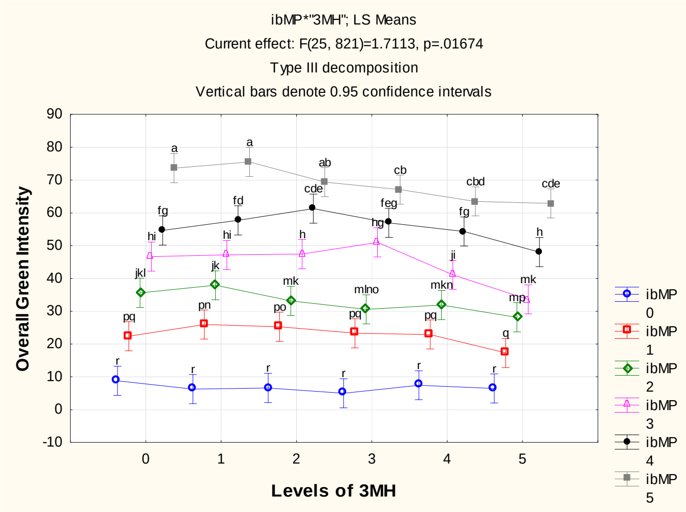

Figure 3.18 Effect of different 3MH and ibMP levels on the intensity of the overall green attribute.

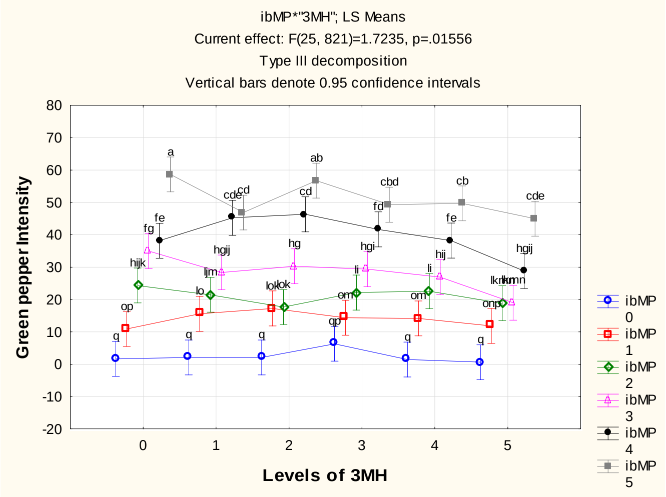

Figure 3.19 Effect of different 3MH and ibMP levels on the intensity of the green pepper attribute.

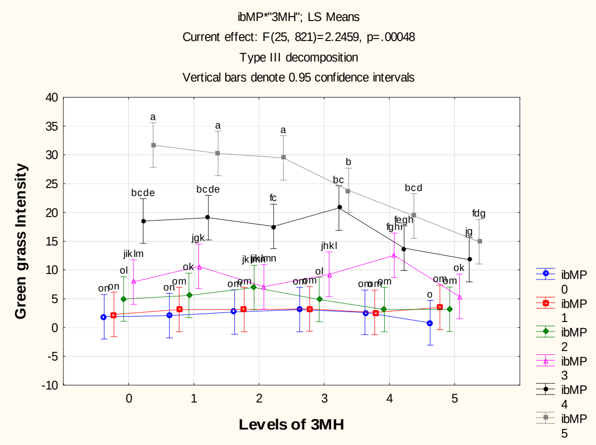

Figure 3.20 Effect of different 3MH and ibMP levels on the intensity of the green grass attribute.

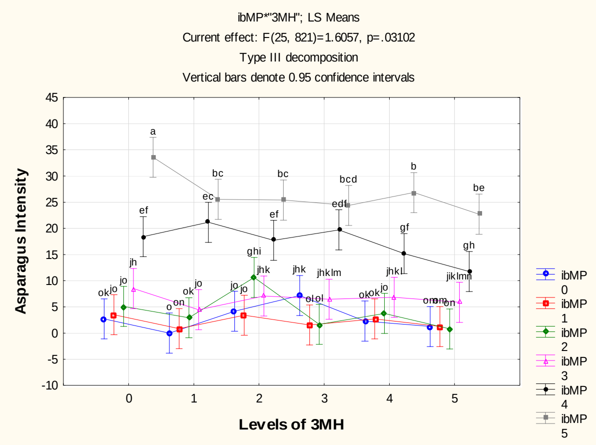

Figure 3.21 Effect of different 3MH and ibMP levels on the intensity of the asparagus attribute.

A two-dimensional network was also compiled of all the data using Cytoscape as well. This is a novel approach for the evaluation of sensory data. Cytoscape is mostly used to integrate biomolecular interaction networks (Shannon *et al.,* 2003). Various sub networks were identified as being the most important and showing the most interactions. Three main sub networks are presented which showed the most interactive connections. These are the sub networks where 3MH is kept constant at its highest (0-5, Figure 3.22), second highest concentration (0-4, Figure 3.23) and ibMP is kept constant at its highest concentration (5-0, Figure 3.24.). The Cytoscape sub networks compare the concentration that is kept constant (the centre point) to the other interaction samples. The attributes that are affected by the samples and the samples that significantly differ from the centre point with a certain fold decrease are shown on the sub network. The fold decrease is represented by a gradual colour scale but to visually represent the degree of decrease better, shapes were used. The fold decrease in the figures was represented by a circle (1-1.39), hexagon (1.4-1.9) and square (1.9-2.2) and the intervals chosen were based on the colour of the fold decrease. It was seen that at higher concentrations a higher decrease in the intensity of most of the attributes were found.

When the 3MH level was kept constant at its highest concentration (level 5) differences in the perception of the attributes passion fruit, overall tropical, guava and grapefruit were seen across various ibMP concentrations (Figure 3.22). The passion fruit attribute was shown to be the easiest suppressed by increasing ibMP concentrations. Decreases in the intensity of passion fruit perceived were already seen at level 1 of ibMP. The passion fruit attribute was also decreased the most at the highest concentrations of ibMP. The attribute overall tropical was affected from level 2 of ibMP. The grapefruit and guava attributes were shown to be less affected by increasing ibMP concentrations. The perception of grapefruit was only decreased from level 3 of ibMP and upward and that of guava's only from level 4 or higher of ibMP.

When 3MH was kept constant at its second highest concentration less interaction was seen (Figure 3.23). The attributes passion fruit, overall tropical, grapefruit and guava were all suppressed at level 2 and 5 of ibMP. Level 4 of ibMP also decreases the guava and overall tropical attributes. Level 3 of ibMP did not show significant interaction with level 4 of 3MH. This can indicate that with sample 3-4 the balance between the green and tropical attributes was so similar that no significant suppression has been observed by the panel.

In the sub network where the level of ibMP was kept constant at its highest concentration various interactions were observed (Figure 3.24). The asparagus attribute was suppressed at all levels of 3MH except level 3. The perception of the green pepper attribute only decreased at levels 1, 3 and 5 of 3MH. The green grass attribute's perception was decreased from level 3 of 3MH onward. The fold decrease also became larger as the concentrations of 3MH increased for the green grass attribute. The overall green attribute seems to be the least affected by increasing 3MH concentrations and interaction was only seen at level 5 of 3MH. Certain intermediate levels (5-2, 5-3) were not shown on the sub network for some of the attributes. This might be due to an equilibrium reached during the mutual suppression of 3MH and ibMP. The interaction at these combinations might not be humanly perceived because of this equilibrium. Similar trends were seen using Cytoscape and the ANOVA graphs but because the statistical approach differs minor variations were observed. The Cytoscape sub networks were compiled using a very specific approach and focussed on one central concentration where as the ANOVA graphs showed a more general approach and compared all the concentrations. However, the minor discrepancies observed did not change the outcome of the results.

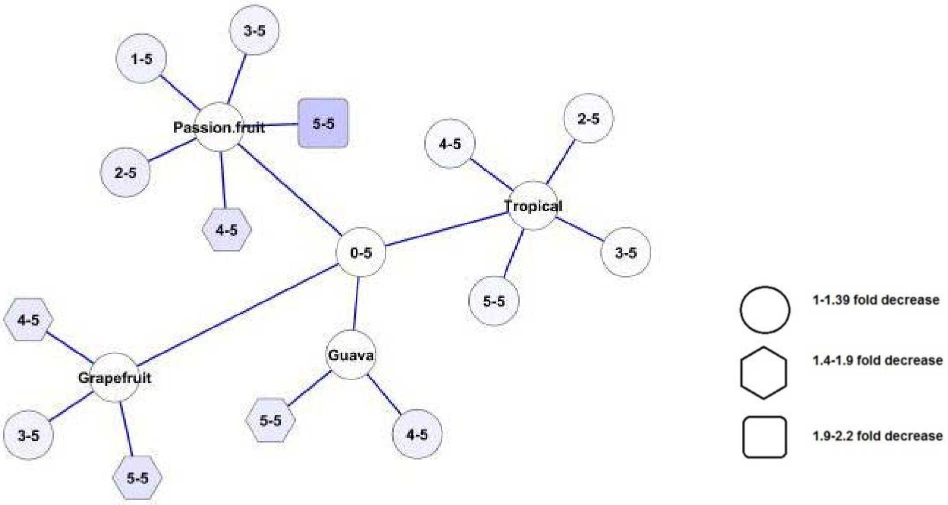

Figure 3.22 The Cytoscape sub network showing the effect of different levels of ibMP on the attributes associated with 3MH at level 5.

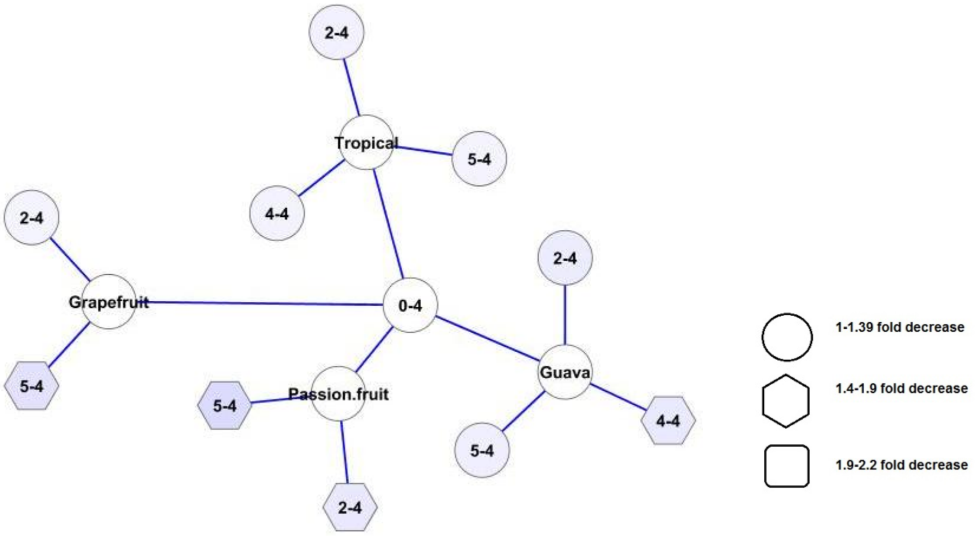

Figure 3.23 The Cytoscape sub network showing the effect of different levels of ibMP on the attributes associated with 3MH at level 4.

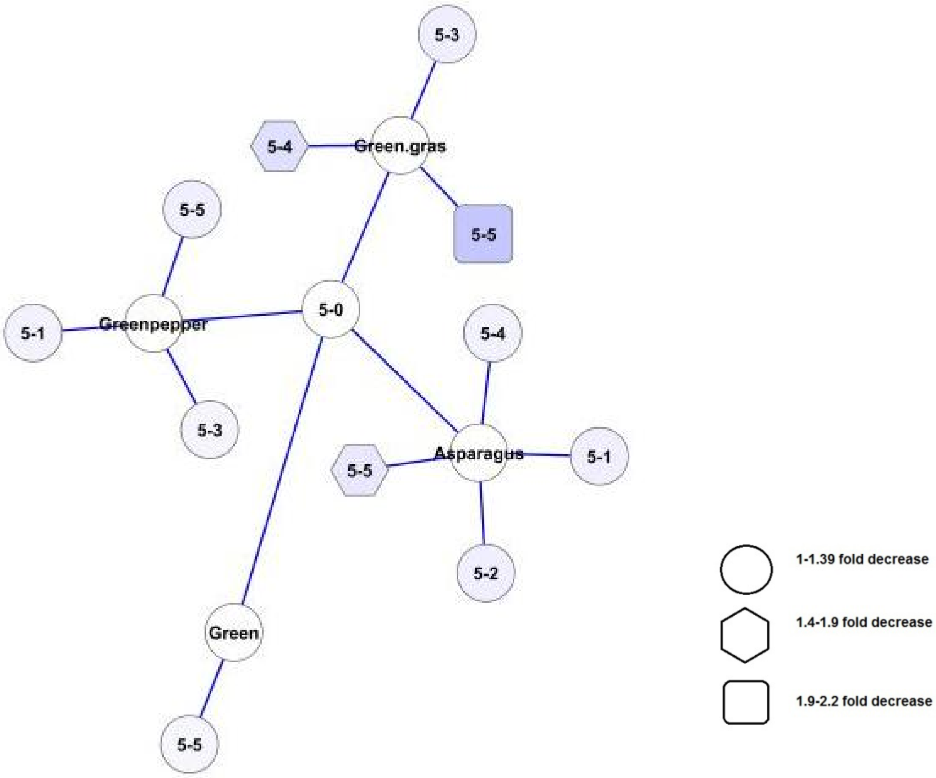

Figure 3.24 The Cytoscape sub network showing the effect of different levels of 3MH on the attributes associated with ibMP at level 5.

This work showed clear suppression between ibMP and 3MH at certain levels. Previous research has found evidence of the suppressive nature of ibMP (Campo *et al.*, 2005; King *et al.,* 2011) and the concept of mutual suppression has been suggested by King *et al.,* (2011). Campo *et al*., (2005), found that by adding an increasing concentration of a methoxypyrazine mixture (ibMP, ipMP and sbMP) to dearomatized wine spiked with 3MHA the tropical fruit notes decreased (Campo *et al.,* 2005). Another study found that when the ibMP concentration was doubled to 20 ng/L and the thiol concentration (3MH, 3MHA and 4MMP) kept constant the overall fruit aroma and tropical attributes decreased significantly (King *et al.,* 2011). The suppressive effect was seen as decreases in the intensity of attributes associated with ibMP and 3MH respectively. King *et al.* (2011) also found that 3MH increased citrus flavour and tropical attributes (King *et al.,* 2011). Esters have been found to enhance tropical aromas associated with thiols (King *et al.,* 2011; Benkwitz *et al.,* 2012a), while phenols were also shown to influence the perception of flavours associated with ibMP and certain thiols (Lund *et al*., 2009a). A recent study found that ibMP is suppressed by *β*-damascenone but that some attributes associated with the volatile thiols were enhanced by it (Benkwitz *et al.,* 2012a).

Wine is a complex mixture of a vast number of compounds which may affect the perception of a certain flavour. The matrix effect of wine on its perception should not be underestimated (Ferreira *et al.,* 2000; Saenz-Navajas *et al.,* 2010). Certain attributes associated with 3MH and ibMP seem to be less sensitive towards suppression than others. This also needs to be assessed using 3MHA and 4MMP in combination with important methoxypyrazines in wine, as these two thiols have low detection limits in wine (Dubourdieu *et al.,* 2006). Further investigation is required to explain this phenomenon, as the lexicon of wine is important for marketing purposes. It is envisioned that this study could further help to clarify the role that these two impact aroma compounds play at different levels in the sensory attributes of Sauvignon blanc wine.

3.4 Conclusion

It was established in this study that ibMP and 3MH suppressed each other at certain concentrations. Specific attributes of one compound was suppressed at certain concentrations of the other, with some attributes being more sensitive than others. The largest interaction was seen when the highest levels of these two compounds were assessed. By looking at a wide range of concentrations of 3MH and ibMP greater understanding was obtained of the concentrations at which suppression occurred and which attributes were affected by it. Further research is needed to investigate the individual and combined effect of other thiols and methoxypyrazines to fully understand Sauvignon blanc aroma. The current study could contribute valuable information on the understanding of Sauvignon blanc flavour related to its chemical composition. Commercial wine farms in South Africa are aiming to produce Sauvignon blanc wines with either green or tropical styles or a mixture of these. It could be proposed that manipulating the vineyard and wine to produce higher concentrations of methoxypyrazines and volatile thiols may lead to suppression of a certain aroma in the wines. Further research in this field could predict optimal concentrations of certain compounds to create a specific style of Sauvignon blanc wine.

3.5 References

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3.6 Appendix A

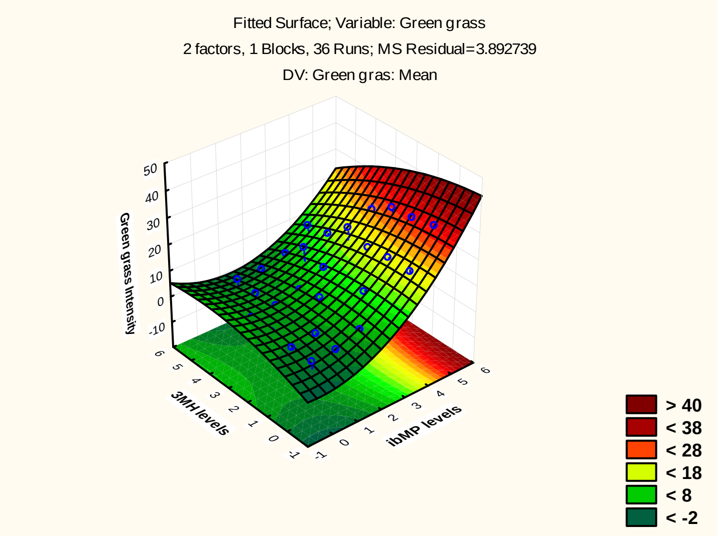

Figure 3.7 Response surface graph of the green grass attribute obtained from samples spiked with 3MH and ibMP at various levels.

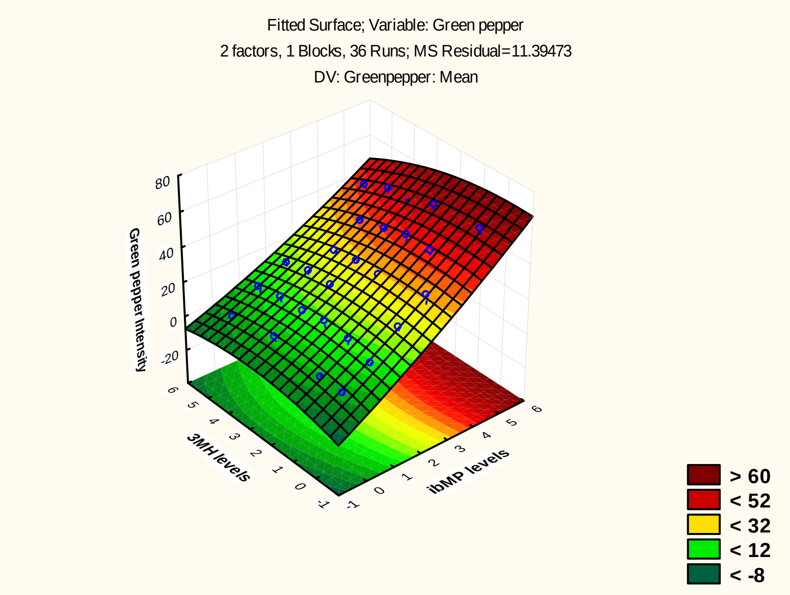

Figure 3.8 Response surface graph of the green pepper attribute obtained from samples spiked with 3MH and ibMP at various levels.

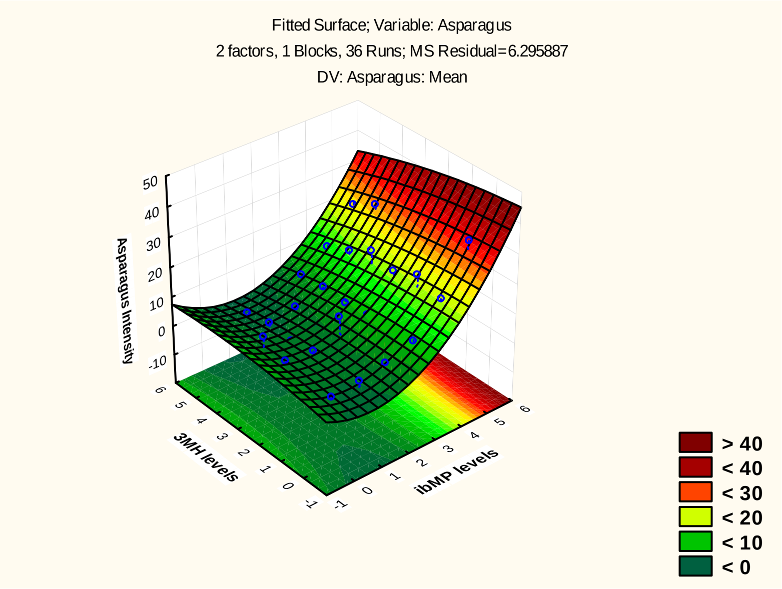

Figure 3.9 Response surface graph of the asparagus attribute obtained from samples spiked with 3MH and ibMP at various levels.

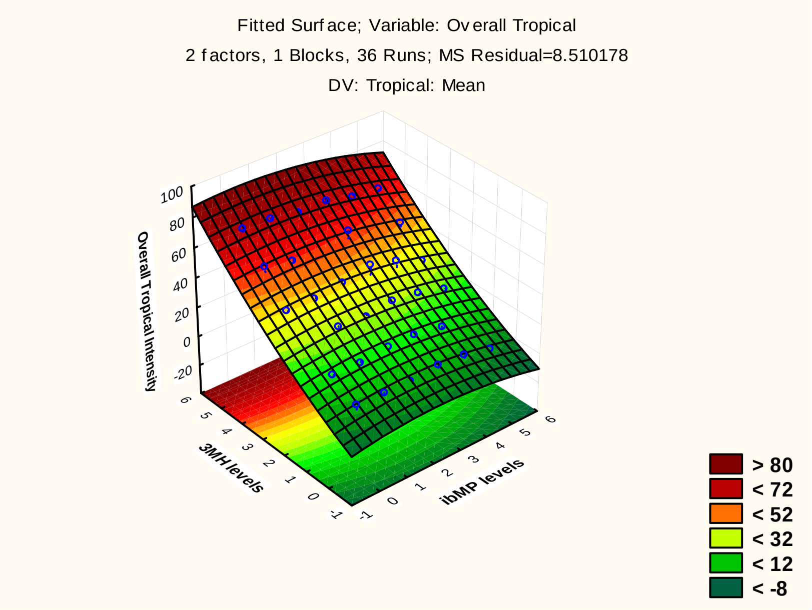

Figure 3.10 Response surface graph of the overall tropical attribute obtained from samples spiked with 3MH and ibMP at various levels.

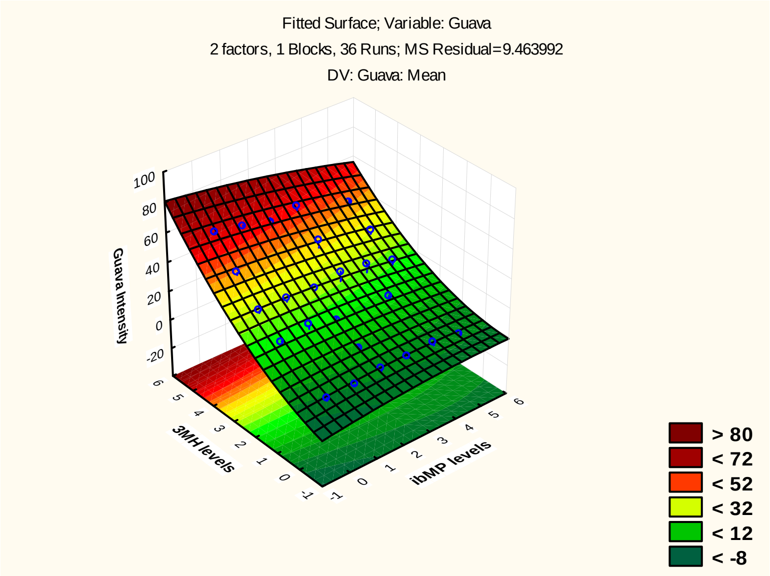

Figure 3.11 Response surface graph of the guava attribute obtained from samples spiked with 3MH and ibMP at various levels.

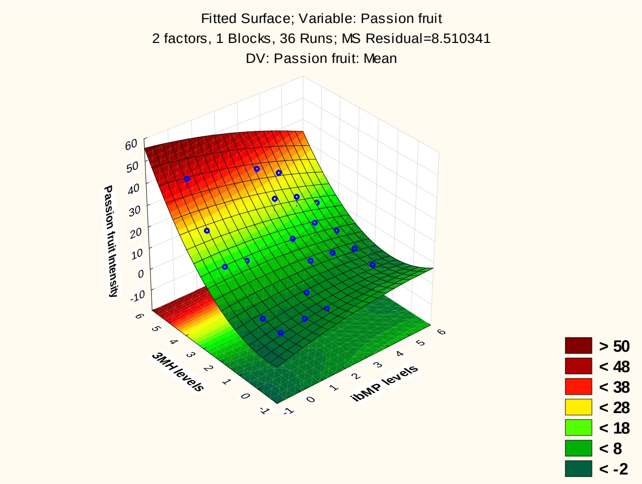

Figure 3.13 Response surface graph of the passion fruit attribute obtained from samples spiked with 3MH and ibMP at various levels.

Chapter 4

Research note

Preliminary results on interaction between volatile thiols and 2-methoxy-3-isobutylpyrazine in commercial South African Sauvignon blanc wines

4. Research note: Preliminary results on interaction between volatile thiols and 2-methoxy-3-isobutylpyrazine in commercial South African Sauvignon blanc wines

4.1 Introduction

In the South African wine industry Sauvignon blanc wines are becoming increasingly popular. Often either tropical or green flavours are perceived in Sauvignon blanc wine. The tropical flavours are strongly influenced by the volatile thiols 4-mercapto-4-methylpentan-2-one (4MMP), 3-mercaptohexan-1-ol (3MH) and 3-mercapto-hexylacetate (3MHA) (Tominaga *et al.,* 1998). Methoxypyrazines contributes towards the green style of which 2-methoxy-3-isobutylpyrazine (ibMP), 2-methoxy-3-isopropylpyrazine (ipMP) and 2-methoxy-3-*sec*-butylpyrazine (sbMP) are dominant in Sauvignon blanc wines (Lacey *et al.,* 1991; Marais, 1994). Recently South African wine producers started to move away from one dimensional wines and are striving to produce more complex Sauvignon blanc wines. Volatile thiols and methoxypyrazines, together with all the other flavour compounds in Sauvignon blanc wine, have the potential to act together to create a balanced product.

Although extensive knowledge on methoxypyrazine levels in South African wines are available, very little is known on the volatile thiol concentrations. Marias *et al.,* 2004 have found ibMP concentrations ranging from one to 14 ng/L in South African Sauvignon blanc wines (Marais *et al.*, 2004), while another study found concentrations ranging from 0.4 ng/L to 44 ng/L (Alberts *et al.,* 2009). A study done in New Zealand compared the volatile thiol content of six South African Sauvignon blanc wines from Stellenbosch to those from other countries. Levels ranging from 1013 to 2955 ng/L for 3MH and 10 to 119 ng/L for 3MHA were found (Lund *et al.*, 2009b). Another New Zealand study compared Sauvignon blanc wines from around the world and measured six South African wines as well. Average values of 38 ng/L for 3MHA, 1526 ng/L for 3MH and 6.6 ng/L for 4MMP were measured (Benkwitz *et al.,* 2012b). Although these results are relevant, a larger pool of South African Sauvignon blanc wines needed to be assessed for their volatile thiol concentrations.

Although the volatile thiols and methoxypyrazines are impact compounds of Sauvignon blanc wines, other aroma compounds also play a role. Various studies have looked at the sensory interaction between the aroma compounds of Sauvignon blanc wines (Campo *et al.,* 2005; King *et al.*, 2011; Benkwitz *et al.,* 2012a). The methoxypyrazines and volatile thiols could mutually suppress the aroma attributes associated with each other (King *et al.,* 2011). Certain esters were found to increase certain attributes associated with volatile thiols (King *et al.,* 2011; Benkwitz *et al.,* 2012a), while the volatile thiols could suppress the character of esters (Campo *et al.,* 2005; King *et al.,* 2011). A recent study showed that the character of the volatile thiols was enhanced by *β*-damascenone and that the herbaceous aromas of ibMP could be suppressed by it (Benkwitz *et al.,* 2012a). Lund *et al.,* (2009a) showed that the polyphenols could also suppress the perception of certain volatile thiols, esters and methoxypyrazines (Lund *et al.,* 2009a). The current study investigated the volatile thiol and methoxypyrazine content of commercial South African Sauvignon blanc wines and their sensorial expression. Descriptive analysis was also used to generate various sensory characteristics of the wines. The possible sensory interaction between volatile thiols and 2-methoxy-3-isobutylpyrazine was investigated in commercial Sauvignon blanc wines as well.

4.2 Materials and methods

4.2.1 Wine Selection

It was decided to focus mainly on premium and ultra premium South African Sauvignon blanc wines, but four less expensive Sauvignon blanc wines were also included for comparison (Table 4.1). The 20 premium and ultra premium Sauvignon blanc wines were chosen based on a few factors including prizes won at wine tasting competitions and the reputation of the producer. In total 24 Sauvignon blanc wines were chosen from various wine producing areas in South Africa. The wines were all unwooded and from the 2011 vintage.

Table 4.1 The wines investigated and the codes assigned during sensory and chemical analyses

Producer	Code
Altydgedacht	Alt
Neethlingshof	Neet
Phizante Kraal	PZK
Diemersdal	Diem
Bergkelder Fleur du Cap	FdC
Spier Lonely Blue Gum	Gum
Neil Ellis	NE
Vergelegen	Verg
Hermanuspietersfontein No.3	HPF
Dawid Nieuwoudt Ghost Corner	DN
Cederberg Cellar Driehoek	Drie
Groot Constantia	GC
Springfield Life from Stone	Spr
Klein Constantia	KC
Anura	Anu
Graham Beck Pheasant's Run	Gbec
Tokara	Tok
Kleine Zalze	Dzal
De Grendel	Dgre
Overhex Soulo	Hex
Du Toitskloof *	Dtoit
Robertson *	Rob
Two Oceans *	2Oc
Flutterby *	Flut

* Less expensive wines used.

4.2.2 Volatile Thiol Analysis

The sample preparation and analysis for the volatile thiols (3MH, 3MHA and 4MMP) was done according to Suklje *et al.,* 2012, which is based on the method first described by Tominaga *et al.,* in 1998. Deuterated standards of 3MH and 3MHA were used as internal standards for the corresponding thiols and 4-methoxy-2-methyl-2-mercaptobutane (4MMB) was used as internal standard for 4MMP. The internal standards (25 µL) were added to 50 mL of wine sample with 5 mL of *p*-hydroxymercuribenzoic acid (*p*HMB) and 0.5 mL butylated hydroxyanisole (BHA). The *p*HMB acted as the binding agent when the solution was put through the anion exchange column consisting of Dowex (Sigma-Aldrich). A 50 ml of 50 mM cysteine solution was used to elute the thiols from the column before extraction with dichloromethane. The samples were concentrated using a Rotavapor R-114 (Büchi, Switzerland) to a final volume of 30 to 50 µL. The concentrated samples were analyzed on a Gas Chromatograph (Agilent Technologies 7890A) with a Mass Spectrometry (MS) detector (Agilent Technologies 5975C) upgraded with a Triple-Axis Detector. A HP Innowax column (60 m x 0.25 mm x 0.25 µm) was used with an injection temperature of 240 °C. The oven temperature per minute is shown in table 4.2. The carrier gas used was Helium and the total run time per sample was 81 minutes.

Table 4.2 The temperature programming during the sample run time.

Time (minutes)	Temperature (°C)
0	0
5	50
26.67	115
27.54	150
48.88	205
53.38	250
78	50
81	0

4.2.3 Methoxypyrazine analysis

Methoxypyrazine analysis was outsourced to an accredited laboratory (VinLAB Pty Ltd, Stellenbosch, South Africa). Gas chromatography (Varian 3900GC) with a mass spectrometry (Varian, Saturn 2100T) as detector was used to perform the analysis. A COMBIPAL-xt auto sampler was used together with a split/splitless 1177 injection port. The separation was done using a CPWAX52 column (30 m x 0.25 mm x 0.25 µm) from Aligent J&W. Helium was used as the carrier gas at 1.3 mL/min. A temperature of 220 ºC was used for the injection port while the MS transfer line was set at 245 ºC. The oven temperature was initially set at 50 ºC for 5 min then increased to 110 ºC at 5 ºC/min before it was ramped to 245 ºC at 25 ºC/min. The two methoxypyrazines measured were ibMP and ipMP.

4.2.4 Sensory descriptive analysis

The conditions under which tasting took place were the same as described in Chapter. 3, paragraph 3.2.4. A panel of nine women with prior experience of sensory testing was used to analyse the wines. Training was done for a total of eight hours. The panel was trained for the following aroma attributes generally found in South African Sauvignon blanc wines; overall green, green pepper/grassy, canned beans/peas, asparagus, overall tropical, passion fruit, guava, pineapple, litchi, melon/paw paw, citrus/grapefruit, stone fruit and sweaty/cat urine. The following taste attributes were also used; overall green, overall tropical, sour taste and sweet. Reference standards were used for training (Table 4.3).

Table 4.3 Reference standards used during training of descriptive analysis panel.

Attributes	Reference Standards
Green pepper/Grassy	Fresh green pepper [ab]
Canned beans/peas	Canned beans and peas (Koo) [ab]
Asparagus	Canned asparagus (Koo) [ab]
Overall Tropical	Tropical fruit juice (Ceres) [a]
Stone fruit	Fresh nectarine [ab]
Litchi	Canned litchi (Koo) [ab]
Melon/paw paw	Fresh melon and paw paw [a]
Pineapple	Fresh and canned pineapple (Koo) [ab]
Passion fruit	Fresh passion fruit [ab]
Guava	Fresh guava [ab]
Citrus/Grapefruit	Fresh grapefruit [ab]

[a] fresh fruit reference

[b] fresh fruit soaked in dearomatized Sauvignon blanc

A total of 24 wines were evaluated with descriptive analysis. Six to nine samples were tested daily in triplicate. Testing was done in four sessions lasting two hours each. A 15 minute break was taken between repetitions to avoid palate fatigue. Wines were evaluated both orthonasally and retronasally.

4.2.4 Statistical analyses of data

PanelCheck® software was used to assess panel performance and perform principal component analysis (PCA) on the sensory data of the wines (Version 1.4.0, Nofima, Ås, Norway). Analysis of variance (ANOVA) was used to determine the significance of the sensory attributes evaluated during descriptive analysis. Column graphs of the volatile thiols and ibMP were generated using Microsoft Office Excel 2007 (Microsoft Corp., Redmond, WA). Regression analysis using a moderator was done to investigate interaction effects between chemical compounds and sensory attributes in the wines. Range plots were drawn using standardized scores for the x- and y-axes. High and low values of the moderator were calculated based on average maximum and minimum volatile thiol and ibMP concentrations (Statistica, version 10, Statsoft Inc., Tulsa, USA).

4.3 Results and Discussion

A principal component analysis (PCA) bi-plot was compiled using the data obtained from the sensory descriptive analysis (Figure 4.1). In the PCA bi-plot 65.7% of the variance was explained by PC1 and PC2. Separation along PC1 (46.1%) was observed between the green attributes; overall green, green pepper/grassy, asparagus and canned beans on the right side and the tropical attributes; overall tropical, guava, passion fruit and citrus/grapefruit attributes on the top left side of the bi-plot. The separation seen along PC2 explained 19.6% of the variance and showed the variation between the wine samples. ANOVA was used to confirm significant differences between wine samples for all attributes (results not shown). The DN, HPF, Hex, Spr, and Tok wines correlated with the overall green and green pepper/grassy attributes. The mean attribute scores of only two wines were significantly higher for the canned beans/peas attribute, namely the Spr and Tok wines. The Spr wine was the only wine shown to be significant different from the other wines based on the asparagus attribute (group A, figure 4.1). The wines that correlated with the overall tropical, guava, passion fruit and citrus/grapefruit attributes were Drie, Gbec, Alt, Dgre, Diem, Neet, PZK and Dzal (group B, figure 4.1). The average intensity scores of some attributes were too low to contribute significantly to the aroma profile of the wines, namely; litchi, melon/paw paw and sweaty/cat urine. These attributes are located in the centre of the PCA bi-plot and showed intensities lower than 15%.

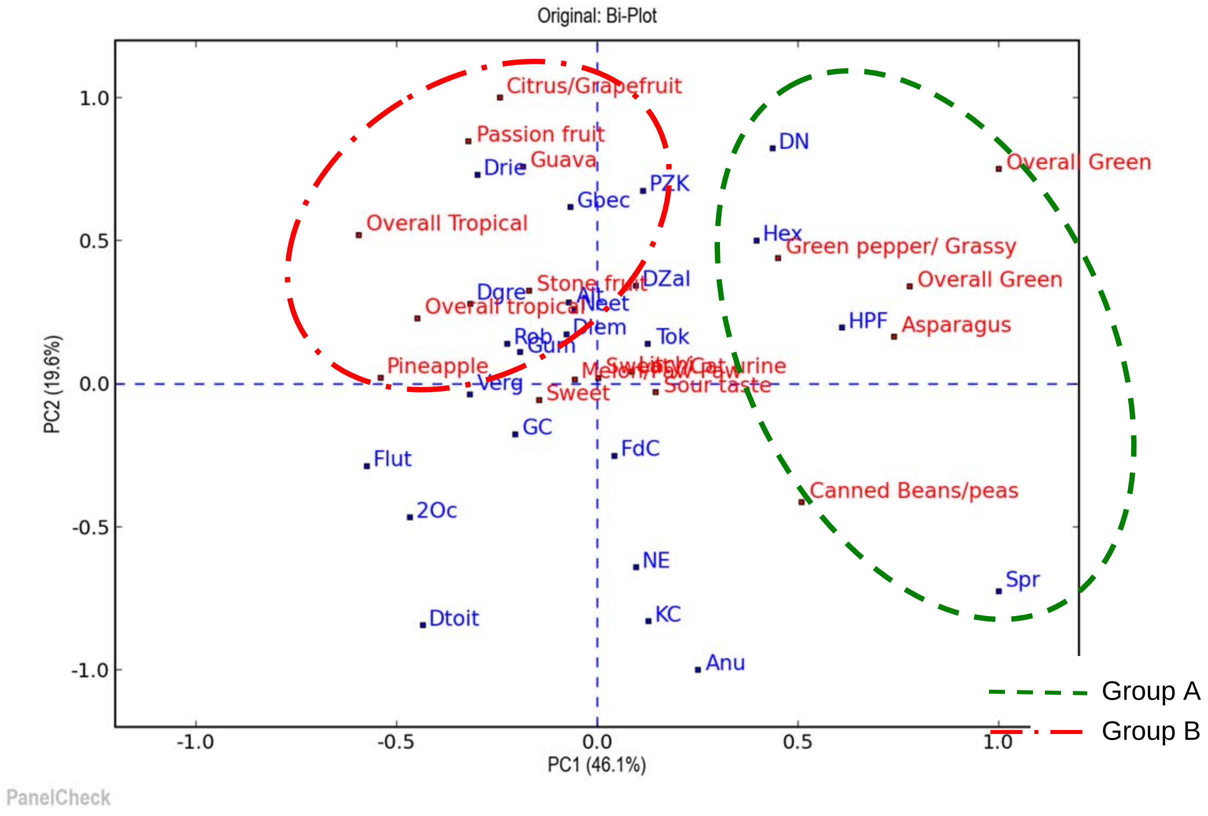

Figure 4.1 PCA Bi-plot of the sensory data of the commercial wines.

Some of the wines that correlated with the green attributes also had high methoxypyrazine concentrations in the wines (Figure 4.2). Certain wines containing high concentrations of

4MMP (Figure 4.3), 3MHA (Figure 4.4) and 3MH (Figure 4.5) also showed correlation with overall tropical, guava, passion fruit and grapefruit attributes on the PCA bi-plot. However certain wines with high concentrations of both volatile thiols and methoxypyrazines showed sensory expression for only one of the groups such as Spr. Average concentrations of 4MMP (10.1 ng/L, Figure 4.3) and 3MHA (157.96 ng/L, Figure 4.4) for the South African wines were found to be similar to Sauvignon blanc wines from New Zealand, Australia, France and Chile (Benkwitz *et al.,* 2012b). Average 3MH (969.8 ng/L, Figure 4.5) concentrations were lower in South African wines than in Sauvignon blanc wines from the above-mentioned countries and the USA (Lund et al., 2009b; Benkwitz *et al.,* 2012b). The ibMP (Figure 4.2) concentrations measured in the current study rnged from 1.2 to 40.1 ng/L and corresponded to previous studies done on South African Sauvignon blanc wines (Alberts *et al.,* 2009).

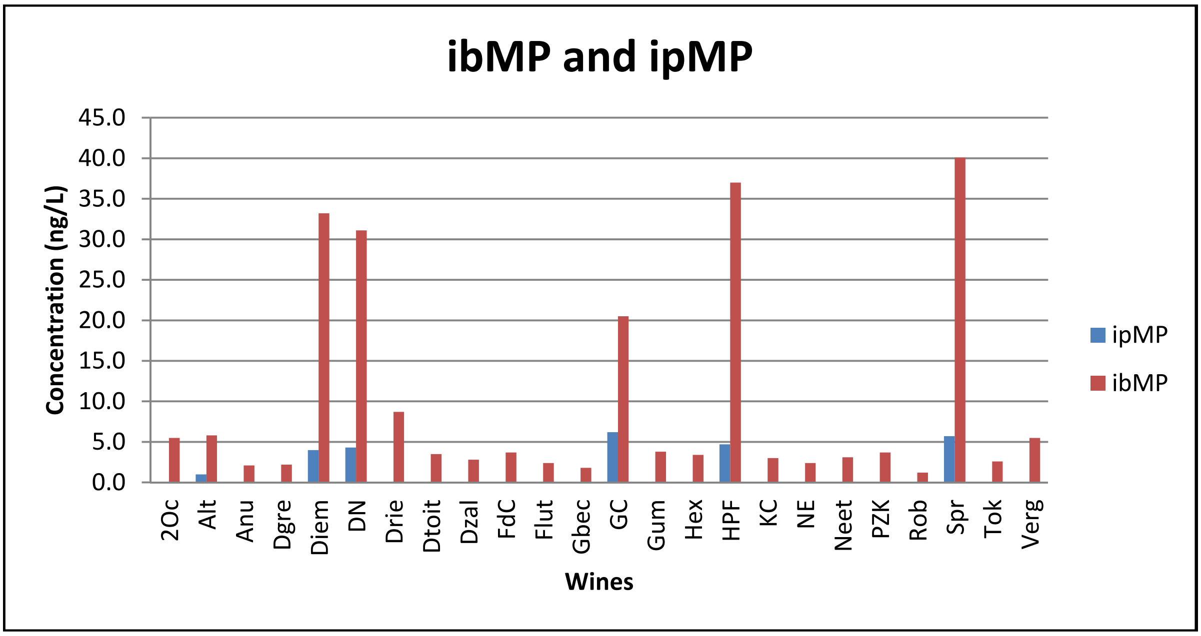

Figure 4.2 IbMP and ipMP concentrations measured in the wines. Levels lower than 1 ng/L were not detected.

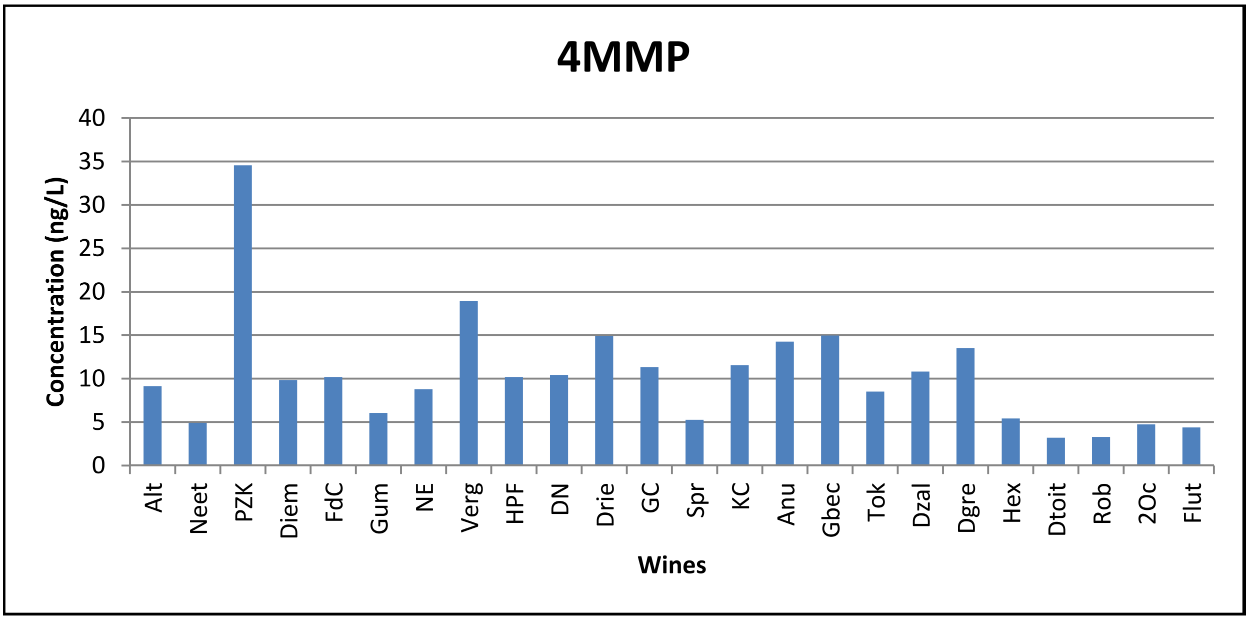

Figure 4.3 4MMP concentrations measured in the wines.

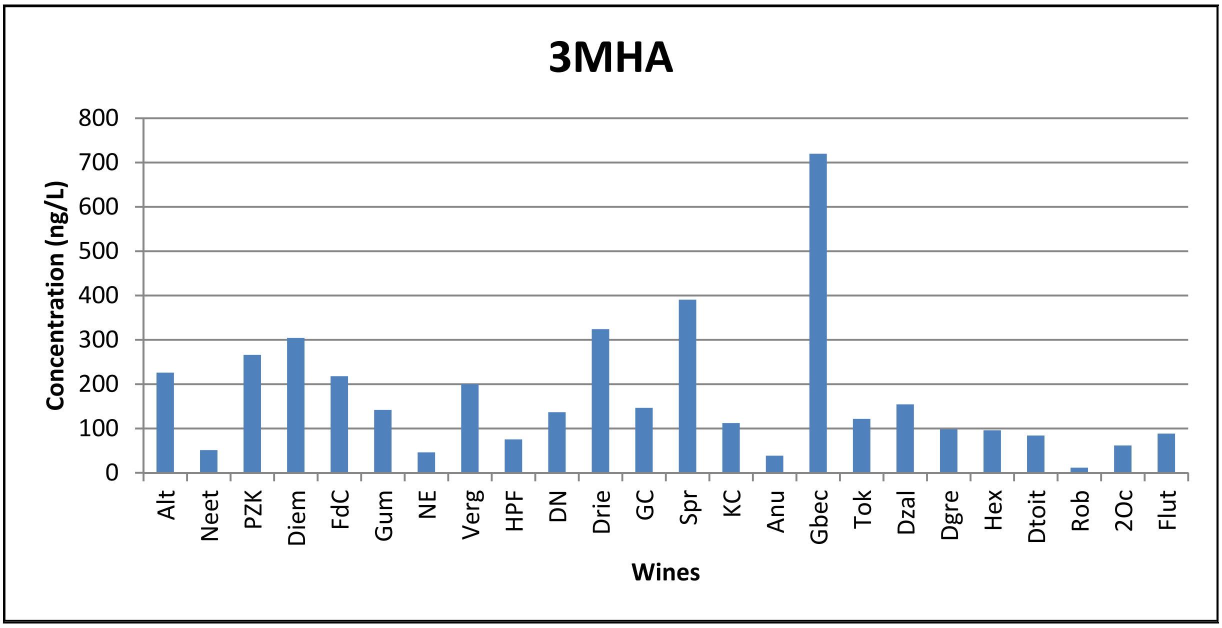

Figure 4.4 3MHA **concentrations** measured in the wines.

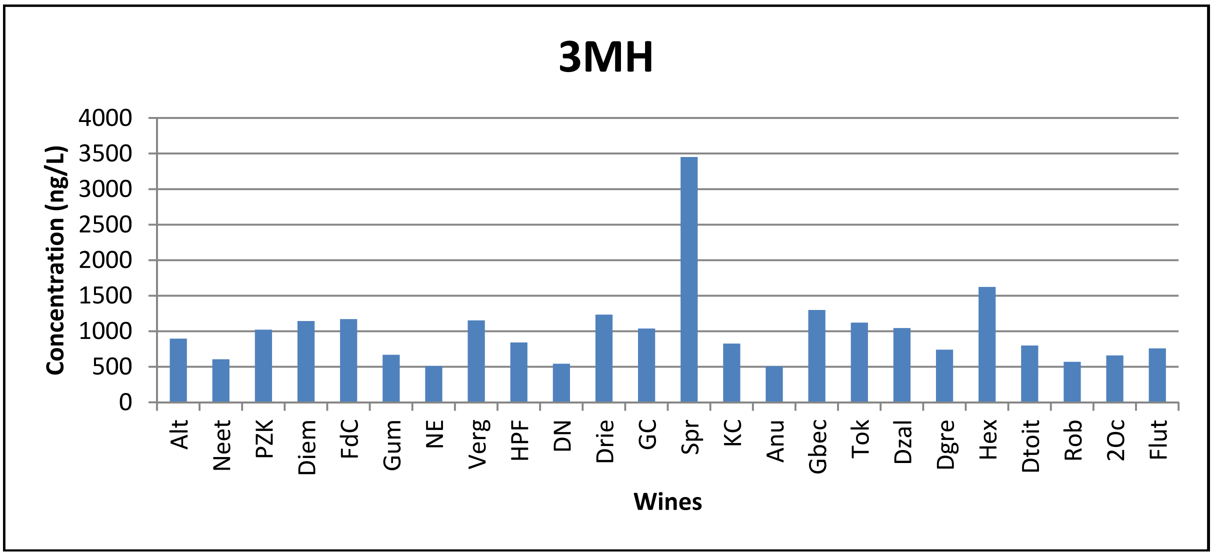

Figure 4.5 3MH concentrations measured in the wines.

The possible sensory interaction between ibMP and the volatile thiols were investigated using regression analysis with a moderator. The main sensory attributes associated with ibMP in the past were overall green, green pepper/grassy, asparagus and canned beans/peas (Allen *et al.,* 1991; Lacey *et al.,* 1991; Marais, 1994; Ebeler & Thorngate, 2009; Coetzee & Du Toit, 2012). Those associated with the volatile thiols were reported to be overall tropical, guava, grapefruit and passion fruit (Swiegers *et al.,* 2005; Swiegers *et al.,* 2006; Roland *et al.,* 2011; Coetzee and Du Toit, 2012) which were also confirmed in Chapter 3. Table 4.3 shows the independent variables, moderators, dependant variables and their effect on the R^2 values. R^2 values were calculated with and without the interaction effect. The p-values indicated where R^2 values differed significantly because of the effect of the interaction. Only interactions that showed significant p-values are shown in the table and range plots were compiled to illustrate these interactions. The x-axis showed the general increase in the independent variable (ibMP or volatile thiols) and the y-axis the increase in the dependant variable (attributes). The effects of the moderator at high and low values on the various attributes are shown in the plots.

Table 4.4 Table of the regression analysis performed on the volatile thiols and ibMP with and without interaction.

Independent Variable	Moderator	Dependent Variable	R^2 with interaction	R^2 without interaction (individual variance only)	p-value
ibMP	4MMP	Overall Green	0.4063	0.1029	0.00
ibMP	4MMP	Green pepper/ Grassy	0.2035	0.0016	0.03
ibMP	4MMP	Asparagus	0.3876	0.0232	0.00
3MH	ibMP	Guava	0.1382	0.0012	0.07
3MH	ibMP	Passion fruit	0.1493	0.0227	0.08
3MH	ibMP	Citrus/Grapefruit	0.4282	0.1555	0.00
3MHA	ibMP	Overall Tropical	0.1895	0.0023	0.03
3MHA	ibMP	Citrus/Grapefruit	0.3463	0.0183	0.00

The ibMP was first used as the independent variable assessing its effect on the green attributes. The volatile thiols were used as moderators to determine the interaction effect they had on the attributes associated with ibMP. It was found that the overall green attribute (Figure 4.6) showed an increase with increasing ibMP concentration when the 4MMP levels were low. However, at higher 4MMP concentrations a decrease in the correlation between ibMP and the overall green attributes was observed. The green pepper/grassy (Figure 4.7) and asparagus attributes (Figure 4.8) showed similar results at high 4MMP concentrations. This could be due to the extremely low detection limit of 4MMP (Tominaga *et al.,* 1998) Similar results were found for another thiol in Chapter 3 of this study between ibMP and 3MH. King *et al.,* (2011) also found that a combination of all three volatile thiols suppressed the attributes associated with ibMP.

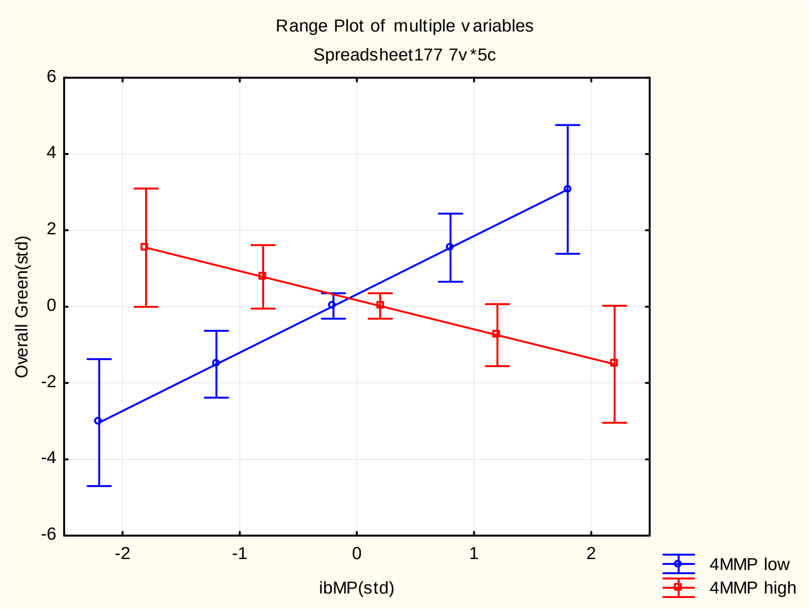

Figure 4.6 Effect of high and low 4MMP concentrations on the correlation of ibMP with the overall green attribute.

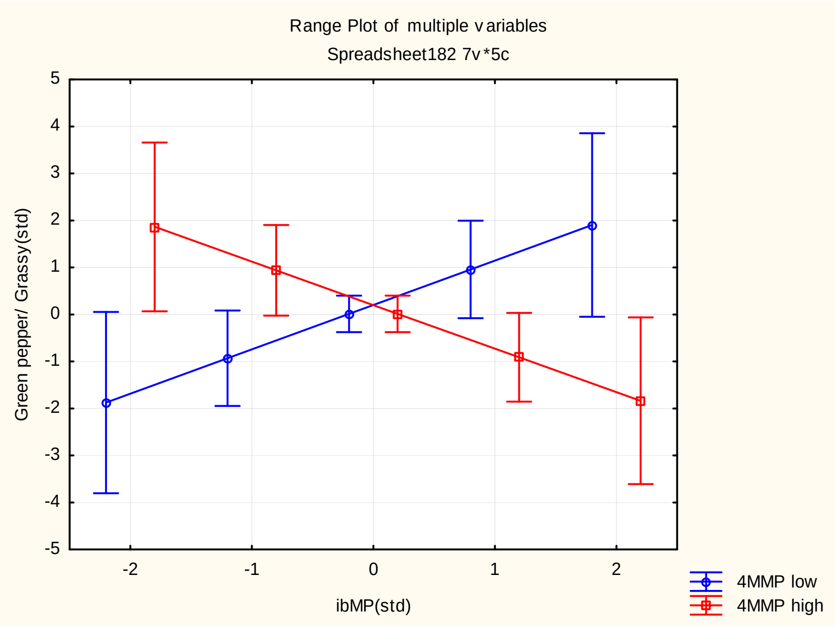

Figure 4.7 Effect of high and low 4MMP concentrations on the correlation of ibMP with the green pepper/grassy attribute.

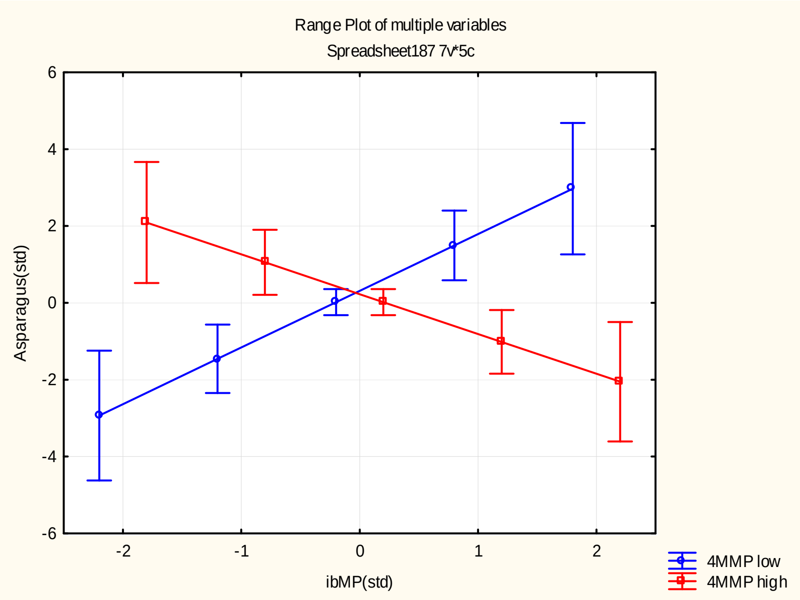

Figure 4.8 Effect of high and low 4MMP concentrations on the correlation of ibMP with the asparagus attribute.

The 3MH, 3MHA and 4MMP were also used as the independent variable to assess the effect of each of the volatile thiols on the tropical attributes with the ibMP as moderator. The guava (Figure 4.9), passion fruit (Figure 4.10) and citrus/grapefruit (Figure 4.11) attributes associated with 3MH showed suppression at high ibMP concentrations. In the case of 3MHA suppression was found for the overall tropical (Figure 4.12) and citrus/grapefruit (Figure 4.13) at high ibMP concentrations.

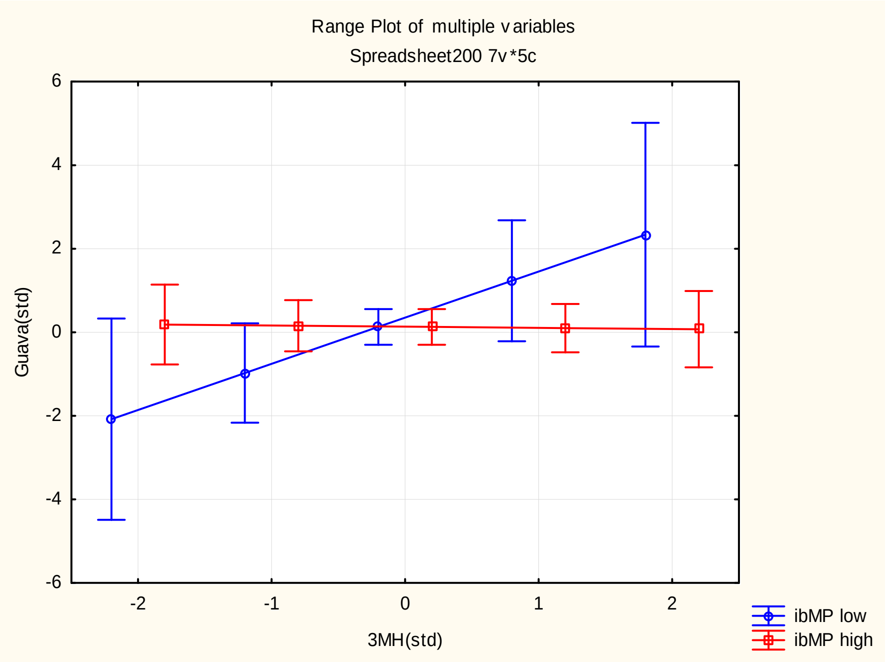

Figure 4.9 Effect of high and low ibMP concentrations on the correlation of 3MH with the guava attribute.

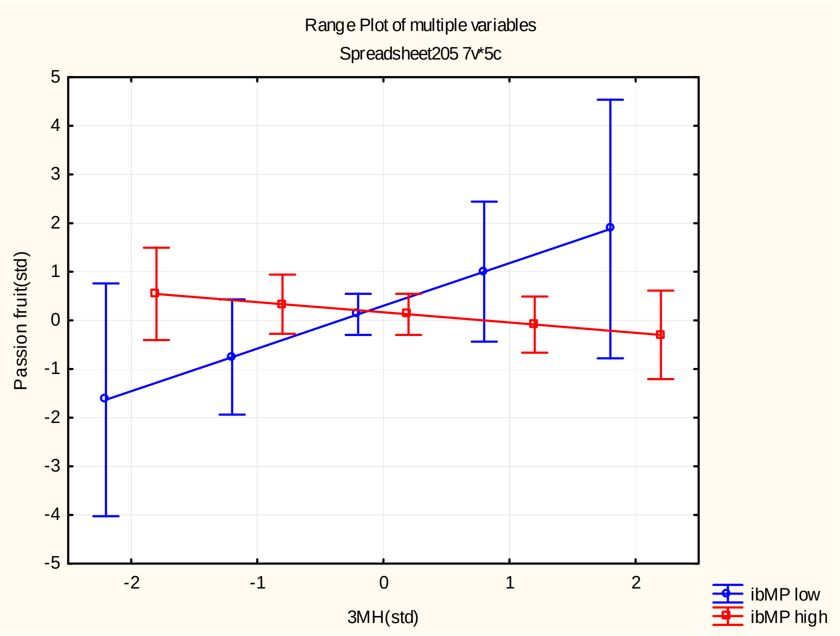

Figure 4.10 Effect of high and low ibMP concentrations on the correlation of 3MH with the passion fruit attribute.

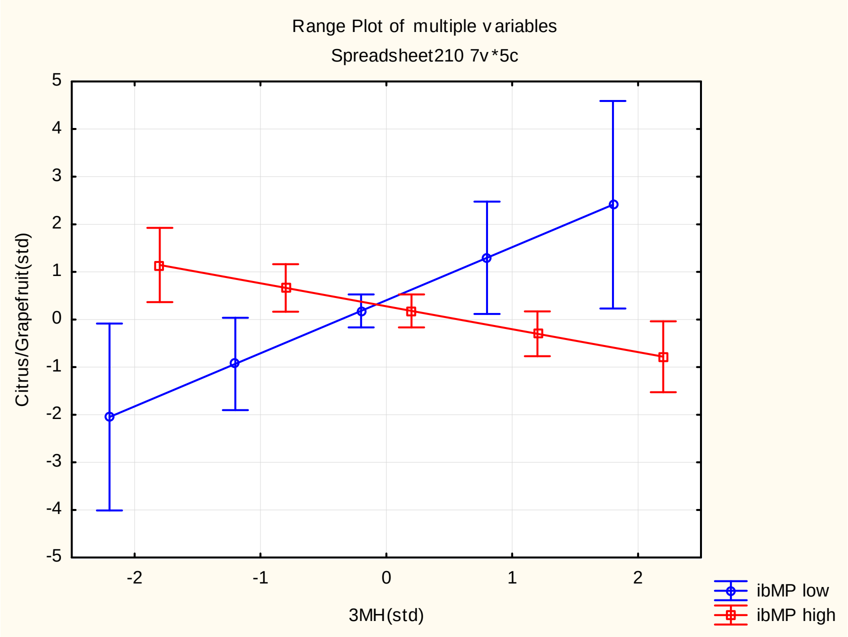

Figure 4.11 Effect of high and low ibMP concentrations on the correlation of 3MH with the citrus/grapefruit attribute.

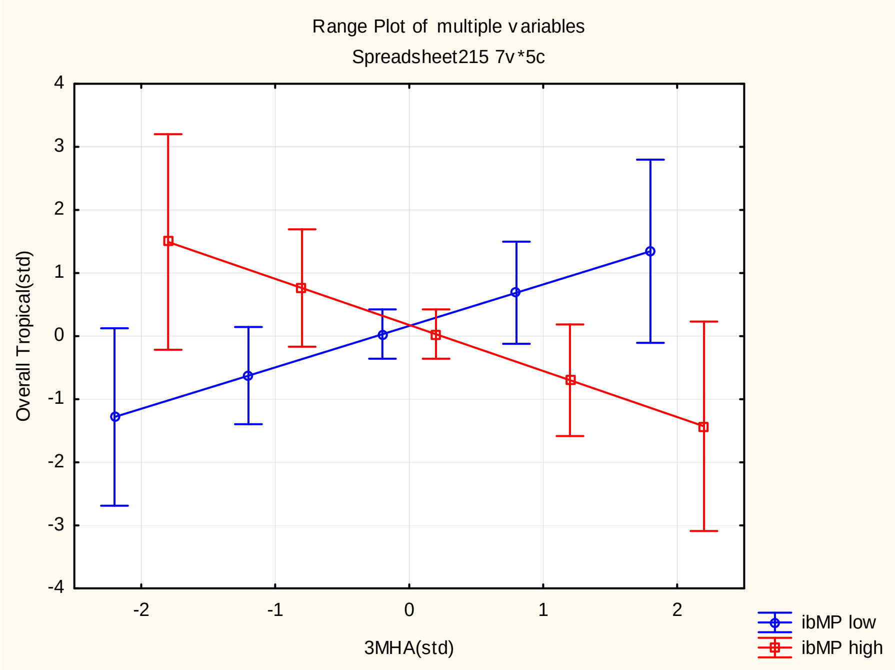

Figure 4.12 Effect of high and low ibMP concentrations on the correlation of 3MHA with the overall tropical attribute.

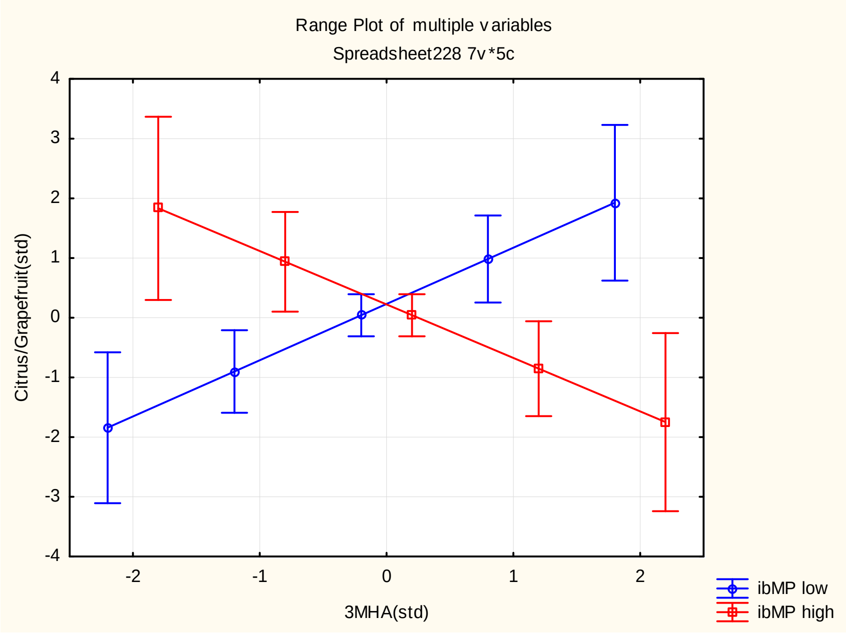

Figure 4.13 Effect of high and low ibMP concentrations on the correlation of 3MHA with the citrus/grapefruit attribute.

This showed the mutual suppressive trend between ibMP and the volatile thiols on some aroma attributes of each other. This was confirmed by previous research in this study (Chapter 3) as well as by King *et al.,* (2011). However, during both these studies known concentration were added to dearomatized wine while the current study was done on commercial Sauvignon blanc wines. This showed for the first time that the mutual suppressive effect of the volatile thiols and ibMP on each other can be seen in commercial South African Sauvignon blanc wines. However, one should keep in mind that a wine matrix is very complex, with various compounds, both volatile and non-volatile, influencing sensory characteristics of wines. Volatile compounds such as esters and certain norisoprenoid are known to influence the sensory perception of both volatile thiols and ibMP in Sauvignon blanc (King *et al.,* 2011; Campo *et al.,* 2005; Benkwitz *et al.,* 2012a). Non-volatile compounds such as phenolics have also been found to affect the perception of the volatile thiols, ethyl decanoate and ibMP (Lund *et al.,* 2009a). These various studies showed the complex interactive effects between the compounds found in Sauvignon blanc wines, but it seems that interactions between volatile thiols and ibMP in model or dearomatized wines also occur to a certain extend in commercial wines. Further research is thus needed to fully assess all the possible interactions in the complex Sauvignon blanc wine matrix.

4.4 Conclusion

Sauvignon blanc wine is a very complex matrix with various chemical compounds influencing the aroma. Mutual suppressive trends between the volatile thiols and a methoxypyrazine in commercial South African Sauvignon blanc wines were reported for the first time. However, there are various other aromatic interactions that need further investigation in Sauvignon blanc wines. Esters, monoterpenes, norisoprenoids and polyphenols could affect Sauvignon blanc aroma as well as influence the expression of the volatile thiols and methoxypyrazines. Further research could focus on these complex interactions at various levels in Sauvignon blanc wines and how these are reflected in commercial wines. Other factors influencing the volatile composition of Sauvignon blanc wines such as origin, vineyard practices and fermentation conditions could also be investigated.

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Chapter 5

General discussion and conclusions

5. General discussion and conclusions

5.1 Conclusions and future prospects

Sauvignon blanc wines are one of the most complex white wines cultivars used to produce wines with various aroma nuances. The specific character of Sauvignon blanc wines can be attributed to a variety of chemical compounds and research is in a premature stage to fully understand the complex interaction between them. The two main groups contributing to Sauvignon blanc wine aroma are the volatile thiols and methoxypyrazines. The volatile thiols have been found to enhance the tropical style of Sauvignon blanc wines while the methoxypyrazines contribute to the green style (Buttery *et al.,* 1969; Allen *et al.,* 1991; Lacey *et al.,* 1991; Darriet *et al.,* 1995; Tominaga *et al.,* 1996; Tominaga *et al.,* 1998; Coetzee & Du Toit, 2012).

In chapter 3 it was found that 2-methoxy-3-isobutylpyrazine (ibMP) correlated with the green attributes such as overall green, green pepper, grassy and asparagus. The overall tropical, guava, passion fruit and grapefruit attributes correlated with increasing 3-mercaptohexan-1-ol (3MH) concentrations. However, the expression of the volatile thiols and methoxypyrazines has been found to be complex. It was seen that high concentrations of ibMP suppressed the tropical attributes associated with the volatile thiols. The green attributes correlating with ibMP were also suppressed by high 3MH concentrations. The concentrations where interaction occurred are in line with levels found in Sauvignon blanc wines from various countries (Lund et al., 2009; Benkwitz *et al.,* 2012). This showed that ibMP and 3MH have the potential to mutually suppress each other in Sauvignon blanc wines from different origins. Most of the interaction was seen at higher concentration which shows that over expression of a certain chemical compound could lead to suppression of another. Similar results were reported by King *et al.,* (2011) between ibMP and a combination of thiols.

In chapter 3 it was observed that the degree of suppression differed between the attributes associated with ibMP and 3MH. Some attributes were more affected and showed a greater decrease in their sensory expression than others. The specific concentrations at which suppression occurred also sometimes differed for between attributes. The way aromas are perceived by humans is a very complex and still largely unexplained field. Olfactory receptors can perceive a combination of aromas, but some compounds can interact more strongly than others (Swiegers *et al.,* 2005). This could explain the differences seen in sensory perception of the attributes and differences in the degrees of suppression. Olfactory receptors can also be inhibited by some aroma compounds leading to competition between compounds at the binding sites (Swiegers *et al.,* 2005). This could be a possible explanation for the interaction seen between 3MH and ibMP.

Commercial South African Sauvignon blanc wines were evaluated in Chapter 4. The sensory composition of the wines was determined using descriptive analysis and their volatile thiols and the methoxypyrazines concentrations measured using GC-MS. It was found that the volatile thiols were present above their detection thresholds for all the wines measured. This showed that the volatile thiols are an important contributor to South African Sauvignon blanc aroma. The concentrations of 4MMP and 3MHA measured in this study corresponded to with levels found in international Sauvignon blanc wines, although 3MH concentrations were found to be lower (Lund et al., 2009; Benkwitz *et al.,* 2012). The ibMP concentrations were observed to be similar to levels previously seen in South African Sauvignon blanc wines (Alberts *et al.,* 2009). The interaction between the volatile thiols and ibMP was assessed using regression analysis. The trend between the volatile thiols and a methoxypyrazine to also mutually suppress each other was shown for the first time in commercial Sauvignon blanc wines.

Both the volatile thiols and methoxypyrazines have extremely low detection thresholds meaning they can be detected in Sauvignon blanc wines at very low concentrations. These components have also been found to be present in South African Sauvignon blanc wines at concentrations far above their detection thresholds. This can explain why suppression between the volatile thiols and methoxypyrazines can occur at certain levels. Current wine making procedures are implemented to achieve the optimal concentration of volatile thiols and methoxypyrazines to produce a complex wine. This, however, may also lead to suppression of aroma compounds instead of increasing complexity, but this aspect needs further investigation.

More research is needed to fully understand the complex interaction between the chemical compounds contributing to Sauvignon blanc aroma. The interaction between the volatile thiols and methoxypyrazines and other aroma compounds such as esters, monoterpenes and norisoprenoids could still be further investigated in model wine and Sauvignon blanc wine matrices. The mechanism of the interaction between the volatile thiols and methoxypyrazines need to be further investigated and the possible role of olfactory perception examined. Future studies could also focus on further describing the sensory and chemical profile of South African Sauvignon blanc wines from different wine regions. The volatile thiol concentration of Sauvignon blanc wines from various South African wine regions could be compared. This study contributed to knowledge on South African Sauvignon blanc wines, but also demonstrated the complex character of Sauvignon blanc wines which still needs to be further explained.

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