Chemical evaluation and sensory relevance of thiols in South African Chenin Blanc wines

by

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Declaration

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Summary

South African Chenin Blanc is gaining recognition for its high quality both domestically and abroad. As the most widely-planted cultivar in the country, there is interest in research which can provide additional knowledge to producers and further increase Chenin Blanc wine quality. One of the sensory modalities contributing to wine quality is wine aroma, which is studied through sensory analysis and the chemical quantification of volatile compounds. Commercially-available South African Chenin Blanc wines had been characterized previously for a variety of chemical compounds, but not for thiols. Thiols, including 3-mercaptohexan-1-ol (3MH) and 3-mercaptohexyl acetate (3MHA), are volatile sulphur compounds which are important to the 'tropical' and 'green' aromas of many wines, especially Sauvignon Blanc. The main aims of this research were to chemically characterize 3MH and 3MHA levels in a variety of commercially-available dry South African Chenin Blanc wines and explore the sensory contribution of these compounds to Chenin Blanc wine aroma. Chapter 3 reported the chemical analysis results of 3MH and 3MHA in South African Chenin Blanc Wines and explored trends within the chemical results. Chapters 4, 5, and 6 addressed the sensory relevance of thiols to South African Chenin Blanc wines.

In Chapter 3, both 3MH and 3MHA were quantified in South African Chenin Blanc wines at levels above their odour thresholds. The average levels found were 893 ng/L for 3MH and 23 ng/L for 3MHA, with ranges of 380-2929 ng/L for 3MH and 0-305 ng/L for 3MHA. Significant differences were found for 3MHA levels by wine age, vine age, wood contact, price, and lees contact were found, while 3MH only differed significantly for wine origin.

In Chapters 4 and 5, the sensory contribution of thiols was analysed through interaction studies. In Chapter 4, interactions of a thiol (3MH), an ester (ethyl hexanoate), and a terpene (linalool) in partially-dearomatized Chenin Blanc wine were analysed by descriptive analysis. Interaction effects were identified, such as the antagonism between the 'tropical' attributes of 3MH and the 'floral' character of linalool. The second interaction experiment, reported in Chapter 5, analysed combinations of 3MH and 3MHA in different matrices by projective mapping (PM) with intensity. This study showed that the perception of thiols was affected by the volatile and non-volatile wine matrix. The addition of an intensity measure to the ultra flash profiling step of the method provided more detailed data, which made the rapid sensory method better suited to interaction studies. In all sensory studies, wines with high thiols, especially high 3MHA, were described with 'tropical' and 'green' terms

In Chapter 6, polarized projective mapping (PPM) was used to characterize commercial South African Chenin Blanc wine aroma, and sensory results were compared with extensive volatile chemical analyses. Results showed a sensorial and chemical opposition between wooded and unwooded wines. The levels of 3MHA in the wines correlated with the unwooded wines and thiol-related descriptors. PPM was applied for the first time to wine, validating a method which increases the maximum sample size of wines in rapid sensory analysis.

The results of this research made contributions to the sensorial and chemical characterization of South African Chenin Blanc wines, as well as the validation of PPM and PM with intensity in wine. The knowledge that thiols are present in Chenin Blanc wines, together with existing research on practices affecting thiols can help inform viticultural and oenological decisions in the future of Chenin Blanc winemaking.

Opsomming

Suid-Afrikaanse Chenin Blanc begin toenemende erkenning geniet as hoë gehalte wyne plaaslik sowel as in die buiteland. As die mees aangeplante kultivar in Suid-Afrika is daar ‘n behoefte aan navorsing wat addisionele kennis aan verbouers kan verskaf om die kwaliteit van Chenin blanc wyn te bevorder. Een van die sensoriese modaliteite wat bydrae tot wynkwaliteit is wynaroma. Wynaroma kan bestudeer word met behulp van sensoriese analise en chemiese kwantifisering van vlugtige verbindings. Kommersieel beskikbare Suid-Afrikaanse Chenin blanc wyne is voorheen gekarakteriseer in terme van ŉ verskeidenheid chemiese verbindings. Hierdie analises het egter nie tiole ingesluit nie. Tiole, insluitende 3-merkaptoheksan-1-ol (3MH) en 3-merkaptoheksielasetaat (3MHA) is vlugtige swaelverbindings wat ‘n belangrike rol speel in terme van ‘tropiese’ en ‘groen’ aromas van verskeie wyne, veral Sauvignon Blanc. Die hoofdoelwitte van hierdie navorsing was om die vlakke van 3MH en 3MHA chemies te bepaal vir ‘n verskeidenheid kommersieël-beskikbare droë Suid-Afrikaanse Chenin Blanc wyne asook die verkenning van die sensoriese bydrae wat hierdie verbindings tot Chenin blanc aroma maak. Hoofstuk 3 rapporteer die chemiese analise resultate van 3MH en 3MHA in Suid-Afrikaanse Chenin blanc wyne en verken die tendense daarvan. Hoofstukke 4, 5 en 6 bespreek die sensoriese relevansie van tiole in Suid-Afrikaanse Chenin blanc wyn.

In Hoofstuk 3 word resultate gewys waar beide 3MH en 3MHA gekwantifiseer is bo hul aroma opsporingsdrumpels. Die vlakke wat gevind is, was 380-2929 ng/L, met ‘n gemiddeld van 893 ng/L, vir 3MH en 0-305 ng/L, met ‘n gemiddeld van 23 ng/L, vir 3MHA. Beduidende verskille is gevind vir 3MHA vlakke met betrekking tot die ouderdom van die wyn, houtbehandeling, prys, en gismoerkontak terwyl 3MH vlakke slegs beduidend verskil het met betrekking tot die oorsprong van die wyn (‘wine of origin’).

In Hoofstukke 4 en 5 is die sensoriese impak van tiole ondersoek met behulp van interaksie studies. In Hoofstuk 4 is die interaksie van ‘n tiol (3MH), ‘n ester (etielheksanoaat) en ‘n terpeen (linaloöl) in Chenin Blanc wyn wat gedeeltelik ontgeur is met behulp van beskrywende sensoriese analise geanaliseer. Interaksie effekte is geïdentifiseer soos antagonisme tussen ‘tropiese’ eienskappe van 3MH en die ‘blomagtige’ karakter van linaloöl. Die tweede interaksie eksperiment, bespreek in Hoofstuk 5, is uitgevoer om kombinasies van 3MH en 3MHA in verskillende matrikse met behulp van projeksiekartering met intensiteit te analiseer. Hierdie studie het gewys dat die persepsie van tiole geaffekteer word deur die vlugtige en nie-vlugtige wynmatriks komponente. Die toevoeging van ‘n intensiteitsmeting tot die beskrywende stap van projeksiekartering het aanleiding gegee tot meer detail in die datastel, wat die vinnige sensoriese evalueringsmetode beter aangepas het vir interaksiestudies. Tydens al die sensoriese eksperimente is wyne met hoër tiole, veral hoë 3MHA, beskryf as ‘tropiese’ en ‘groen’.

In Hoofstuk 6 is gepolariseerde projeksiekartering gebruik om kommersiële Suid-Afrikaanse Chenin Blanc wyne se aroma te karakteriseer. Sensoriese resultate is vergelyk met uitgebreide chemiese analise van ‘n wye verskeidenheid van vlugtige komponente in wyn. Resultate het ‘n sensoriese en chemiese opposisie tussen gehoute en ongehoute wyne uitgewys. Die vlakke van 3MHA in die wyne het met ongehoute wyne en tiool-verwante beskrywende sensoriese terme gekorreleerd. Gepolariseerde projeksiekartering is vir die eerste keer gebruik om die sensoriese eienskappe van wyne te beskryf, dus is ‘n metode gevalideer waar ‘n groter aantal wyne tydens ‘n vinnige sensoriese evalueringsmetode as te vore geëvalueer kan word.

Die resultate van hierdie studie het bydraes gelewer tot die sensoriese en chemiese karakterisering van Suid-Afrikaanse Chenin Blanc wyne, sowel as die validasie van gepolariseerde projeksiekartering en projeksiekartering met intensiteit vir die sensoriese evaluering van wyn. Die kennis tiole teenwoordig is in Chenin Blanc wyne te same met die bestaande navorsing oor praktyke wat die vlakke van tiole in wyne beïnvloed, kan help om wingerd- sowel as wynkundige besluite in toekomstige Chenin blanc wynbereiding te rig.

This thesis is dedicated to my family and loving fiancé who supported and encouraged me, and dealt with international calls at strange hours.

Biographical sketch

Christine Wilson was born in Hayward, California in the United States on 10 April 1991. She attended Gravenstein Elementary School and Hillcrest Middle School, and graduated from Analy High School in 2009. Christine obtained her B.S. in Viticulture and Enology in 2013 from the University of California, Davis. In 2015, Christine enrolled for an MScAgric in Oenology at the Department of Viticulture and Oenology, Stellenbosch University.

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Preface

This thesis is presented as a compilation of 7 chapters.

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Chapter 1

Introduction and research aims

Chapter 1 : Introduction and research aims

1.1 Introduction

Chenin Blanc is South Africa's 's most planted grape, and it has a long history in the country's wine industry. Much of the Chenin Blanc grown in South Africa does not end up as a varietally-labelled bottle of wine (SAWIS, 2016). Nevertheless, the portion of Chenin Blanc which is sold as varietal wine is being recognized domestically and internationally for its excellent quality (CMB Results, 2015; Atkin *et al.*, 2016). Because of the wide availability of this grape and the high-quality wines it can produce, there is great potential for Chenin Blanc to increase industry revenues and represent South Africa as an iconic national wine. Research which provides insight and knowledge to Chenin Blanc producers can support the industry in further improving the quality of their wines and realizing the full potential of this grape variety.

Different cultivars are known to have different sensory characteristics, and one of the sensory modalities through which these differences are perceived is the sense of smell. Wine aroma is an important part of wine appreciation as one of the intrinsic factors which consumers use to judge wine quality. The human perception of wine aroma results from the mixture of volatile compounds in a wine, and is affected by interactions between the volatiles, and with the non-volatile components of the wine matrix (Polášková *et al.*, 2008; Styger *et al.*, 2011; Sáenz-Navajas *et al.*, 2012). These volatile aroma compounds can drive differences between different varieties and styles of wine, allowing for volatile "fingerprinting" and identification of impact compounds (Fischer, 2007; Polášková *et al.*, 2008).

South African Chenin Blanc wines have been previously profiled for volatile compounds, including alcohols, fatty acids, acetate esters, ethyl esters, and terpenes (Lawrence, 2012). However, thiols as a class of aroma compounds have not been extensively analysed in Chenin Blanc. Thiols are a group of sulphur-containing volatile compounds which are important to the 'tropical' aromas of many wines, especially Sauvignon Blanc (Dubourdieu *et al.*, 2006; Herbst-Johnstone *et al.*, 2011; Roland *et al.*, 2011; Coetzee & Du Toit, 2012; Coetzee *et al.*, 2015). Thiols are found in wine at levels on the order of ng/L, but nonetheless are potent odorants because of their extremely low odour thresholds. Though they have been identified in several different wine varieties (Guth, 1997; Tominaga *et al.*, 2000; Murat *et al.*, 2001; López *et al.*, 2003), research since 2003 has mostly focused on thiols in Sauvignon Blanc. Thiols could be important to the 'tropical' and 'guava' aromas of Chenin Blanc wines, but characterization of thiol levels in Chenin Blanc wines has not been performed due to the difficulty of the analysis. These difficulties result from the labile nature of these compounds and the challenges of quantifying part per trillion levels (Jeffery, 2016), as well as the potentially hazardous use of mercury compounds in some methods (Tominaga *et al.*, 1998; Tominaga & Dubourdieu, 2006). More recently, new methods have been validated, giving researchers more options for thiol analysis (Chen *et al.*, 2013; Piano *et al.*, 2015).

To relate chemical data to the human sensory experience of wine, chemistry should be paired with sensory evaluation. The field of wine sensory science is vibrant and offers a wide variety of sensory methodologies that can be employed to understand the role of thiols in the aroma of Chenin Blanc wines. Additionally, there are opportunities to develop and explore new sensory methodologies to suit specific experimental objectives. Greater understanding of the compounds responsible for differences in wine aroma is reached through the combination of chemical and sensory analysis. Additional knowledge about the complex interactions of volatiles with one another and the non-volatile matrix further enriches this understanding. Sensory interaction studies involving the perception of thiols in Sauvignon Blanc wines have been published (King *et al.*, 2011; Van Wyngaard *et al.*, 2014; Coetzee *et al.*, 2015), but none tailored to Chenin Blanc wines have been performed. Ultimately, this knowledge can be utilized by the industry to tailor their viticultural and oenological practices to craft Chenin Blanc wines with their desired sensory characteristics.

1.2 Research aims

The main aims of this project were twofold:

- Chemical characterization of 3MH and 3MHA levels in a variety of commercially-available dry South African Chenin Blanc wines.
- To explore the sensory contribution of these compounds to Chenin Blanc wine aroma through various sensory experiments using commercial wines, spiked wines, and model solutions.

Additional aims of the research were:

- Observe the interaction of thiols with each other and other classes of compounds within the Chenin Blanc matrix.
- To explore the use of rapid sensory methods in interaction studies which involved thiols.
- Validate the use of Polarized Projective Mapping, a rapid reference-based sensory method, on wine.
- To address the importance of the matrix to the results of interaction studies.
- Generate hypotheses for further study by exploring trends in the thiol results of Chenin Blanc wines.

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Chapter 2

Literature review

Background on thiols, South African Chenin Blanc, and sensory analysis methods

Chapter 2 : Literature Review - Background on thiols, South African Chenin Blanc, and sensory analysis methods

2.1 Introduction

Wine appeals to senses through its colour, aroma, and taste. Perhaps more than any other food or beverage, people enjoy and share wine through communicating its sensory properties. Since it is a sensorially complex and variable product, wine is often described in a great degree of detail by wine experts and consumers. The perception of wine aroma is important to the overall impression of a wine, and contributes largely to an individual's experience and liking. Wine's sensory properties are discussed in terms of taste descriptors such as 'sweetness' and 'sourness', mouth feel descriptors including 'astringency' and 'body', as well as aroma descriptors like 'peach', 'red fruit', and 'citrus'. Even for consumers who don't have the experience required to communicate specific aroma attributes, their perception of aroma still influences their experience of the wine.

The aroma of wine, which is a vital piece of wine's enjoyment, is a result of the volatile aroma compounds present in the wines. The volatile compounds contribute to its aroma and flavour through ortho- and retro-nasal olfaction, respectively. This aroma is the result of complex interactions between different volatile chemical compounds, the wine matrix, and each individual's body and brain chemistry. In an oenology environment, wine aroma research seeks to understand this system in a variety of ways; it focuses on studying the origin of different compounds and the reactions that occur during winemaking, characterizing the volatile composition of wines, and evaluating the perception of different compounds (at varying concentrations, in different matrices, and in interaction with other compounds). Results obtained through wine aroma research can ultimately contribute to wine quality by broadening a winemaker's knowledge and ability to produce wines with desired aroma characteristics.

A budding area of wine aroma research is the class of sulphur compounds known as "thiols". Thiols (or mercaptans) are compounds which are named for their sulphahydryl (-SH) group. However, in the case of wine, by convention, there is a distinction made between the two names. "Mercaptan" is used to refer to sulphur compounds which have negative aromas and are considered as wine faults, such as the infamous hydrogen sulphide (H_2S) which smells of 'rotten eggs'. Conversely, "thiols" refer to compounds with pleasant, generally 'tropical' aromas which contribute positively to wine aroma. They are extremely powerful compounds because of their low odour thresholds, and are thought to be important to the aroma of different cultivars, though the vast majority of thiol research has been performed on Sauvignon Blanc wines.

As the most widely-planted grape in South Africa (SAWIS, 2016a), Chenin Blanc is of great interest to researchers and the South African wine industry. Chenin Blanc aroma has been investigated in terms of fatty acids, ethyl and acetate esters, terpenes, and higher alcohols (Lawrence, 2012; Van Antwerpen, 2012), but knowledge of thiol levels in Chenin Blanc wines is extremely limited. A better understanding of the typical levels and perception of this class of aroma compounds in Chenin Blanc wines will contribute to the chemical and sensory profiling of the variety. This knowledge could ultimately help aid in further improving the quality of these wines.

Throughout the following chapters, the chemical analysis of certain thiols contributing to the positive fruity and herbaceous aroma nuances in South African Chenin Blanc wines was performed, followed by sensory experiments to help explain the contribution of these thiols to Chenin Blanc aroma. Accordingly, this literature review will begin by describing the importance of Chenin Blanc within the South African wine industry. This is followed by a focus on available thiol research, which will be discussed in the context of all varieties, as well as Chenin Blanc specifically. The available Chenin Blanc aroma research will also be detailed. Next, the different sensory methodologies utilized throughout this thesis will be discussed in terms of appropriate applications, advantages and disadvantages, and the different statistical analyses used to interpret the results.

2.2 The importance of Chenin Blanc to the South African wine industry

Chenin Blanc, historically known as Steen in South Africa (Singleton *et al.*, 1975), is one of the nation's oldest and most important wine grapes. It was previously known as a "cheap and cheerful" workhorse variety, where quantity preceded quality. However, focus has shifted toward vinifying high-quality Chenin Blanc wines (Loubser, 2008). This quality is being recognized in competitions around the world. A South African Chenin Blanc wine was recently awarded the "Overall Best White Wine" at an international competition containing over 8000 wines in 2015 (CMB Results, 2015). Additionally, the South African Chenin Blanc category was recently featured in the respected wine magazine, *Decanter* (November, 2016 issue), with one Chenin Blanc being the first South African wine to achieve a score of 98 points in the publication (Atkin *et al.*, 2016; Sherwood, 2016).

The following South African wine industry statistics were published in December, 2015 by the S A Wine industry Information & Systems NPC (SAWIS, 2016a). In terms of prevalence, Chenin Blanc is very important to the South African wine industry. Of the wine grapes planted in South Africa, white varieties occupy a greater combined vineyard area (53,849 ha), than red grape varieties (44,748 ha). Out of all grape varieties, including table grapes, Chenin Blanc is the most widely planted in South Africa, with 17,965 ha planted, representing 18.2% of the total wine grape area. The next most planted grape variety, Colombard, comparatively only occupies 11,839 ha (12.0%). Because of this availability, Chenin Blanc has a stable role as the "workhorse" grape of the industry. The proportion of vineyards planted to Chenin Blanc has remained relatively constant over time between 2008 (18.6%) and 2015 (18.2%). Due to Chenin Blanc's relatively high yield, it represents an even greater proportion of South African wines in terms of tonnage crushed for winemaking purposes (341,625 tons, 23% during the 2015 harvest) (SAWIS, 2016a).

Though Chenin Blanc is by far the leading cultivar in terms of plantings and tons crushed, there are fewer 750 mL bottles sold as "Chenin Blanc" (~4,300,000) than those labelled as "Dry White" (~16,000,000) or "Sauvignon Blanc" (~13,500,000) (SAWIS, 2016a). Due to Chenin Blanc's high yield and availability, it is also used as a base for distillation into brandy and wine spirits, as well as exported in bulk. Additionally, it is frequently blended with other varieties rather than bottled as a varietal wine (SAWIS, 2016a).

The only available data which can demonstrate the economic importance of Chenin Blanc comes from producer cellars which have bought the grapes that they use. Data from SAWIS (2016b) shows that the commercial value of Chenin Blanc in terms of price per ton sold to producer cellars (who accounted for 86.4% of tons of Chenin Blanc crushed in 2015) has increased slightly from R1889/ton in 2013, to R1960/ton in 2014, and to R1974/ton in 2015. This is just under the 2015 average value for all white grapes sold to producing cellars of R2076/ton. Considering only the

86.4% of Chenin Blanc grapes (274,611 tons) sold to producer cellars, this already accounts for a total value of over R577,000,000. This demonstrates that this cultivar is economically important to the industry.

Overall, as the most available cultivar in the country which also has the potential to produce internationally recognized wines, Chenin Blanc is a very important facet of the South African wine industry. Chenin Blanc's value can be expected to increase further as it gains greater recognition in the domestic and international markets and the quality continues to improve.

2.3 The role and prevalence of thiols in wines

2.3.1 General Introduction to Thiols

The most notable property of thiols is their exceptionally low odour thresholds (Table 2.1). Even though they are found in wines at very low concentrations compared to most other volatiles, the fact that they can be sensed in the range of ng/L makes them extremely powerful aroma compounds (Roland *et al.*, 2011; Coetzee & Du Toit, 2012). These low thresholds are in the same order as that of pyrazines such as 3-isobutyl-2-methoxypyrazine (IBMP), which contributes to the 'green' character of many wines (Roujou de Boubee *et al.*, 2000). In comparison, the odour thresholds of most volatile aroma compounds are orders of magnitude higher, in the range of µg or mg/L (Francis & Newton, 2005).

Within the family of thiols, there have been many compounds identified in a variety of food products (Vermeulen *et al.*, 2005; McGorrin, 2011). A subset of thiols, varietal thiols, are thiols which are derived from odourless precursors already present in the grapes (Roland *et al.*, 2011). Of the varietal thiols, the three currently recognized as most important to wine will be discussed here, namely 4-methyl-4-mercapto-pentan-2-one (4MMP), 3-mercaptohexan-1-ol (3MH) and 3-mercaptohexyl acetate (3MHA) (Roland *et al.*, 2011). Regarding the nomenclature of thiols, according to new rules the "mercapto-" prefix should be replaced with "sulphanyl", making these compounds 4-mercapto-4-sulfapentan-2-one (4MSP), 3-sulfanylhexan-1-ol (3SHA), and 3-sulfanylhexyl acetate (3SH). In this document, however, the traditional names are used, as the new names are yet to be widely adopted.

4MMP has the lowest odour threshold of the three at 0.8 ng/L (Table 2.1), and its aroma is traditionally described as 'box tree', 'blackcurrant' (Darriet *et al.*, 1995; Guth, 1997a), 'broom' (Bouchilloux *et al.*, 1998), and 'cat urine' (Dubourdieu *et al.*, 2006). It has also been described as 'green', 'mint', and 'exotic fruits' (Pet'ka *et al.*, 2006). 3MH has an odour threshold of 60 ng/L (Table 2.1) and is described as 'passion fruit' and 'grapefruit' (Tominaga *et al.*, 1998). These descriptors are supported by the fact that 3MH has been identified in the passion fruit itself (Engel & Tressl, 1991). The third compound, 3MHA, has an odour threshold of 4.2 ng/L (Table 2.1). It best described as 'box tree' (also known as 'box hedge'), but also as 'grapefruit' and 'passion fruit' (Tominaga *et al.*, 1996; Dubourdieu *et al.*, 2006), as well as 'guava' and 'gooseberry' (Swiegers & Pretorius, 2007). Somewhat problematically, 'box tree' is a culturally-specific term unfamiliar within South Africa, where 'guava' and 'gooseberry' are more likely to be used.

When a certain aroma in a product can be pinpointed as coming from one single compound, it is called an "impact compound" (Polášková *et al.*, 2008). Because of wine's complexity, only a few impact compounds have been identified in wine, and among them are two thiols (Polášková *et al.*, 2008). 4MMP has been identified as an impact compounds for Sauvignon Blanc and Scheurebe wines (Guth, 1997a). To date, 3MH has been shown to be a characteristic odorant of Grenache Rosé (Ferreira *et al.*, 2002), Petite Arvine (Fretz *et al.*, 2005), and Semillon (Tominaga *et al.*, 2006). 3MH and 3MHA are impact compounds in New Zealand Sauvignon Blanc wines (Benkwitz *et al.*, 2012). In the same way that the monoterpene linalool has been shown to give Muscat wines their 'floral' aroma, and rotundone gives Shiraz its characteristic 'black pepper' note, these thiols are responsible for the characteristic 'tropical' aromas of these wines (Polášková *et al.*, 2008).

Table 2.1 Odour thresholds and descriptors of 4MMP, 3MH, and 3MHA

Compound	Abbreviation	odour threshold (ng/L)	Descriptor
4-mercapto-4-methylpentan-2-one	4MMP	0.792[1]	box tree, cat urine
3-mercaptohexan-1-ol	3MH	60[2]	passion fruit, grapefruit
3-mercaptohexyl acetate	3MHA	4.2[3]	box tree, grapefruit, passion fruit

[1](Darriet *et al.*, 1995) in model wine
[2](Tominaga *et al.*, 1998) in model wine
[3](Tominaga *et al.*, 1996) in 10% ethanol

Most research has focused on the analysis of thiols in Sauvignon Blanc wines. Worldwide ranges in Sauvignon Blanc wines are 4-40 ng/L for 4MMP, 26-18,000 ng/L for 3MH, and 0-2500 ng/L for 3MHA (Coetzee & Du Toit, 2012). The levels quantified in South African Sauvignon Blanc wines are lower, with 500-3500 ng/L 3MH and 10-720 ng/L 3MHA in a sample of 24 1-year-old wines (Van Wyngaard, 2013), and 718-2260 ng/L 3MH and 19-1029 ng/L 3MHA in another sample of 18 wines (Piano *et al.*, 2015).

Though thiols have mainly been measured in Sauvignon Blanc wines, they have been shown to be important to other varieties as well. Shown in Table 2.2 is a summary of reported results for 4MMP, 3MH, and 3MHA concentrations in varieties other than Sauvignon Blanc. Many varieties have been studied, from the more well-known Gewürztraminer and Cabernet Sauvignon, to the more obscure Devín and Marmajuelo (Table 2.2). Additional thiols have been analysed in these varieties, such as benzyl mercaptans in Champagne and Chardonnay (Tominaga *et al.*, 2003; Capone *et al.*, 2016), and 2-furanmethanethiol in Petit Manseng (Tominaga *et al.*, 2000a), red Bordeaux blends (Tominaga *et al.*, 2000b), and Champagne wines (Tominaga *et al.*, 2003). For practical reasons, however, only the three most important thiols are included in Table 2.2.

Table 2.2 Levels of 4MMP, 3MH, and 3MHA reported in non-Sauvignon Blanc wines

Variety/Type	4MMP (ng/L)		3MH (ng/L)		3MHA (ng/L)		# of Samples	Wine Origin	Citation
	Range	Mean	Range	Mean	Range	Mean			
Bacchus	--	25	nq	--	nq	--	1	France	(Schneider *et al.*, 2003)
Bordeaux Clairet	--	--	68-1362	761	0-8.6	4.2	10	Bordeaux, France	(Murat *et al.*, 2001)
Bordeaux Rosé	--	--	80-2256	517	0-19.8	6.6	20	Bordeaux, France	(Murat *et al.*, 2001)
Bordeaux red blend (Cab. Franc, Cab. Sauvignon, Merlot)	--	--	<60-4560	--	--	--	9	Bordeaux, France	(Blanchard *et al.*, 2004)
Bordeaux red blend (Cabernet Sauvignon and Merlot)	--	--	10-5000	--	1-200	--	12	Bordeaux, France	(Bouchilloux *et al.*, 1998a)
Champagne (Chardonnay, Pinor noir)	--	--	250-640	--	--	--	18	Champagne, France	(Tominaga *et al.*, 2003)
Chardonay	~0-6.5	--	~70-2700	--	~4-80	--	106	Australia	(Capone *et al.*, 2016)
Colombard	nd	nd	432-1053	--	21-63	--	2	Southwest, France	(Tominaga *et al.*, 2000) a
Devín	--	14	--	--	--	40	1	Malokarpatský, Slovakia	(Peťka *et al.*, 2006)
Gewürztraminer	nd-15	7.3	1336-3278	--	0.5-5.7	--	5	Alsace, France	(Tominaga *et al.*, 2000)
Gewürztraminer	--	<10	--	--	--	--	1	Ballrechten-Dottingen, Germany	(Guth, 1997a)
Gual*	--	32.8	--	1020	--	380	1	Tenerife, Spain	(López *et al.*, 2003)
Listán*	--	<10.4	--	258	--	320	1	Tenerife, Spain	(López *et al.*, 2003)
Maccabeo	--	5	--	--	--	--	1	Somontano, Spain	(Escudero *et al.*, 2004)
Malvasía*	--	18.4	--	108	--	116	1	Tenerife, Spain	(López *et al.*, 2003)
Marmajuelo*	--	38.4	--	2640	--	1284	1	Tenerife, Spain	(López *et al.*, 2003)
Muscadet	nd	nd	63-445	--	nd-6	--	10	Nantes, France	(Schneider *et al.*, 2003)
Muscadet	--	"absent"	--	"slight"	--	"absent"	1	France	(Tominaga *et al.*, 1998)
Muscat d'Alsace	7-30	35.3	124-898	--	nd	--	5	Alsace, France	(Tominaga *et al.*, 2000a)
Petite Arvine	--	--	212-6112	--	--	--	11	Valais, Switzerland	(Fretz *et al.*, 2005)
Petit manseng (sweet)	nd	nd	828-4468	--	nd-101	--	3	Jurançon, France	(Tominaga *et al.*, 2000a)
Pinot blanc	0.7-1.1	0.9	88.5-248	--	nd	--	2	Alsace, France	(Tominaga *et al.*, 2000a)
Pinot gris	nd-1.9	1.8	312-1042	--	nd-51	--	5	Alsace, France	(Tominaga *et al.*, 2000a)
Riesling	nd-7.6	2.2	407-970	--	nd-6.4	--	5	Alsace, France	(Tominaga *et al.*, 2000a)
Rioja blend (Tempranillo, Grenache, Graciano)	nq	--	nq	--	nq	--	2	Rioja and Jumilla, Spain	(Aznar *et al.*, 2001)
Scheurebe*	--	400	--	--	--	--	1	Ballrechten-Dottingen, Germany	(Guth, 1997a)
Semillon (botrytized)	8.5-40	21.2	4048-5969	--	nd	--	3	Barsac, France	(Tominaga *et al.*, 2000a)
Sylvaner	0.3-0.5	0.4	58.4-146	--	nd	--	3	Alsace, France	(Tominaga *et al.*, 2000a)
Verdello*	--	32.8	--	420	--	1148	1	Tenerife, Spain	(López *et al.*, 2003)

*Concentrations for these varieties has been back-calculated from given odour active values (OAVs) and odour thresholds
(--) this measurement was not performed
The concentration of 4MMP reported in (Guth, 1997b) is corrected to 400 ng/L in Table 2.2, from the originally reported µg/L, as discussed in the literature (Roland et al., 2011).

Concentrations of 4MMP vary from not detected in several wines to 400 ng/L (Table 2.2) in Scheurebe, which is a very wide range considering its odour threshold of 0.8 ng/L (Table 2.1). Levels of 3MH vary between 10 ng/L in a Cabernet Sauvignon to 6122 ng/L in Petite Arvine, with a similarly high level in botrytized Semillon. Interestingly, 3MH seems ubiquitous to wine because in all cases where 3MH was measured, it was detected. On the other hand, 3MHA levels were low or not detected in many of the wines measured, with a few outstanding cases. The range of 3MHA in these wines spans from not detected in some varieties to 1284 ng/L in one Marmajuelo wine (Table 2.2). The maximum level of 4MMP reported in Table 2.2 (400 ng/L) exceeds the maximum of the range found in Sauvignon Blanc wines of 88 ng/L (Mateo-Vivaracho *et al.*, 2010), by several times and should be confirmed with a larger sample set and current analytical techniques. The levels of 3MH and 3MHA shown in Table 2.2 are within the ranges reported for Sauvignon Blanc (Coetzee & Du Toit, 2012).

The results summarized in Table 2.2 cannot be taken as typical values for thiols in other varieties, because in most cases the thiol measurements were performed in fewer than 10 wines. The fact that thiols are present in many non-Sauvignon Blanc wines at levels above their odour thresholds calls for more importance to be placed on this family of compounds during chemical characterization of wines.

2.3.2 Thiols in Chenin Blanc

There is very little available research on the presence and potential importance of thiols in Chenin Blanc wines. To illustrate this point, a search using Google Scholar was performed on August 16, 2016, where <“thiols” “Sauvignon blanc”>, returned 1,220 results, while <“thiols” “Chenin blanc”>, returned just 187 results. While the 187 results include both the “thiols” and “Chenin blanc” within their text or references, almost none of the literature includes the analysis of thiols *in* Chenin Blanc wines. Additionally, some of the literature including Dubourdieu *et al.*, 2006 have cited Tominaga *et al.*, 2000 as having demonstrated the presence of thiols in Chenin Blanc. However, Tominaga *et al.*, 2000 actually only discusses Gewürztraminer, Pinot gris, Riesling, Muscat d’ Alsace, Sylvaner, Pinot Blanc, Colombard, Petit Manseng, and botrytized Semillon.

The story of Chenin Blanc and thiols is surprisingly old, and begins with the first paper where the presence of thiols in wine was hypothesized. The presence of a thiol in Chenin Blanc was first inferred indirectly in 1981 by du Plessis & Augustyn before thiols were ever identified in wine. They speculated that Chenin Blanc and Colombard’s characteristic ‘guava’ aroma could be a result of 4MMP. By adding copper sulphate (which converts volatile mercaptans (thiols) to a non-volatile form), to Chenin Blanc and Colombard wines, the authors were able to show a significant decrease in ‘guava’ aroma. Additionally, a neutral base wine spiked with 4MMP was identified by judges as Chenin Blanc or Colombard, and was described as ‘guava’, ‘fruity’, ‘sweaty’, and ‘catty’. Shortly thereafter, alternate sources of this ‘guava’ aroma were proposed in another publication. The ‘guava’ aroma of Chenin Blanc wines was also associated with other compounds, particularly ethyl butyrate, and the ratio of ethyl butyrate to ethyl decanoate and ethyl octanoate (Van Rooyen *et al.*, 1982).

It was over a decade later when a thiol (4MMP) was first identified in Sauvignon Blanc wines (Darriet *et al.*, 1995), and subsequent thiol research has focused heavily on Sauvignon Blanc. This focus on Sauvignon Blanc wines and the difficulty of measuring thiols has resulted in a large gap in the research of thiols in Chenin Blanc.

The only paper to-date which includes analysis of thiols in Chenin Blanc comes from the Department of Viticulture and Oenology (DVO) at Stellenbosch University, and reports on levels found in a few experimental wines (Weightman, 2014; Aleixandre-Tudo *et al.*, 2015). In three wines that were exposed to different skin contact treatments, the authors found levels of 3MH between 365 – 554 ng/L, and 3MHA between 0 – 35 ng/L. In the study, 3MHA levels and the perception of 'fruitiness' were found to decrease in Chenin Blanc wines which were subjected to skin contact before and during fermentation. While this confirms that thiols are present above odour thresholds in some Chenin Blanc wines, typical thiol levels in commercial wines are yet to be assessed.

According to our knowledge, no comprehensive research has been published which measured thiols in a variety of Chenin Blanc wines. The lack of research on thiols in Chenin Blanc is likely due to the difficulty of quantifying thiols in wine. Because thiols are found in such low concentrations in wine and are very volatile and sensitive to oxidation (Blanchard *et al.*, 2004; Sarrazin *et al.*, 2010), they are challenging to quantify. The analytes must be highly concentrated and preserved during extraction, while at the same time removing interferences. Additional difficulties with analysis come from the lack of availability of ideal internal standards, and the undesirable use of potentially hazardous materials like *p*-hydroxymercuribenzoate during sample preparation (Chen *et al.*, 2013). Improvements in thiol quantitation methods in terms of derivatizing agents and extraction techniques have made this analysis more feasible (Chen *et al.*, 2013; Piano *et al.*, 2015), and a survey of thiols in Chenin Blanc wines is called for.

2.4. South African Chenin Blanc aroma research

World-wide, there is little wine research dealing specifically with Chenin Blanc. Even within research on South African wines, there are studies which include a number of varieties, but still exclude Chenin Blanc (Louw *et al.*, 2010). Much of the available research on South African Chenin Blanc comes from the University of Stellenbosch. The main aroma research questions explored include sensory and/or chemical profiling of styles, profiling bush vine wines, and studying the effect of different vinification parameters, such as the use of oak (Botha, 2015), skin contact (Weightman, 2014; Aleixandre-Tudo *et al.*, 2015), or different yeasts (Reynolds *et al.*, 2001; Jolly *et al.*, 2003).

Since Chenin Blanc is a neutral grape and is well-suited to a variety of production methods, the resulting wines range from fresh with a crisp acidity to rich and heavy. This variety causes South African consumers to not know what to expect when buying a Chenin Blanc. To address consumer confusion, style classifications were implemented. The three different styles of dry Chenin Blanc wines, as recognized by the Chenin Blanc Association of South Africa (CBA) are Fresh & Fruity (FF), Rich and Ripe - Unwooded (RRUW), and Rich & Ripe – Wooded (RRW) (CBA, 2016).

Sensory profiling has shown that panels have been unable to consistently distinguish the three styles (Bester, 2011; Hanekom, 2012; Van Antwerpen, 2012). A study on dry Chenin Blanc wines also found that wines separated into two groups: FF/RRUW and RRW, with the RRW wines well-separated from the others, but FF and RRUW wines forming a continuum (Bester, 2011). The RRUW wines were described with 'earthy/light' descriptors, while the FF wines were described as 'fresh fruit', 'tropical', 'sweet' and 'floral', and the RRW wines were associated with 'buttery/caramel', 'sweet' and 'ripe fruits' descriptors (Bester, 2011). In a different study, a descriptive analysis of 42 Chenin Blanc wines was paired with a sorting study on a subset of 21 wines, and style separation was found difficult in both experiments (Van Antwerpen, 2012). In the sorting task, as in Bester (2011), again RRW wines separated from the FF and RRUW wines, which were mixed (Van Antwerpen, 2012). In this case, the RRW wines were described as ‘wood’, ‘sweet’, ‘honey’, and ‘complex’, while the RR and RRUW wines were described as ‘fruity’, ‘tropical’, ‘green’, ‘citrus’, and ‘floral’ (Van Antwerpen, 2012). In the descriptive analysis, FF wines described as ‘fresh fruit’ and ‘tropical’, opposed RRW wines described as ‘rich fruit’, and ‘wood’, with RRUW spanning the space between the other two groups (Van Antwerpen, 2012).

In one study (Hanekom, 2012) on bush vine Chenin Blanc wines using descriptive analysis, the wines separated by age with younger wines being associated with the FF style described as ‘fresh fruity’, ‘tropical’ and ‘vegetative’, and older wines with the RRW/RRUW styles described as ‘ripe/cooked fruit’, ‘woody’, ‘sweet associated’ and ‘rich fruit’. There was no separation found between wooded and unwooded wines. This agreed with the grouping within a PCA of the chemical analysis, which showed ethyl and acetate esters (ethyl butyrate, 2-phenylethyl acetate, isoamyl acetate and hexyl acetate) associated with the FF group, and a ‘floral’ monoterpene, linalool, associated with the RRW/RRUW group (Hanekom, 2012).

Chemical analysis using gas chromatography – mass spectrometry (GC-MS) and gas chromatography – flame ionization detection (GC-FID) is better able to differentiate between the different styles than sensory analysis (Lawrence, 2012). In this study, FF wines were associated with acetate esters which have ‘banana’, ‘pear’, ‘honey’ and ‘rose’ aromas (isoamyl acetate and 2-phenylethyl acetate). These results agree with those found for FF wines by Hanekom (2012). The RRUW wines were associated with ethyl butyrate, ethyl hexanoate and two terpenes (geraniol and β-ionone), which give ‘apple’, ‘strawberry’, ‘violet’, ‘rode’ and ‘geranium’ aromas. The last group, RRW was associated with compounds classically derived from malolactic fermentation giving ‘buttery’, ‘creamy’, and ‘toasty’ aromas, namely ethyl lactate, diacetyl, and acetoin (Lawrence, 2012). This differentiation between styles was less clear in another chemical profiling of 105 dry and semi-dry South African Chenin Blanc wines, with a continuum from FF to RRUW to RRW (Van Antwerpen, 2012). This study found that FF wines had higher levels of isoamyl acetate and ethyl hexanoate, RRUW wines had higher levels of the monoterpene limonene which smells of ‘orange’, and RRW wines has higher levels of ethyl lactate and diethyl succinate (Van Antwerpen, 2012).

Other aspects of Chenin Blanc aroma have been studied in a few cases. It was found that high shipping temperatures (37 °C) result in a decrease in ‘tropical’ and ‘fruity’ aromas in Chenin Blanc wines, and an increase in ‘over-aged’ aroma (Du Toit & Piquet, 2014). These same aromas can also be influenced by the yeast used to ferment the wines. The use of *Candida pulcherrima* in combination with *Saccharomyces cerevisiae* during fermentation of Chenin Blanc did not affect levels of esters compared to controls fermented with *S. cerevisiae* alone, though the sensory analysis showed the highest ‘guava’ levels in the wines fermented with *C. pulcherrima* (Jolly *et al.*, 2003).

Investigations into the sensory effect of oak and alternative oak products on Chenin Blanc wines (Botha, 2015) showed unoaked wines were described as ‘lemon’, ‘grapefruit’, ‘pineapple’ and ‘passionfruit’, while wines aged in 5th-fill Sylvain Reserve barrels were described as ‘peach’, ‘grapefruit’, ‘guava’ and ‘dried fruit’. Wines matured in new barrels were described as ‘dried fruit’, ‘marmalade’, ‘oak’, ‘caramel’ and ‘vanilla’, while stave treatments were described as ‘raisin’, ‘caramel’, ‘toffee’, ‘honey’, and ‘burnt/smoked wood’. This study also demonstrated some evolution of aromas over time, with the unoaked wine evolving from ‘citrus’ and ‘pineapple’ after 4 months of aging, to ‘baked apple’, ‘banana’, and ‘dried peach’ after 6 months of aging, and ‘passionfruit’, ‘dried apple’ and ‘orange blossom’ after 9 months of aging (Botha, 2015).

Generally, it seems that differentiating Chenin Blanc wines styles based on aroma is challenging with the current style classifications. Assessors are in all cases able to differentiate between FF and RRW wines, but RRUW wines form a continuum between the other styles, or group with the FF or RRW wines. The differences in sensory analysis found may be attributed to the different sensory methodologies used, different sample sets, or different groups of assessors. The chemical analysis indicates that certain esters like isoamyl acetate and 2-phenylethyl acetate are associated with FF wines, while some monoterpenes may characterize RRUW wines and RRW wines can be associated with malolactic fermentation-derived characters like diacetyl and diethyl succinate.

While sensory and chemical profiling of Chenin Blanc wine has been performed in a variety of different ways, almost none of the research has taken thiols into account, as discussed in section 2.3.2. Much of the research has been focussed on style classification, with less focus on viticultural or oenological parameters affecting Chenin Blanc.

2.5. Sensory methods

Ultimately, wine is meant to be enjoyed as a social and sensory experience. Due to the complexity of the wine matrix, this sensory experience is difficult to predict solely from chemical analysis (Campo *et al.*, 2005). Estimates of the number of volatile aroma compounds which contribute to wine aroma have risen from several hundred (Blanchard *et al.*, 2004) to at least 1000 different compounds (Francis & Newton, 2005; Polášková *et al.*, 2008). Though it has been shown that in each wine, just a few compounds (termed impact compounds) are responsible for the dominating aroma characteristics, pinpointing which compounds are important to measure in different wines is a daunting task (Polášková *et al.*, 2008). One of the main challenges of chemical profiling other than the analysis itself, is assuring that all relevant compounds are measured. This is one reason why it is extremely difficult to predict the sensory perception of a wine from its chemical composition.

Odour active values (OAVs), also referred to as Aromatic Index (Blanchard *et al.*, 2004), are one tool used to contextualize chemical results by translating them into a number that may indicate potential sensory impact. OAVs are calculated as the concentration of a compound divided by its odour threshold, and a value above 1 is considered “odour active”. However, this index is not perfect, as a high OAV does not mean in all cases that the compound will be important, or even perceived in the wine due to matrix effects (Escudero *et al.*, 2004). Even if all the relevant compounds in a wine are measured, their perception can be altered by different wine matrices and interact with one another in unexpected ways (Swiegers *et al.*, 2005; Polášková *et al.*, 2008; Barkat *et al.*, 2012). For these reasons the chemical analysis of wine in isolation, though valuable, is of limited use.

Chemical analysis of wine aroma should not be performed alone, but rather paired with sensory analysis to increase the relevance of research. While humans are variable and imperfect instruments, the selectivity of the human nose has the incredible ability to detect at least 10,000 odorants (Axel, 1995). Connecting chemical and sensory data allows the researcher to explain the sensory relevance of their findings, and contextualize them in terms of the human experience. This connection between sensory and chemical data can be performed statistically by creating a model by regression methods such principal component regression (PCR) and partial least squares regression (PLS) (Næs *et al.*, 2010). In cases where regression cannot be used, associations between chemical compounds or classes and the sensory properties of different wines can also be explored with principle component analysis (PCA).

The field of sensory analysis offers many methodologies that are suited to different experimental applications. In cases where researchers are interested profiling relatively similar products, a technique like descriptive analysis is more appropriate. Other methods such as sorting, projective mapping, flash profile, or polarized sensory positioning are more rapid and suited to analyse products that are less complex, or have large sensorial differences (Valentin *et al.*, 2012). The appropriate method is also selected based on the goal of the experiment and what type of data is required. Some methods are more suited to generating descriptors and the intensities thereof, while others are more focused on grouping the wines by similarity and representing sensory distances. The sensory methodologies relevant to this thesis, along with their applications, advantages, and disadvantages are discussed in the following sections.

2.5.1. Descriptive analysis in wine sensory research

Descriptive analysis (DA), also known as conventional profiling, is seen as the “gold standard” for sensory analysis as it provides detailed, quantitative information and is good for describing even small differences between products (Lawless & Heymann, 2010). It is a consensus training method which evaluates differences between products in terms of descriptor intensities. As a well-validated traditional method, DA is often used as a point of comparison for new methodologies (Bester, 2011; Chollet *et al.*, 2011; Hanekom, 2012; Hopfer & Heymann, 2013; Torri *et al.*, 2013). It has helped validate Projective Mapping (PM, see section 2.5.2.2) with a set of wines including Chenin Blanc wines with two other varieties from France (Pagès, 2005). DA or versions thereof can be applied during product development, sensory characterization of products (*e.g.* types of wines, or the effect of treatments), and volatile compound interaction studies (Lawless & Heymann, 2010; Coetzee *et al.*, 2015).

In the general descriptive analysis method, 8 to 12 panellists are led by the panel leader through a series of training sessions which familiarize panellists with the product set and teach them to rate the intensity of important sensory attributes (Lawless & Heymann, 2010). The training sessions focus on generating a concise list of product descriptors, familiarizing panellists with the descriptors through the use of aroma reference standards, and practicing judging the intensity of those descriptors (Lawless & Heymann, 2010). The samples used during training can either be the same products to be evaluated in the testing session, or a set of products with similar sensory characteristics. Once the panel is sufficiently trained, the panellists are presented with the products in a randomized order, and asked to rate the intensity of each descriptor on a scale of 1-100 for each product.

Panel performance can be evaluated by means of univariate and multivariate statistical methods in a workflow proposed by (Tomic *et al.*, 2010) using the PanelCheck software program (http://www.panelcheck.com), Several statistical tools can be used to evaluate panel performance. Tucker-1 plots help to evaluate discrimination ability of individual panellists for each descriptor, and Manhattan plots can give a picture of the panellists’ individual performances (Tomic *et al.*, 2010). Analysing panel performance in this way is important during the training process to be able to identify areas whether there is consensus among the panellists and whether individual panellists are repeatable and are able to perceive each attribute. and after the testing to confirm that the panellists performed satisfactorily. DA data is typically analysed by analysis of variance (ANOVA) to determine which descriptors have significantly different intensities between products, and check for repetition and judge effects (Lea *et al.*, 1997). The data can be visually represented by principle component analysis (PCA). PCA is a multivariate analysis that creates a two-dimensional representation of the data in a way that the maximum amount of variance is explained (Lawless & Heymann, 2010). Canonical variate analysis (CVA) can also be used, which allows for calculation of significantly discriminating dimensions, and 95% confidence intervals (Heymann *et al.*, 2014).

The advantage of DA is that it is well-established and reliable, and commonly performed in sensory laboratories around the world. For products with subtle sensory differences, or in cases where researchers want to evaluate small differences in intensity, descriptive analysis is accepted as the best method. It also uses a trained panel, which assures that the language and descriptors used are understood in the same way by all assessors. Disadvantages of the method mainly come from its costly and time-intensive nature, due to the many training sessions involved. Additionally, attaining consensus between panellists can be difficult. A subtle drawback has to do with the way the samples are presented. In DA, a monadic sequential presentation is used (each product is compared with the previous product and evaluated within the context of the "product space"). This relies more on memory and training than the holistic presentation used in other methods, where all the products are compared to one another at the same time.

2.5.2. Rapid methods

Though descriptive analysis provides reliable, quantitative descriptions of products through its carefully structured procedures, the disadvantages of time and cost involved can be prohibitive. To address these issues, the family of "rapid methods" has been developed which require little-to-no training, and have increased greatly in popularity over the last decade (Valentin *et al.*, 2012). They aim to provide quick, free-form, and intuitive ways of performing sensory analysis.

Rapid methods can be separated into descriptive methods, which aim to describe the sensory attributes of products, and discriminative methods, whose goal is to group wines by similarity or dissimilarity. Some popular descriptive rapid methods include free choice profile, flash profile, and ultra flash profile, while discriminative rapid methods include sorting, Napping®, projective mapping, polarized sensory positioning, polarized projective mapping, and sorted napping (Valentin *et al.*, 2012). Frequently, discriminative and descriptive methods are combined (such as projective mapping with ultra flash profiling) to gain information on which products are similar or dissimilar, as well as what attributes drive those differences.

While researchers must be careful not to prioritize convenience of data gathering over quality of data or fit of the method, rapid methods have been shown to give acceptable or high-quality data in many instances (Dehlholm *et al.*, 2012b; Valentin *et al.*, 2012), especially when it comes to products with large differences or when researchers are more interested in the discrimination between products than quantifying attributes (Delarue & Sieffermann, 2004). Rapid methods can be used in combination with conventional descriptive analysis to enhance the depth of gathered data, or used on their own. With refinement and development of more rapid methods, they are becoming very popular and important tools in the sensory scientist's arsenal. The specific rapid methods relevant to this work are explained below.

Ultra flash profiling (UFP)

Ultra flash profile (UFP) is a descriptive method which gives the important characteristics of each product assessed. It is frequently used as an accompaniment to discriminative rapid methods to enrich the data obtained. UFP is a simplification of other earlier descriptive methods. Originally, free choice profile (FCP) (Williams & Langron, 1984) was adapted to flash profile (FP) (Dairou & Sieffermann, 2002; Delarue & Sieffermann, 2004), which later became UFP. Flash profile is an adaptation of free choice profile in which panellists are asked to freely describe the products, the descriptors are pooled by the experimenter, and finally the products are ranked for each descriptor separately. From Flash Profile, a variation called ultra flash profile (UFP) was proposed, which simplifies the process to better accompany rapid methods since it is an intuitive one-step process (Perrin *et al.*, 2008). This method removes the step of pooling, as well as the ranking procedure. It simply requires that panellists describe the products with their own criteria, and the aggregation of the terms is done by the experimenter after the testing. This type of data which generates a contingency table is analysed by correspondence analysis (CA) (Perrin & Pagès, 2009).

This method is intuitive, particularly for the wine industry where people are accustomed to describing wines by listing sensory attributes. It is useful as a complementary descriptive technique, as many of the rapid methods do not include descriptive data about the products being described. For example, as projective mapping (see section below) alone only provides positional data, it is frequently combined with UFP (Dehlholm *et al.*, 2012b). The main disadvantage of this method is the difficulty of simplifying the data into a useful format. Since the panellists are allowed to freely describe the products, the list of descriptors for complex products can be very long with many semantic and linguistic synonyms. These terms must be combined by the experimenter, which introduces a definite element of subjectivity. This is mitigated by condensing the list in consultation with other experimenters in the sensory field following a set of rules. It also only provides a matrix of binary data, with a “0” if a descriptor is not present for a particular product, and a “1” if it is, rather than the detailed intensity scale used in DA.

Napping® and projective mapping (PM)

Napping® and projective mapping (PM) are discriminative methods, specifically (dis)similarity methods which use the degree of similarity or dissimilarity between products as the basis of discrimination. Projective mapping (PM), was introduced to sensory science by Risvik *et al.* in 1994 as a way to allow panellists to evaluate each product as a whole, instead of dissecting and rating individual descriptors one at a time. In this method, assessors arrange products on a two-dimensional surface (usually 40 cm x 60 cm), where the physical distance between products represents their degree of similarity or dissimilarity. It is extremely free-form in nature, as the criteria used by the judges is not restricted. PM has been applied to wines, including Chenin Blanc in several cases (Pagès, 2003, 2005; Morand & Pagès, 2006; Perrin *et al.*, 2008; Torri *et al.*, 2013; Heymann *et al.*, 2014; Weightman, 2014; Botha, 2015).

While projective mapping and Napping® are very similar methodologies, the difference outlined in Dehlholm *et al.* (2012b), explains that Napping® is a form of projective mapping, which restricts the space to 40 cm x 60 cm and collects descriptive data by performing UFP in the same session. Since the Napping®/projective mapping methods were first applied to wine (Pagès, 2003, 2005), they have gained in popularity and several variations have been developed, such as partial napping (Dehlholm *et al.*, 2012b), sorted napping (Pagès *et al.*, 2010), and polarized projective mapping (Ares *et al.*, 2013).

The data matrix obtained in PM is formatted as rows of products, with columns of (X,Y) coordinates separated by judge (Pagès, 2005). Data of this type must be analysed by multivariate techniques. Originally, generalized Procrustes analysis (GPA) was used for the data (Risvik *et al.*, 1994), but multiple factor analysis (MFA) (Escofier & Pagès, 1994) has become the preferred statistical analysis method (Pagès, 2005; Morand & Pagès, 2006). MFA essentially performs multiple principle component analyses (PCAs) on a number of tables of variables, and then combines them into one global, multidimensional map (Abdi *et al.*, 2013).

Interpretation of the MFA is not as straightforward as interpreting an ANOVA of DA results. Since MFA is a two-dimensional representation of three-dimensional data, it is important to consider the $\cos^2$ of each product in each dimension (Husson *et al.*, 2011). The $\cos^2$ shows how close the vector of each product is to the plane of the consensus map and gives information about the quality of the product's representation on the map. Particularly for the case of products which are better represented in another dimension, these products' placement in the MFA may be misleading. Additionally, confidence ellipses are a convenient tool to aid in the interpretation of the MFA representation. Dehlholm confidence ellipses run on a parametric bootstrapping method creating virtual panels with replacement, and provide information about the reliability of the actual configuration of groupings (Dehlholm *et al.*, 2012a). An increased multidimensionality in the data with PM as compared to DA has been seen, due to the unlimited and undefined factors considered in the analysis (Perrin *et al.*, 2008). As a result, it may be important to consider the additional dimensions of the resulting MFA beyond just two dimensions.

There are several advantages of the PM technique. As no training sessions are required and evaluation can take place in one isolated session, PM is a cost and time-effective method. The flexibility afforded by the single testing session especially makes it convenient to use experts with limited availability (Pagès, 2005). Another major advantage is the fact that it can be used with many different types of panellists, whether they be consumers, experts or industry (Valentin *et al.*, 2012). From a psychological perspective, the method is holistic in two ways: all of the products are evaluated at the same time, and the product is evaluated as a whole instead of dissecting it into several descriptors that must be rated (Pagès *et al.*, 2010; Dehlholm, 2012).

In terms of disadvantages, in the basic PM technique, no descriptive data is obtained, so the resulting map can only be discussed in terms of similarity and dissimilarity, and the sensory drivers of groupings are unexplained. The solution to this issue is simply to combine it with a descriptive method, such as Ultra Flash Profiling (Perrin *et al.*, 2008). However, the disadvantages of UFP discussed in the section above still apply. It also may be cognitively difficult for some assessors to perform (Perrin *et al.*, 2008; Hopfer & Heymann, 2013). Difficulty arises for the experimenter as well in interpreting the MFA, and the cautions discussed above must be considered. A significant drawback of PM is that the space on the map is finite and so the number of products is limited to around 12, greatly limiting the utility of this technique (Pagès, 2005).

Polarized sensory positioning (PSP)

In this section, the aspects of polarized sensory positioning (PSP) which apply to the next section on polarized projective mapping are explained. PSP is also a similarity-based method, but rather than comparing all the samples to one another as in PM, each sample is evaluated in terms of degree of similarity to three pre-selected reference samples ("poles") (Teillet *et al.*, 2010). The samples are rated on scales ranging from "exactly the same" to "totally different". This design gives the major advantage of being able to compare products evaluated in separate sessions (Teillet, 2014). The use of poles provides a point of reference which is kept constant between different evaluations. This essentially allows large sample sets to be analysed over several sessions. Currently, aggregation of data has only been performed with PSP using results from an incomplete block design, rather than separate evaluation sessions (Teillet, 2014). The main disadvantages of this method are that the product comparisons are just one-to-one, rather than holistic as in PM, and the discriminative capacity is lowered for samples that are very similar to the poles (Ares *et al.*, 2013).

PSP has been applied to mineral waters (Teillet *et al.*, 2010), make-up foundations (De Saldamando *et al.*, 2013), yogurts (Cadena *et al.*, 2014), chocolate-flavoured milks, vanilla milk desserts, and orange-flavoured powdered drinks (Ares *et al.*, 2015). The applicability of this method to wine research has recently been explored (Crous, 2016).

Polarized projective mapping (PPM)

Polarized projective mapping (PPM) is a new discriminative method which combines PM and PSP (Ares *et al.*, 2013). As in PM, it is based on the physical arrangement on a two-dimensional plane, where product (dis)similarity is considered as the distance between objects. However, this method also incorporates the concept of poles from PSP. It is performed by pre-locating the three "poles" on an A3 (60cm x 40 cm) sheet of paper, and asking panellists to arrange the products around the poles, with the degree of physical distance between products representing the degree of sensory difference (Ares *et al.*, 2013). Like PM data, PPM data is analysed by MFA or GPA. However, as the assessors do not place the poles onto the sheet themselves, they should not contribute to the construction of the MFA and are treated differently during the statistical analysis (personal communication, Dr. Gastón Ares, 2015). Instead of including their coordinates in the table used to perform the MFA, they are treated as supplementary individuals and projected onto the MFA once it has been created.

From PSP, it takes the idea of using poles in order to aggregate results from different sessions. This gives the experimenter the ability to analyse a larger sample set than is currently possible with PM, removing one of PM's main disadvantages. This advantage should not be understated and makes PPM an exciting new technique, but so far data aggregation has not been performed. The method has only been applied to orange-flavoured powdered drinks in just three studies (Ares *et al.*, 2013; De Saldamando *et al.*, 2015a, 2015b). It has not yet been validated for more complex product categories, such as wine. Necessary areas of method development are the importance of the location of the poles on the paper, and PPM's applicability to more sensorially complex products (Ares *et al.*, 2013). Further exploration and validation of this method in the future could prove its usefulness to the field of sensory science.

2.6. Conclusions

As an important cultivar for the South African wine industry, Chenin Blanc has the potential to produce excellent wines. However, relatively few scientific publications have specifically focused on Chenin Blanc. While Chenin Blanc wines have been sensorially and chemically profiled to an extent, sensory evaluation has shown difficulty classifying different styles of Chenin Blanc, and chemical profiles have not included thiols in their analyses. Considering that thiols have been shown to be present at levels above their odour thresholds in many varieties, it can be hypothesized that they may be important to Chenin Blanc wines as well.

The information gained through chemical analysis of wines is enriched when paired with sensory analysis, as it is important to understand the human perception of volatile aroma compounds. This relationship is complex because the perception of volatile aroma compounds can change based on their concentration and the matrix in which they are found. Considering this, carefully-designed sensory experiments are necessary to be able to describe the impact of particular chemical compounds on wines. Some of the methods which can be used to do so were detailed in this chapter. Each methodology has its advantages and disadvantages, and new methods are being developed to address the limitations of current methods. Method development and validation is important, as it leads to advances in sensory science and provides a larger array of tools for wine researchers to use. A combination of thiol analysis of South African Chenin Blanc wines and appropriate sensory analyses, as presented in the remainder of this thesis, would help to fill in gaps in the current knowledgebase of the aroma of these wines.

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Chapter 3

Research Results

Thiol levels in dry South African Chenin Blanc wines

Chapter 3 : Thiol levels in dry South African Chenin Blanc wines

3.1 Introduction

The importance of thiols to Sauvignon Blanc wines around the world has been established and, as detailed in Chapter 2, investigations have extended to other varieties as well (Chapter 2, Table 2.2). In the other varieties analysed, thiols have been found, though concentrations of individual compounds differ between varieties (Mateo-Vivaracho *et al.*, 2010). The presence and importance of thiols to Chenin Banc wine, however, has not been investigated though it has been speculated. In fact, in 1981, du Plessis and Augustyn hypothesized the presence of 4-mercapto-4-methylpentan-2-one (4MMP) in the variety before varietal thiols were ever identified in wine.

Chenin Blanc is generally considered as a neutral variety, and is easily moulded by the winemaker's choices. Chenin Blanc can be fermented in stainless steel to preserve its freshness and fruitiness, but the cultivar also lends itself to different vinification techniques like barrel fermentation, barrel maturation, and lees aging. Despite the different winemaking practices resulting in different wine styles, Chenin Blanc is often characterized as having 'guava', and 'tropical' notes, though the chemical(s) responsible for this aroma haven't been established yet. Du Plessis and Augustyn (1981) based their original paper on this 'guava' character, and found that it disappeared along with varietal typicity with the addition of Cu_2SO_4 to the wines. This led to the hypothesis that a sulphur-containing compound like 4MMP could be responsible for the 'guava' character.

Thanks to advancements in the field of analytical chemistry and the development of methods suited to the quantitation of thiols in wine, this analysis has become more feasible for researchers to perform. The presence of thiols in Chenin Blanc wines were confirmed for the first time recently in three experimental wines in a study investigating skin contact (Aleixandre-Tudo *et al.*, 2015), but the thiol measurements were part of a large set of chemical analyses and were not the focus of the investigation. Thiols have been reported in Chenin Blanc in this case, but the three wines were made from one batch of grapes and no study has been undertaken to investigate thiol levels in Chenin blanc commercial wines. For this reason, in this study, 65 commercial dry South African Chenin Blanc wines were analysed for their thiol concentrations. The thiols measured are 3-mercaptohexyl acetate (3MHA), which has aromas of 'passion fruit', 'grapefruit', and 'box tree' (Tominaga *et al.*, 1996) and 3-mercaptohexan-1-ol (3MH), which is described as 'passion fruit' and 'grapefruit' (Tominaga *et al.*, 1998). The usefulness of data mining exercises, as emphasized by Valente (2016), prompted a mining of trends within the thiol results. The data collected was used to explore correlations between various characteristics of the wines (such as use of barrels in vinification and vine age) and thiol levels.

3.2 Materials and methods

3.2.1. Samples

In total, 65 commercially-available dry South African Chenin Blanc wines were analysed over two years. Twenty-five were measured in 2015, and 40 in 2016. At the time of analysis, the ages of the wines were as follows: 1-year (n=48), 2-years (n=13), and 3-year (n=4) old. Samples were purchased from retailers and wine farms within the Western Cape. Information on each wine for the data mining portion of this study was obtained from the bottle labels, fact sheets, and communications with tasting room managers and winemakers.

3.2.2. Chemical analysis method

Two volatile thiols, 3-mercaptohexan-1-ol (3MH) and 3-mercaptohexyl acetate (3MHA) were analysed according to the method published by Piano *et al.* (2015). The method used a liquid-liquid extraction, followed by concentration of the samples and derivatization before injection into the ultra-performance liquid chromatography mass spectrometer (UPLC-MS/MS) using the settings described in the original method cited above.

3.2.3. Statistical analysis

Categorical factors were analysed with one-way analysis of variance (ANOVA), and the quantitative factors were analysed by Spearman's correlation. The significance threshold was set at α=0.05. Any categories with five or fewer individual observations, such as a few regions of origin, were removed from the analysis due to insufficient sample size. As the thiol results of the 65 wines did not follow a normal distribution, the thiol results were subjected to a logarithmic transformation before all statistical analyses. Levene's test for homogeneity of variance was performed for each ANOVA. If Levene's test was significant (at α=0.01), meaning the variances of the groups were not equal, the more conservative Games-Howell post hoc test was used to illustrate significant differences. In cases where Levene's test was not significant, Fisher's least significant difference (LSD) was used.

3.3 Results and discussion

3.3.1. Thiol results

Considering that the wines measured were a mixture of ages, which may have an impact on thiols, the thiol analysis results (Table 3.1) are subdivided by age of the wine at the time of measurement.

Table 3.1 3MHA and 3MH levels of dry South African Chenin Blanc wines

	3MHA (ng/L)			3MH (ng/L)		
Age of Wine (Years)	**Min**	**Max**	**Mean ± SD**	**Min**	**Max**	**Mean ± SD**
1 (n=48)	0	305	31 ± 58	380	2929	883 ± 472
2 (n=13)	0	15	1 ± 4	502	1201	804 ± 231
3 (n=4)	0	0	0	887	1937	1302 ± 451
AVERAGE			23 ± 52			893 ± 442

As hypothesized, quantifiable levels of both 3MHA and 3MH above their odour thresholds (4.2 and 60 ng/L, respectively, (Tominaga *et al.*, 1996, Tominaga *et al.*, 1998) were found in the wines (Table 3.1). The average level of 3MHA was similar to the levels of the three experimental Chenin Blanc wines previously published (Aleixandre-Tudo *et al.*, 2015), but the average level of 3MH found here was twice as high. This difference is likely due to the very small sample size of the previous study. For both thiols, there is a large standard deviation of concentration relative to the mean. Both the maximum and mean 3MHA levels are much higher in 1-year-old wines then older wines. For the case of 3MH, the maximum value was also found in a 1-year-old wine but unlike 3MHA, the mean values between the 1-year-old wines and 2-year-old wines are similar, and even increase for the 3-year-old wines. This difference in trends between the levels of the two thiols agrees with previous research on New Zealand Sauvignon Blanc showing a quick decrease of 3MHA over time, while 3MH slowly increases (Herbst-Johnstone *et al.*, 2011).

Comparing the 1-year-old Chenin Blanc wines to a set of 24 1-year-old South African Sauvignon Blanc wines (Van Wyngaard, 2013), the average of 3MHA in the Chenin Blanc wines is much lower (31 ng/L in Chenin Blanc, compared to 158 ng/L in Sauvignon Blanc), but the average level of 3MH is comparable (883 ng/L, compared to 970 ng/L). This means that 3MHA may be more important than 3MH to differences in thiol-related aromas between young Chenin Blanc and Sauvignon Blanc wines from South Africa.

In the context of the 73 non-Sauvignon Blanc wines (both white and red) summarized in Mateo-Vivaracho *et al.*, 2010, these Chenin Blanc results fall within the range observed for 3MHA (<2 ng/L - 425 ng/L). However, the maximum level of 3MH found in this set of Chenin Blanc wines (2929 ng/L, Table 3.1) is higher than their maximum of 2349 ng/L. A summary of all thiol results found in the literature in varieties other than Sauvignon Blanc is detailed in Chapter 2 (Table 2.2), though it should be noted that very few samples were measured in each of those cases.

The sensory perception of individual thiols at different concentrations has been studied using triangle tests with free description (Mateo-Vivaracho *et al.*, 2010). In partially-dearomatized white Maccabeo wine, 3MHA contribute to ‘fruitiness’ and ‘freshness’ of wine at concentrations of 6.4 ng/L, ‘tropical fruit’ character at 25 ng/L, and ‘tropical’ and ‘box tree’ aroma above 50 ng/L. If a similar trend the same holds true for Chenin Blanc wines, 3MHA could have a different sensorial impact at the different levels within the range of 3MHA found in this study (0 ng/L - 305 ng/L, Table 3.1). Illustrated by the mean of 23 ng/L of 3MHA compared to the maximum of 305 ng/L, a few wines had exceptionally higher levels than the rest. In 41 of the wines measured, however, 3MHA was not detected (Appendix A, Table A.1). It is possible that these differences in 3MHA level affect to the ‘fruity’ or ‘tropical’ aroma of the wines, but the sensorial impact of 3MHA in Chenin Blanc is yet to be determined.

For the Chenin Blanc wines measured, 3MH was present at a minimum of 380 ng/L (Table 3.1) which is above the aroma threshold of 60 ng/L, indicating that this thiol was a contributor to the aroma of the wines in this sample set.

Table 3.2 Odour Active Values (OAVs) of thiol measurements in South African Chenin Blanc wines by age at time of measurement

	3MHA (ng/L)			3MH (ng/L)		
Age of Wine (Years)	**Min**	**Max**	**Mean**	**Min**	**Max**	**Mean**
1 (n=48)	0	73	7	6	49	15
2 (n=13)	0	3	0	8	20	13
3 (n=4)	0	0	0	15	32	22
AVERAGE			23			15

An Odour Active Value (OAV) is calculated as the ratio of a compound’s concentration over its odour threshold. It is useful for determining the potential sensory impact of chemical compounds, as a value over 1 is considered “odour-active”. The OAVs were calculated for 3MHA and 3MH in the set of Chenin Blanc wines measured (Table 3.2) using the thresholds of 4.2 ng/L for 3MHA (Tominaga *et al.*, 1996), and 60 ng/L for 3MH (Tominaga *et al.*, 1998). The range of OAVs for both compounds demonstrates that theoretically, thiols could contribute to sensorial differences between these wines. Though the concentrations of 3MHA are lower than 3MH in the wines (Table 3.1), due to 3MHA's lower threshold, it has a higher maximum OAV and may have a higher impact on wine aroma than 3MH (Table 3.2). Though these OAVs indicate that the compounds should be perceived in the wines, they do not necessarily correlate with intensity of sensorial perception, and it is not possible to predict how they would be perceived at different concentrations. These values help to underline the fact that while the concentrations of thiols found are only in concentrations of ng/L, the compounds are still sensorially important to the wine.

3.3.2. Trend exploration

In order to explore the thiol results in greater detail, a series of statistical analyses were conducted to explore correlation between extrinsic factors and intrinsic properties of the with 3MH and 3MHA levels. Readily-available information about the wines was collected from bottle labels, fact sheets, and personal communications, with the purpose of a data mining exercise. Categorical factors were analysed by a series of one-way ANOVAs, and Spearman's correlations were used to analyse the quantitative factors such as lees aging and wine price. The factors selected were:

- Wine age (years)
- Wine origin (W.O. as labelled on the bottle)
- Vine age (young vine vs. old vine 35 years or older)
- Vine trellis system (bush vines vs. trellised vines)
- Wood contact
- Lees aging (months)
- Wine price

It must be emphasized that this is not a controlled study where one factor was varied at a time. In fact, in the real world all of these factors interact with one another. This means any significant differences found cannot be said to be *due to* or *caused by* the factor. Rather, they are *correlated* with the factor. Nevertheless, this type of informal data mining yields interesting information, as it has the potential to help form hypotheses and direct future research.

The thiol data were subjected to a logarithmic transformation prior to statistical analysis, as they were not normally distributed. However, the values before transformation are reported in the tables below so that means and standard deviations represent actual levels in ng/L.

Wine age

As thiols are unstable over time, the age of the wine at the time of analysis was considered. The 2-year-old and 3-year-old wines were combined in this analysis due to small sample sizes. Corresponding with the trend already observed (Table 3.1), for the effect of wine age at the time of measurement, a significant difference was found for 3MHA ($F(1,63)=11.287$, $p<0.05$) (Table 3.3). The levels of 3MHA in 1-year-old wines are significantly higher than the levels for 2- and 3-year-old wines. However, no significant difference was found for 3MH ($F(1,63)=0.514$, $p=0.48$), and in fact the mean was slightly higher for the group of 2- and 3-year-old wines (Table 3.3). As discussed in section 3.3.1, this observation supports the finding that 3MHA is unstable and decreases rapidly over time (Herbst-Johnstone *et al.*, 2011). The wine age factor is also linked with that of wood contact, as older wines measured were likely matured in oak barrels. The results of the ANOVAs for these factors do agree (Table 3.3 and Table 3.7). The instability of 3MHA is important for winemakers to recognize, as it can explain one way in which wine aroma changes during aging. Since the values of 3MHA are probably at a maximum at the end of fermentation and decrease from thereon (Herbst-Johnstone *et al.*, 2011), speculatively the starting 3MHA values of these Chenin Blanc wines may have been even higher just after fermentation.

Table 3.3 ANOVAs of Wine Age vs. 3MHA (ng/L) and Wine Age vs. 3MH (ng/L)

	3MHA		**3MH**	
	F(1, 63)=11.287, *p*=0.0013		**F(1, 63)=0.514, *p*=0.48**	
Wine Age (years)	**3MHA (ng/L) Mean**	**3MHA (ng/L) SD**	**3MH (ng/L) Mean**	**3MH (ng/L) SD**
1 year (n=48)	31[a]	58	883	472
2 or 3 years (n=17)	1[b]	4	921	354
TOTAL (n=65)	23	52	893	442

Different letters indicate statistically significance differences ($p \leq 0.05$) according to Fisher's LSD.

Wine origin

The concept of "regionality" of wine in terms of chemical composition and sensory perception has been studied, but generally in terms of country of origin, rather than regions within a country. Significant differences have been found for different classes of compounds in Malbec from California and Argentina (King *et al.*, 2014) and for Sauvignon Blanc in Austria, New Zealand and France, where significant differences in the thiol 4-mercapto-4-methylpentan-2-one (4MMP) correlated with sensory perception (Green *et al.*, 2011).

In the case of the samples measured in this study, region was considered as the "Wine of Origin" (W.O.), as indicated on the bottle label. Regions with five or fewer observations (Simonsberg-Stellenbosch, Piekenierskloof, Swartland, Lutzville Valley, Cederberg, Robertson, Bottelary) were excluded. For this effect, significant differences were found for both 3MHA ($F(3,50)=5.164$, $p<0.01$), and 3MH($F(3,50)=5.833$, $p<0.01$) (Table 3.4). A significantly higher level of 3MHA was found in the Coastal Region, and levels of 3MH were significantly lower in wines from Stellenbosch. One might think this could be due to the more frequent use of oak contact in Stellenbosch (68% of wines from this region had oak contact, compared to 28.6% of the Coastal Region wines) but, as explained below, oak had a significant effect only on 3MHA levels.

As the W.O. regions are not distinct geographical areas, but rather are nested within one another, these differences should not be attributed to geographic location, climate, or *terroir*. The Western Cape encompasses all the other regions, and the Coastal Region includes the Paarl and Stellenbosch regions. Rather, different marketing strategies, cultivation practices or vinification practices are associated with choosing one W.O. over another. For example, there may be an interaction with price, where lower-cost wines with less oak contact are likely to be large-volume blends of Chenin Blanc grapes from multiple regions, and therefore labelled as Coastal Region or Western Cape.

Table 3.4 ANOVAs of Wine Origin vs. 3MHA (ng/L) and Wine Origin vs. 3MH (ng/L)

	3MHA		3MH	
	F(3, 50)=5.164, *p*=0.0035		F(3, 50)=5.833, *p*=0.0017	
Wine Origin	**3MHA (ng/L) Mean**	**3MHA (ng/L) SD**	**3MH (ng/L) Mean**	**3MH (ng/L) SD**
Stellenbosch (n=25)	12[b]	24	670[b]	231
Coastal Region (n=8)	52[a]	76	1092[a]	499
Western Cape (n=14)	31[a]	35	894[a]	348
Paarl (n=7)	6[b]	15	1015[a]	220
TOTAL (n=54)	22	40	835	347

Different letters indicate statistically significance differences ($p \leq 0.05$) according to Fisher's LSD.

Vine age (young vines vs. old vines, 35 years or older)

For the definition of vine age, both the Chenin Blanc Association of South Africa and an authority on South African old vines, Rosa Kruger (http://www.iamold.co.za) were consulted. The more conservative definition of 35 years or older was used for this study. For the effect of vine age, significant differences were found for levels of 3MHA ($F(1,63)=8.923$, $p<0.01$), but not for 3MH (F(1,63=0.935, $p=0.34$) (Table 3.5). The effect of vine age on Chenin Blanc has not been published yet, but there is growing interest for this research topic. Old vines may be more likely be bush vines (Hanekom, 2012), and more likely to undergo a more oxidative aging process in barrel.

Table 3.5 ANOVAs of Vine Age vs. 3MHA (ng/L) and Vine Age vs. 3MH (ng/L)

	3MHA		3MH	
	F(1, 63)=8.923, *p*=0.0040		F(1, 63)=0.935, *p*=0.34	
Vine Age	**3MHA (ng/L) Mean**	**3MHA (ng/L) SD**	**3MH (ng/L) Mean**	**3MH (ng/L) SD**
old vine 35+ year (n=24)	4[b]	10	816	313
young vine (n=41)	34[a]	62	938	501
TOTAL (n=65)	23	52	893	442

Different letters indicate statistically significance differences ($p \leq 0.05$) according to Fisher's LSD.

Vine trellis system (bush vines vs. trellised vines)

The wines categorized as "bush vine" were those marketed as bush vine wines either on the bottle or on the fact sheets, and all other wines were assumed to be 'trellised'. This decision was made on the assumption that since "bush vine" wines are well-regarded, farms would put this in their marketing materials. No significance was found for the effect of trellis type on either 3MHA or 3MH levels (Table 3.6). It is possible that thiols are affected by trellis type, but the data collected for this analysis was too general and the specific type of trellising system must be considered. Different trellising systems affect the amount of light and air penetration into the canopy, and canopy conversion to allow greater cordon length has been shown to increase the 'tropical' fruit aroma of South African Chenin Blanc wines (Voischenk & Hunter, 2001). There is a potential that specific types of trellising systems within the "trellised" wines could have significant effects on thiol levels.

Table 3.6 ANOVAs of Trellis Type vs. 3MHA (ng/L) and Trellis Type vs. 3MH (ng/L)

	3MHA		3MH	
	F(1, 63)=0.286, *p*=0.59		**F(1, 63)=0.444, *p*=0.507**	
Trellis Type	**3MHA (ng/L) Mean**	**3MHA (ng/L) SD**	**3MH (ng/L) Mean**	**3MH (ng/L) SD**
trellised (n=52)	25	56	904	437
bush vine (n=13)	15	28	848	477
TOTAL (n=65)	23	52	893	442

Different letters indicate statistically significance differences ($p \leq 0.05$) according to Fisher's LSD.

Wood contact

Wines fermented in tank and bottled without any wood contact were considered "unoaked" and wines with any degree of contact (from partial barrel fermentation in old barrels to fermentation and aging in 100% new oak), were considered "oaked". For 3MHA (ng/L), levels were significantly lower in oaked wines ($F(1,63)=11.349$, $p<0.01$) (Table 3.7). This is not surprising, as one of the recognized effects of maturation in oak barrels is a small amount of oxygen transfer, and aeration can reduce thiols through a variety of reactions (Smith *et al.*, 2015). It also may be the case that this difference is due to time, rather than oak contact. Wines that receive wood contact are generally aged longer, leading to more drastic decreases in 3MHA levels before the wines are released. Significant differences were not seen for 3MH ($F(1,63)=0.735$, $p=0.39$) (Table 3.7). Levels of 3MH have been reported to decrease during barrel aging in red wine due to oxidation (Blanchard *et al.*, 2004), though there was no significant difference in the 3MH levels in the case of these Chenin Blanc wines.

Table 3.7 ANOVAs of Wood Contact vs. 3MHA (ng/L) and Wood Contact vs. 3MH (ng/L)

	3MHA		**3MH**	
	F(1, 63)=11.349, *p*=0.0013		**F(1, 63)=0.735, *p*=0.39**	
Wood Contact	**3MHA (ng/L) Mean**	**3MHA (ng/L) SD**	**3MH (ng/L) Mean**	**3MH (ng/L) SD**
oaked (n=29)	6^{b}	15	830	335
unoaked (n=36)	37^{a}	65	944	511
TOTAL (n=65)	23	52	893	442

Different letters indicate statistically significance differences ($p \leq 0.05$) according to Fisher's LSD.

Price

A highly significant negative Spearman's correlation ($p<0.01$) was found between wine price and 3MHA levels, meaning that levels were significantly lower in higher-priced wines (Figure 3.1). The same correlation was not significant for 3MH. Wine price is a complicated factor determined by many different variables, including the degree of precision viticulture employed, vineyard yield, use of high-tech winery equipment, barrel maturation, time aged before release, marketing, and brand value. Of these, the factor considered in the previous ANOVAs is barrel maturation. As oaked wines are costlier to product, the negative correlation between 3MHA and price is in agreement with the lower levels of 3MHA in oaked wines (Table 3.7). Additionally, the expensive wines were more likely to be older due to extended aging in oak, so the price correlation also agrees with the differences in 3MHA due to age of these wine (Table 3.3).

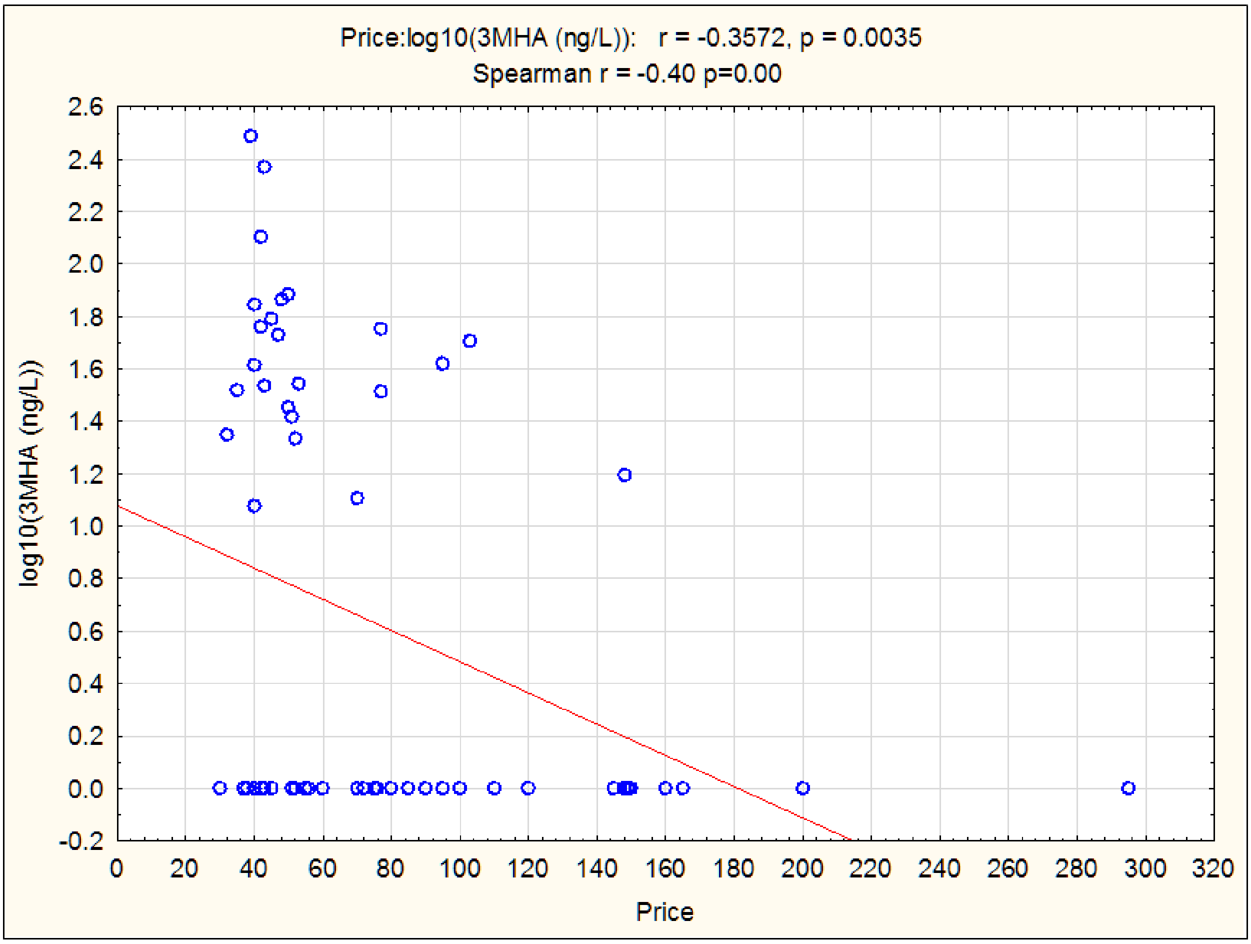

Figure 3.1 Spearman's correlation of price (in ZAR) vs. log-transformed 3MHA (ng/L)

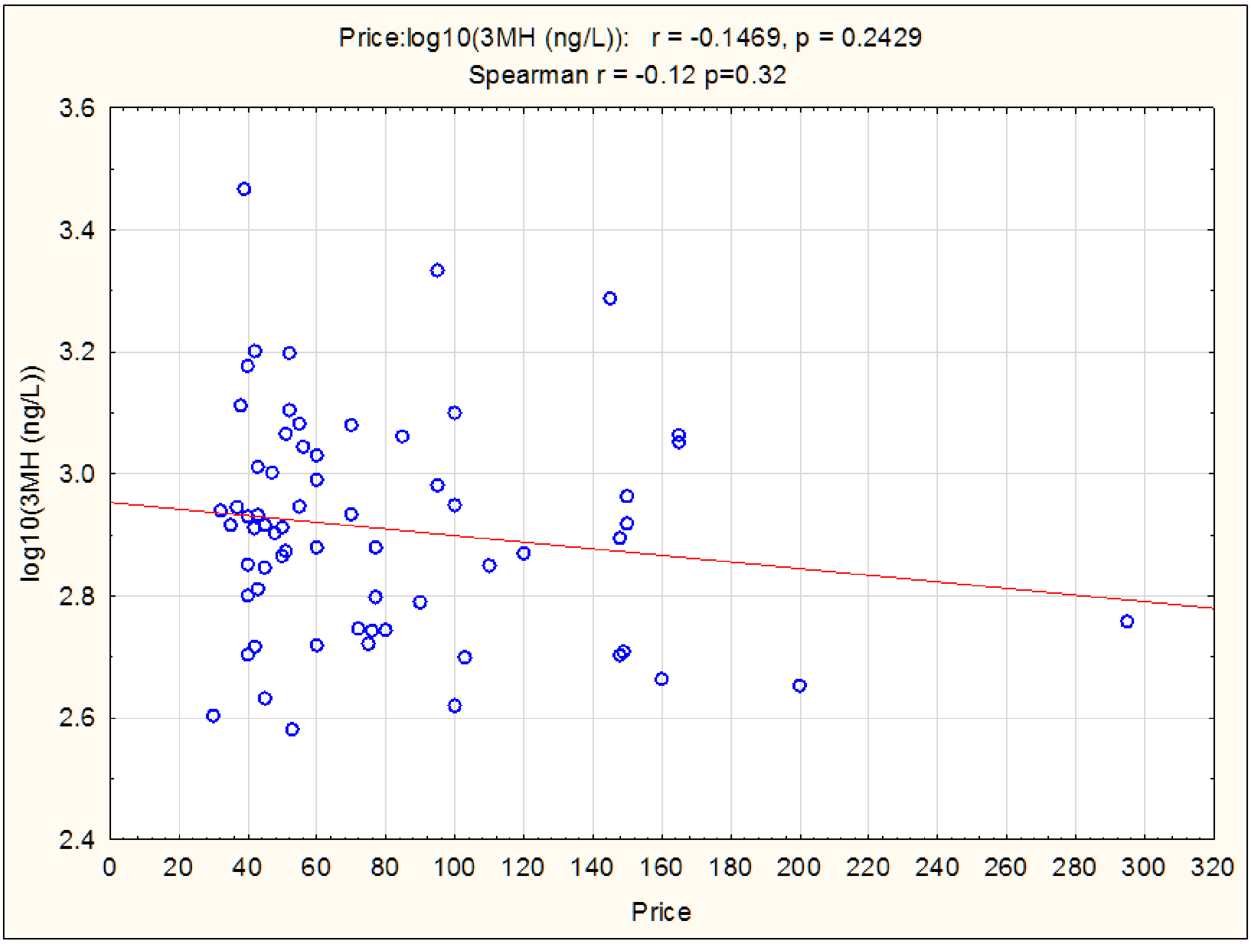

Figure 3.2 Spearman's correlation of price (in ZAR) vs. log-transformed 3MH (ng/L)

Lees contact

A highly significant negative Spearman's correlation was also found between months of aging on lees and 3MHA levels (*p*<0.01), but there was a non-significant (*p*=0.17) correlation between lees aging and 3MH. The reduction of 3MHA is supported by the literature, as lees have been shown to react with SH- groups to move volatile thiols (Vasserot *et al.*, 2003). There could also be an interaction between factors where wines aged on the lees for longer could be more likely aged in oak barrels and released later, and therefore were older at the time of measurement.

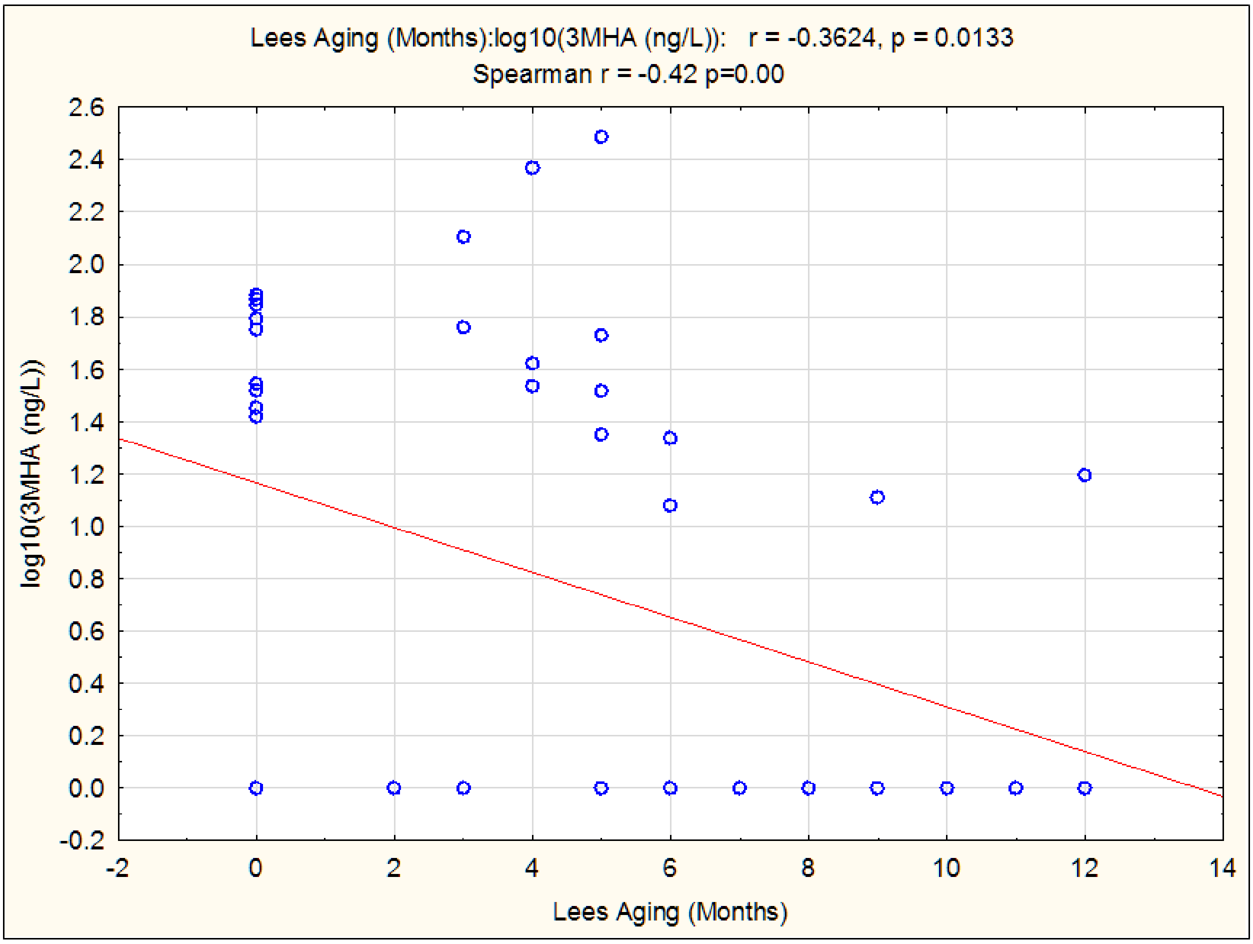

Figure 3.3 Spearman's correlation of lees aging (months) vs. log-transformed 3MHA (ng/L)

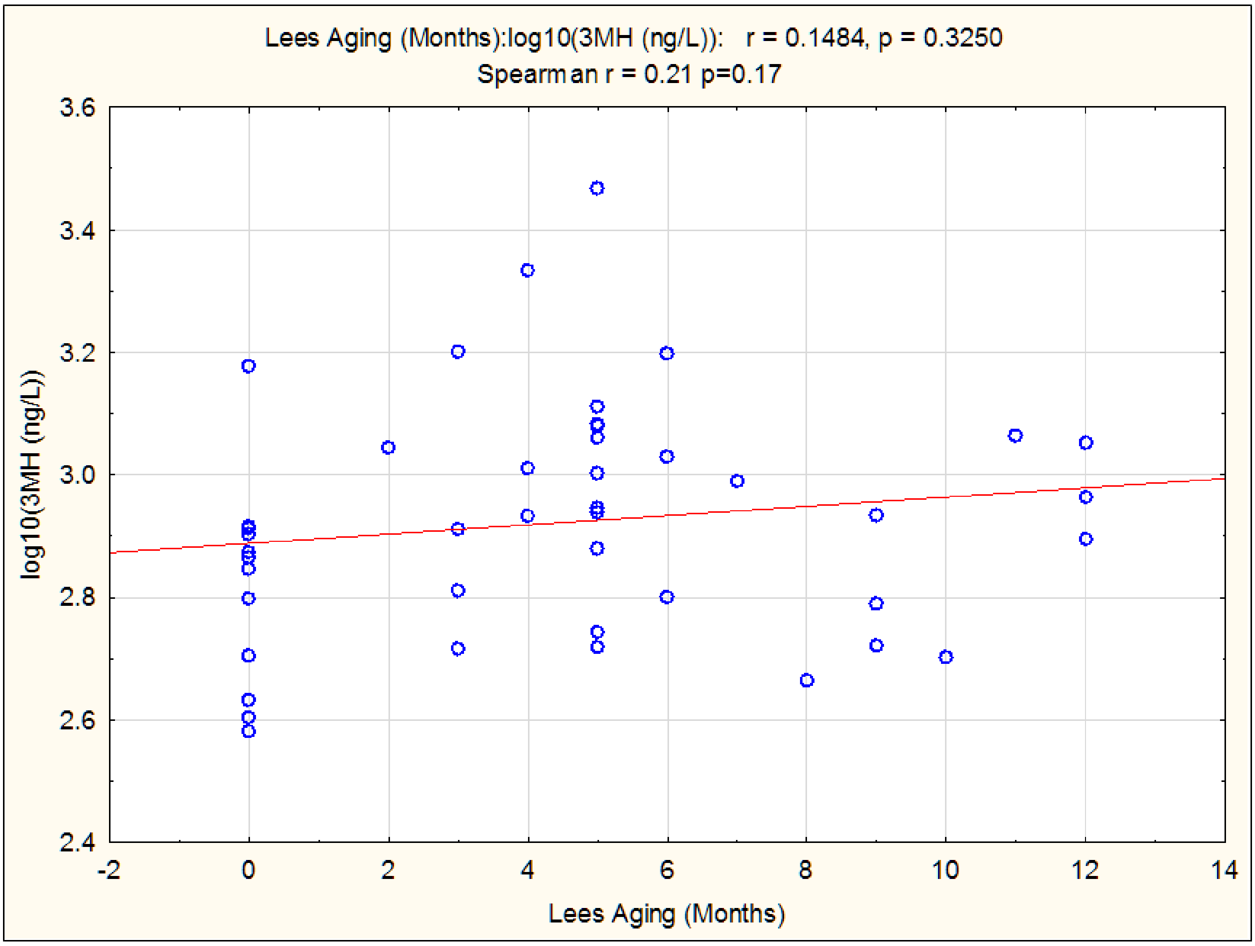

Figure 3.4 Spearman's correlation of lees aging (months) vs. log-transformed 3MH (ng/L)

3.4 Conclusions

The knowledge that thiols are present at appreciable levels in South African Chenin Blanc wines is exciting, as it opens up new avenues of research. Additionally, viticulturists and winemakers can use this knowledge to further their understanding of how their cultivation and vinification practices affect the aroma of Chenin Blanc wines in terms of thiols. Since the analytical method used was only able to measure 3MH and 3MHA, it would be interesting in the future to analyse Chenin Blanc wines for other thiols, including 4MMP.

It is notable that in the data mining step, most of the significant differences in terms of levels were found for 3MHA, and not 3MH. Significant differences were found for 3MHA levels in all cases except trellis type, while the only significant difference for 3MH levels was for wine origin. This illustrated the fact that 3MHA was more variable in these Chenin Blanc wines, but factors affecting 3MH levels are still unclear.

While there are many interacting variables in the data mining analysis which make it difficult to draw concrete conclusions about factors affecting thiols, this research can help form hypotheses for future research projects, for example: To test the effect of wood contact on thiol levels in wine, a study could compare the thiols of wines from the same juice fermented in oak barrels, in tank with oak chips, in tank with no wood contact, and in tank with micro-oxygenation. This study could answer if barrel fermentation or maturation affect thiols, and whether the contact with wood, or just the ingress of oxygen in barrel cause these differences. These wines could further be left to age and measured for thiols again to assess whether lower levels of 3MHA found in older wines (Table 3.3) are due to aging only, oak contact only, or a combination of the two. To truly study the effect of vine age on thiols, plots of different ages with the same trellis system, rootstock, soil type, and clone would have to be located. Grapes from these blocks would be vinified in the same manner and the wines would be then analysed for thiols. In order to study the effect of lees aging on thiols, experimental wines should be allowed to be in contact with the lees longer than a control, and measure the resulting thiol levels. These studies could not only empower winemakers with more knowledge, but could lead to further hypotheses about the nature, behaviour and origin of thiols in wine.

This analysis has established that thiols are present in this set of wines, but the next step is to elucidate the sensory impact of these thiols at the levels found. The initial sensory study by Mateo-Vivaracho *et al.*, 2010 illustrates that thiols are perceived differently at different concentrations, and a thiol's classical descriptor may not be recognizable in wine until well above its odour threshold. This calls for more detailed sensory studies looking at the perception of thiols in Chenin Blanc wines (and other matrices) at different concentrations, which will be addressed in the remaining chapters of this work.

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Appendix A

Thiol levels in South African Chenin Blanc wines

Appendix A. : Thiol levels in South African Chenin Blanc wines

Table A.1 3MHA and 3MH levels of 65 dry, commercially-available South African Chenin Blanc wines.

Wine	Vintage	Age (years)	3MHA (ng/L)	3MH (ng/L)
KZCS	2014	1	33	1024
KZVS	2014	1	32	757
PETIT	2014	1	27	816
CG	2014	1	32	823
SPIER	2014	1	56	1589
RB	2014	1	21	1576
56H	2014	1	53	1004
VIL	2014	1	0	1106
KWV	2014	1	25	746
MB	2014	1	12	856
SIM	2014	1	72	799
SR	2014	1	0	1271
TGG	2014	1	11	630
DG	2014	1	41	2155
LK	2014	1	40	850
KZVS	2015	1	55	626
KZCS	2015	1	232	854
KFP	2015	1	34	380
KFOVR	2015	1	50	498
SP	2015	1	126	813
RB	2015	1	0	1070
BLCM	2015	1	0	822
BCG	2015	1	69	1502
VILSC	2015	1	0	1161
VILBF	2015	1	0	707
REM	2015	1	0	757
SIM	2015	1	75	732
SR	2015	1	0	882
LAP	2015	1	0	553
LAFN	2015	1	0	522
LA30	2015	1	0	449
LZ	2015	1	0	646
CDB	2015	1	0	1148
VL	2015	1	305	2929
DT	2015	1	0	880
NJ	2015	1	61	700
PBVC	2015	1	0	1208
PBPR	2015	1	0	1292
WEL	2015	1	0	428
SVL	2015	1	0	400
SVP	2015	1	21	868
MP	2015	1	0	552
WGHBV	2015	1	0	525

Table A.1 (cont.)

Wine	Vintage	Age (years)	3MHA (ng/L)	3MH (ng/L)
WGHV	2015	1	0	416
WGHLH	2015	1	0	460
WGHRD	2015	1	0	571
LKAP	2015	1	0	504
KAPZ	2015	1	0	519
KZFR	2013	2	15	784
MH	2013	2	0	739
BOO	2013	2	0	957
BBS	2013	2	0	827
KFOVR	2013	2	0	614
DG	2013	2	0	509
LA	2013	2	0	555
RH	2013	2	0	976
KZFR	2014	2	0	502
BBS	2014	2	0	918
RHB	2014	2	0	1157
PBD	2014	2	0	1201
LK	2014	2	0	708
TH	2012	3	0	1258
HB	2012	3	0	1937
BOO	2013	3	0	887
RHB	2013	3	0	1126

Chapter 4

Research results

Interaction effects of 3-mercaptohexan-1-ol, linalool, and ethyl hexanoate on the aromatic profile of South African dry Chenin Blanc wine by descriptive analysis (DA)

Chapter 4 : Interaction effects of 3-mercaptohexan-1-ol (3MH), linalool, and ethyl hexanoate on the aromatic profile of South African dry Chenin Blanc wine by descriptive analysis (DA)

4.1 Introduction

The chemical analysis results presented in Chapter 3 established that both 3-mercaptohexan-1-ol (3MH) and 3-mercaptohexyl acetate (3MHA) can be present in South African Chenin Blanc wines at concentrations many times higher than their odour thresholds. While typical descriptors for 3MH and 3MHA are known (2 2.3.1), that knowledge alone cannot be used to predict the aromatic expression of these compounds in the context of wine. This is because firstly, at different concentrations the sensory perception of volatiles changes not only intensity (López *et al.*, 2003), but also character (Fretz *et al.*, 2005; Mateo-Vivaracho *et al.*, 2010; Van Wyngaard *et al.*, 2014; Coetzee *et al.*, 2015). Secondly, volatile aromatic compounds don't exist in isolation, but rather form a small component of the complex wine matrix. This matrix includes over 1000 other volatiles which can interact with one another, affecting sensory perception (Polášková *et al.*, 2008). It is for this reason that one of the first studies on the 'guava' character of Chenin Blanc by Van Rooyen *et al.* (1982) suggested "…*observing the effect on the guava-like character in neutral wines by altering their composition … By changing one or two factors at a time, further evidence could be collected for a better understanding of the phenomenon*". Similar calls for interaction studies have been echoed by other wine aroma researchers (Francis & Newton, 2005; Polášková *et al.*, 2008).

Some researchers have addressed this by performing interaction studies. A few such studies involved thiols, though these studies were designed to be relevant to Sauvignon Blanc wines (King *et al.*, 2011; Benkwitz *et al.*, 2012; Van Wyngaard *et al.*, 2014; Coetzee *et al.*, 2015). These studies show the enhancing and suppressing effects volatiles can have on one another. For example, in one experiment it was found that 3MHA reduces the 'sweet', 'floral' and 'muscat' character of linalool and 2-phenylethyl acetate, while methoxypyrazines reduce the tropical intensity of 3MHA (Campo *et al.*, 2005). Similar antagonistic interactions between 3MH and pyrazines have been seen in other interaction studies (Van Wyngaard *et al.*, 2014; Coetzee *et al.*, 2015). To our knowledge, no interaction studies with a focus on Chenin Blanc have been published.

Three compounds present in Chenin Blanc wines are 3MH (Chapter 3), ethyl hexanoate, and linalool (Lawrence, 2012). 3MH is typically described as 'passion fruit' and 'grapefruit' and has an odour threshold of 60 ng/L (Tominaga *et al.*, 1998), though with recent interaction studies these descriptors have expanded to include 'guava' and 'green' aromas (King *et al.*, 2011; Van Wyngaard *et al.*, 2014; Coetzee *et al.*, 2015). Ethyl hexanoate has aromas of 'apple peel', and 'fruit' in wine (Francis & Newton, 2005), and an odour threshold of 14 µg/L (Ferreira *et al*., 2000). It was suggested as a possible source for the 'guava' aroma of Chenin Blanc wines (Van Rooyen *et al.*, 1982). Both ethyl hexanoate and 3MH are odourants which have been found in guava fruit (Steinhaus et al., 2009; Pino & Bent, 2013). Linalool is best known for giving a 'floral' character to Muscat wine varieties (Mateo & Jiménez, 2000), but also has aromas of 'citrus', and 'lavender' (Black *et al.*, 2015), and an odour threshold of 25.2 µg/L (Ferreira *et al.*, 2000). Though ethyl hexanoate and linalool oxide have been shown to differentiate between different styles of South African Chenin Blanc wines (Lawrence, 2012), the role of thiols in these wines has not been studied. Additionally, the interactions between these three compounds in Chenin Blanc are not known.

In this work, an interaction experiment was performed by spiking a partially-dearomatized Chenin Blanc wine with combinations of 3MH, ethyl hexanoate, and linalool at various concentrations. Samples were spiked with each individual compound, as well as combinations of all three. The sensory method used to analyse these samples was descriptive analysis (DA). DA is well-suited to quantifying small differences between products by training a panel which is able to rate differences in intensity of descriptors (Lawless & Heymann, 2010). The intensity rating allows for the observation of enhancing and suppressing effects of the three compounds on one another. The comparison of these compounds alone and in combination will allow for description of these three compounds in the South African Chenin Blanc matrix, and identify any enhancing or suppressing effects they may have on one another. Studying the sensory perception of these compounds would help further understand the role of thiols in the context of South African Chenin Blanc wines.

4.2 Materials and methods

4.2.1 Experimental design

Two different descriptive analysis (DA) experiments were performed by the same judges. The first experiment was an interaction study evaluating the three compounds in combination at three different levels, and the second experiment evaluated the same compounds separately. These will be referred to as “combinations” and “singles”, respectively. Only the aroma of the samples was evaluated.

Low, medium, and high levels of ethyl hexanoate (600 µg/L, 1100 µg/L, and 1600 µg/L) and linalool (200 µg/L, 1600 µg/L, and 3000 µg/L) were selected according to published Chenin Blanc chemical analysis data (Lawrence, 2012). The 3MH levels selected (200 ng/L, 1100 ng/L, and 2000 ng/L) fit into the range typically found in South African Chenin Blanc wines (Chapter 3). At all levels, the compounds were present at concentrations above their odour thresholds, and their maximum odour active values were 114 for ethyl hexanoate, 119 for linalool, and 33 for 3MH. The same levels were used for both the combinations and singles.

Since a full factorial design would have resulted in twenty-seven samples for the sensory analysis of the combinations, a central composite design (CCD) was used to reduce the sample set to sixteen (Table 4.1) as proposed by (Esbensen, 2002). The Unscrambler® X (Version 10.2) was used to generate a small inward-facing central composite design with 6 axial samples, 8 cube samples, and 2 centre samples (Figure 4.1). For the singles, each level of each compound was spiked on its own, giving 9 samples in total (Table 4.2).

Table 4.1 Central composite design of combinations showing sample codes and spiking levels. Level 1=low, level 2=medium, level 3=high.

CCD Name	Sample Name	Factor 1 level	Factor 2 level	Factor 3 level	3MH (ng/L)	ethyl hexanoate (µg/L)	linalool (µg/L)
Axial_A(high)	3_2_2	3	2	2	2000	1100	1600
Axial_A(low)	1_2_2	1	2	2	200	1100	1600
Axial_B(high)	2_3_2	2	3	2	1100	1600	1600
Axial_B(low)	2_1_2	2	1	2	1100	600	1600
Axial_C(high)	2_2_3	2	2	3	1100	1100	3000
Axial_C(low)	2_2_1	2	2	1	1100	1100	200
Cube1	1_1_1	1	1	1	200	600	200
Cube2	3_1_1	3	1	1	2000	600	200
Cube3	1_3_1	1	3	1	200	1600	200
Cube4	3_3_1	3	3	1	2000	1600	200
Cube5	1_1_3	1	1	3	200	600	3000
Cube6	3_1_3	3	1	3	2000	600	3000
Cube7	1_3_3	1	3	3	200	1600	3000
Cube8	3_3_3	3	3	3	2000	1600	3000
cp01	2_2_2	2	2	2	1100	1100	1600
cp02	2_2_2	2	2	2	1100	1100	1600

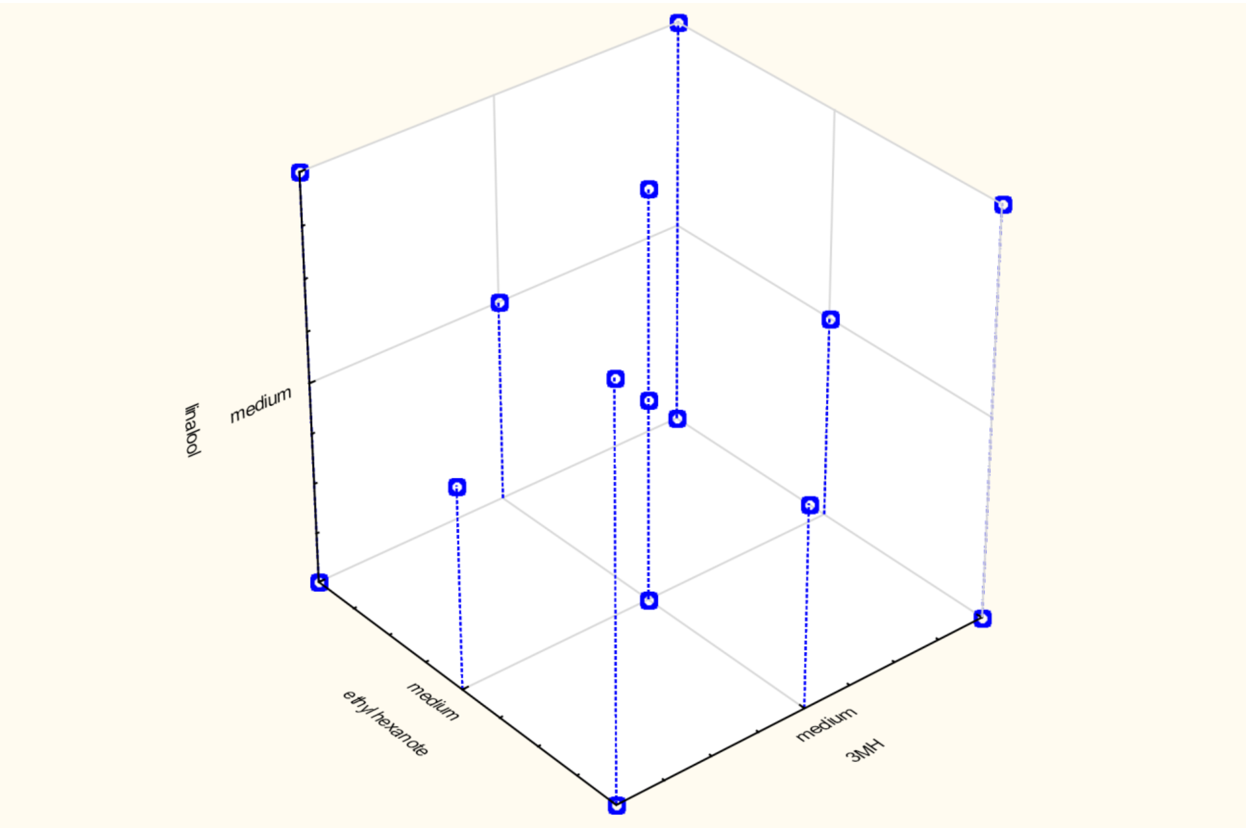

Figure 4.1 Visual representation of the central composite design (CCD) used for the combinations by means of a 3D scatterplot of linalool against 3MH and ethyl hexanoate.

Table 4.2 Sample codes and spiking levels of single compounds. H=3MH, E=ethyl hexanoate, L=linalool.

Sample Name	3MH (ng/L)	ethyl hexanoate (µg/L)	linalool (µg/L)
H_low	200	0	0
H_med	1100	0	0
H_high	2000	0	0
E_low	0	600	0
E_med	0	1100	0
E_high	0	1600	0
L_low	0	0	200
L_med	0	0	1600
L_high	0	0	3000

4.2.2 Samples

A dry unwooded commercially available Chenin Blanc wine was selected based on its neutral aroma, and was treated to obtain a partially-dearomatized base wine. During the treatment and blending steps, the wine was protected from oxidation under N_2 gas. The wine was dearomatized with 5 g/L activated charcoal powder (Merck, Darmstadt, Germany) for 7 hours without agitation, then separated from the charcoal by diatomaceous earth filtration. In a screening session, three researchers chose a blend of 1/3 charcoal-treated wine to 2/3 untreated wine that yielded a neutral wine base with low aromatic intensity.

Dilutions of pure 3MH (98%, Interchim, Montluçon, France), ethyl hexanoate (≥99%, Sigma-Aldrich, St. Louis, Missouri), and (±) linalool (97%, Sigma-Aldrich, St. Louis, Missouri) for spiking were prepared in HPLC-grade ethanol (≥ 99.8%, Sigma-Aldrich, St. Louis, Missouri, United States) and stored at -80 °C for no more than 5 weeks. All samples were prepared by spiking the partially-dearomatized base wine 12 hours prior to training or testing, during which time the samples were stored under N_2 gas at 4°C. The delay between spiking time and evaluation allowed for integration of the aroma compounds into the matrix. Samples were allowed 1 hour to reach room temperature before being served. The levels of 3MH spiked were checked with the method described in Chapter 3, and the ethyl hexanoate, and linalool levels were checked by the methods detailed in 6.

4.2.3 Sensory evaluation

Combinations

The judges were not informed of the nature or goal of the study. The aroma of the spiked partially-dearomatized wine was evaluated over 10 one-hour training sessions spanning a period of three weeks. Each training session alternated between the axial and cube samples to minimize sensory fatigue. During consensus training, descriptors generated by the panellists were defined with aroma reference standards. The use of references helped to familiarize all the panellists with the terms, and standardize their understanding of the descriptors. Initially, 34 reference standards were presented. Throughout the training process, the lexicon was narrowed to 18 descriptors by the panel (Table 4.3).

Table 4.3 Reference standards and corresponding descriptors agreed upon by the panel for both experiments.

Descriptor	Reference Standard
guava	3 cm^3 fresh, ripe guava
pineapple	2 cm^3 fresh pineapple
passion fruit	1/4 of the pulp from a fresh passion fruit
banana	1 cm^3 ripe banana in 10 mL distilled water
peach	3 cm^3 canned peach "KOO"
apple	3 cm^3 fresh green apple with skin
lemon	3 cm^3 fresh fruit (pulp + flesh)
orange	3 cm^3 fresh fruit (pulp + flesh)
grapefruit	3 cm^3 fresh fruit (pulp + flesh)
floral	verbally agreed-upon as an all-encompassing floral category
orange blossom	2 drops solution "Firmenich" on a cotton ball
bergamot/earl grey	1.5 g Earl Grey tea "Five Roses®"
tea	1.5 g black tea "Five Roses®"
artificial sweet	1 g cotton candy
honey	5 mL honey + 10 mL hot water
dusty/mineral	small chip of slate stone, wet with water
tomato leaf	fresh cherry tomato leaves and stalk
cooked veg	5 mL canned green bean brine "KOO" + 5 mL canned asparagus brine "Food Lover's Signature"

For the testing sessions, spiked wines were poured in 20 ± 2 mL aliquots one hour before serving into clear glasses (ISO 3591:1977) and covered with plastic Petri dish lids. Each glass was labelled with a unique, random 3-digit code. All evaluations took place in off-white individual sensory booths in a quiet, well-ventilated, odourless 20 ± 2 °C air-conditioned room (ISO 8589:2007). The sixteen samples were presented in a monadic sequential manner according to a Williams Latin Square design (Macfie *et al.*, 1989). The sample set was broken up into subsets of 5 or 6 glasses, and panellists were given a 5-minute break between subsets to minimize sensory fatigue. Panellists rated the intensity of each descriptor along an unstructured line scale from “none” to “intense” using Compusense® five software (Release 5.6). Two panellists preferred to use paper rather than a computer to rate the samples, and were allowed to do so. Four replications of the combinations were performed, each on a separate day.

Singles

After evaluation of the combinations, the same panel received one training session to practice evaluating samples that were spiked with only one level of one compound at a time. Only one training session was deemed necessary because the singles were inherently less complex, and the descriptors generated and reference standards used for these samples were the same as for the combinations (Table 4.3). Thus, the training for the combinations was deemed sufficient to evaluate the singles as well. Testing was performed following the same methods and procedures as in the combinations. Four replications of the singles set were performed over two days, with a fifteen-minute break between replications to avoid sensory fatigue.

Panellists

The same panel of ten judges aged 23-58 years (1 male, 9 females) participated in both experiments. The judges were members of the community, as well as students and staff of the Department of Viticulture and Oenology at Stellenbosch University. The panellists were recruited based on their willingness to participate and previous experience evaluating South African Chenin Blanc, and were remunerated for their participation. Eight of the ten panellists had previous experience with analysis of Chenin Blanc wines by descriptive analysis.

4.2.4 Statistical analysis

Panel performance was evaluated using PanelCheck (V1.4.2) according to the workflow suggested by Tomic *et al.* (2010). The discriminability and consensus of the panel were evaluated by means of analysis of variance (ANOVA) and Tucker-1 plots. The data structure of both experiments – combinations and singles - were analysed by mixed-model ANOVA. For both experiments, the significance threshold was set at p=0.05. The Fisher's LSD post-hoc test was used to show significant differences. Response-surface plots were created to illustrate two-way interactions in Statistica (Version 13) by doing regression analyses according to the way central composite design (CCD) data is analysed. Principal component analysis (PCA) was also performed to illustrate correlations between attributes and samples. PCA was run on the covariance matrices of both experiments, as suggested by Borgognone *et al.* (2001) in XLSTAT (Version 18.06, Addinsoft). Descriptors included in the PCAs were limited to those with a significant main effect or significant interaction effect in the ANOVAs.

4.5 Results and discussion

Panel performance of both experiments was acceptable, as evaluated by the workflow described above (data not shown). Though the training and testing of the singles took place chronologically after the combinations, the results are presented in the opposite order to explain the attributes associated with the compounds before investigating the interaction between the compounds.

4.5.1 Singles

The panel was able to agree upon differences in aroma between the singles, shown by the very high 93.8% explained variance in the PCA (Figure 4.2). 3MH and linalool had a greater impact on aroma than ethyl hexanoate, as these compounds oppose one another along PC1 of the PCA, which explains 81.8% of the variance in the data. The samples spiked with ethyl hexanoate clustered closer to the centre of the PCA, and did not explain much of the variation between samples. The descriptors which were not significantly different in intensity between the samples were 'pineapple', 'passion fruit', 'banana', 'artificial sweet', and 'honey' (Table 4.4). The non-significant 'passion fruit' descriptor is of note, as it is one of the typical descriptors of 3MH. 'Passion fruit' was perceived in all samples at a similar intensity, though Coetzee *et al.* (2015) found that 'passion fruit' became the dominant descriptor of 3MH in model wine at concentrations above 2000 ng/L. The descriptors with significant differences in intensity between samples were 'guava', 'peach', 'apple', 'lemon', 'orange', 'grapefruit', 'floral', 'orange blossom', 'bergamot/earl grey', 'tea', 'dusty/mineral', 'tomato leaf', and 'cooked veg' (Table 4.4).

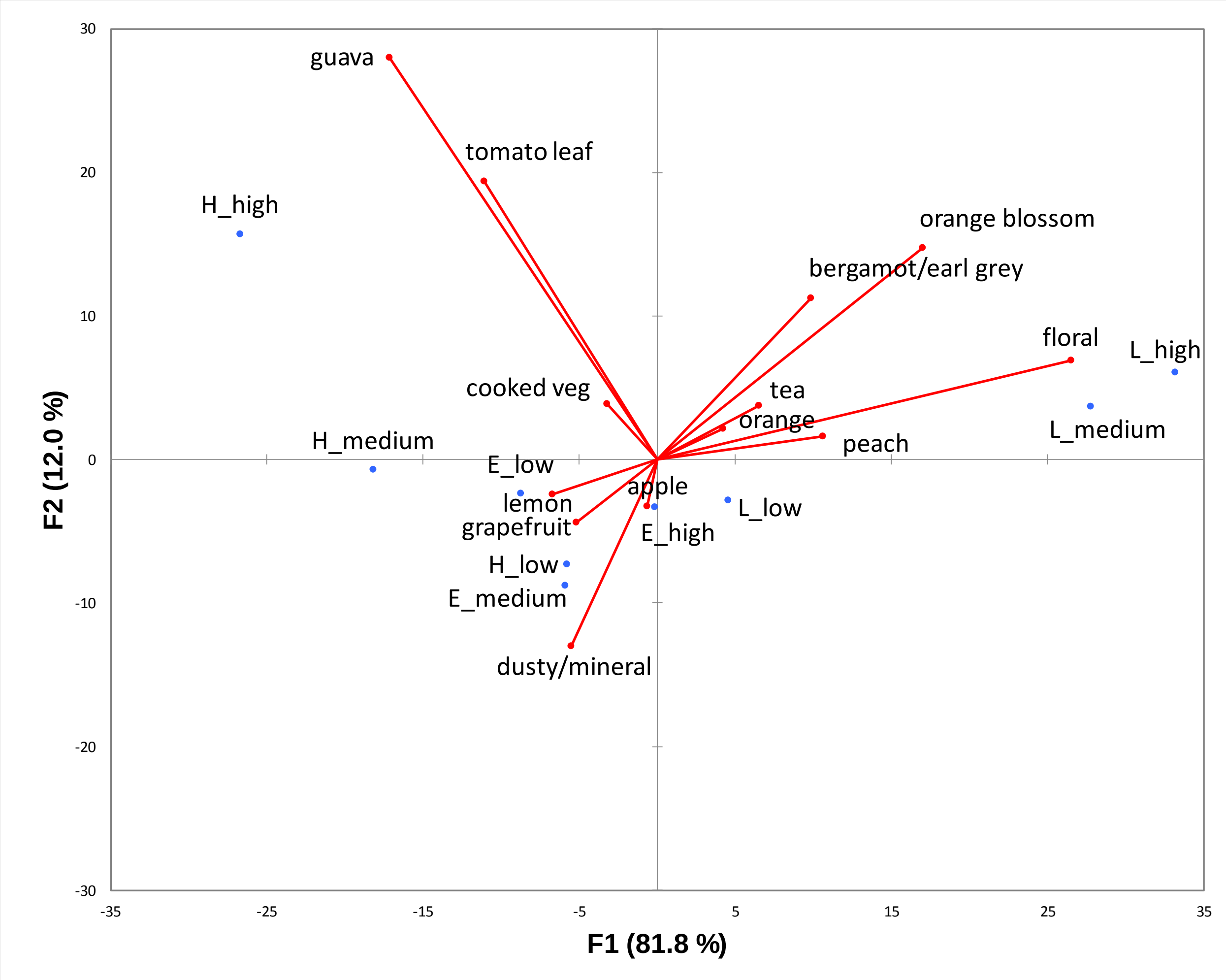

Figure 4.2 PCA of single compound data, showing attributes with a significant main or interaction effect. H=3MH, E=ethyl hexanoate, L=linalool. See Table 4.2 for spiking levels.

Table 4.4 ANOVA results showing F-values for the single compounds.

	Compound	Compound*Level
	df=2	df=4
Sensory Attribute	**F-Value**	
guava	33.230*	11.250*
pineapple	0.320	0.805
passion fruit	0.982	0.358
banana	1.173	0.931
peach	13.433*	1.396
apple	0.562	3.426*
lemon	10.042*	0.950
orange	7.004*	1.985
grapefruit	4.499*	0.635
floral	73.262*	8.619*
orange blossom	50.418*	6.609*
bergamot/earl grey	18.462*	2.848*
tea	9.399*	1.317
artificial sweet	1.415	0.339
honey	0.570	0.087
dusty/mineral	7.531*	3.224*
tomato leaf	17.855*	7.780*
cooked veg	0.465	4.609*

* indicates significance at $\alpha=0.05$

The medium and high levels of 3MH (H_medium, H_high) correlated with 'lemon' in the PCA, as well as the thiol-related descriptors 'grapefruit', 'guava', 'tomato leaf', and 'cooked veg' (Figure 4.2). The association of 3MH with 'tomato leaf' and 'guava' descriptors is in agreement with recent interaction studies in model wine (Coetzee *et al.*, 2015), and dearomatized Sauvignon Blanc wine (Van Wyngaard *et al*., 2014). The powerful effect of high 3MH on 'tomato leaf' and 'guava' intensity is visible in the spider web plot (Figure 4.3). As shown by the graph of the LS means, 'guava' intensity increased at greater concentrations of 3MH, and was significantly higher than all other samples in the H_high sample (Figure 4.4). This pattern is the same for 'tomato leaf', though it was rated at lower average intensities compared to 'guava' (Figure 4.3). Additionally, 'guava' intensities were higher for samples with ethyl hexanoate than for linalool (Figure 4.4), which could indicate either an enhancing effect by ethyl hexanoate or a suppressing effect by linalool on the 'guava' attribute. 'Grapefruit' intensity was also significantly higher in wines spiked with 3MH than with linalool, and intermediate in wines spiked with ethyl hexanoate (Figure 4.5) 'Cooked veg' was rated at lower intensities overall, but behaved similarly to 'guava' with the highest intensity in the H_high sample (Figure 4.6). However it was also high in the L_low and E_low samples The relationship of 'cooked veg' and a similar 'cooked beans' attribute with thiols has been previously established (King *et al.*, 2011; Coetzee *et al.*, 2015). In the PCA, the H_medium sample moved toward the subtle ethyl hexanoate-spiked samples, and the H_low sample was grouped with them (Table 4.2)

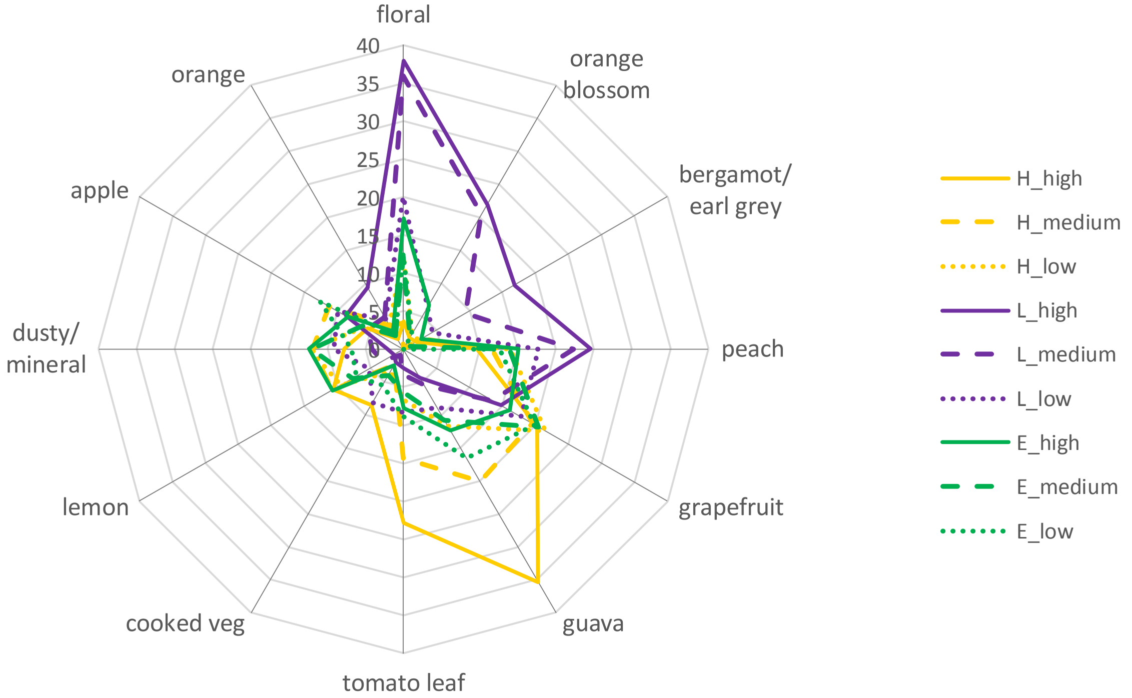

Figure 4.3 Spider web plot showing the aromatic profile of the singles DA samples for descriptors with a significant compound main effect or a significant compound*level interaction at α=0.05.

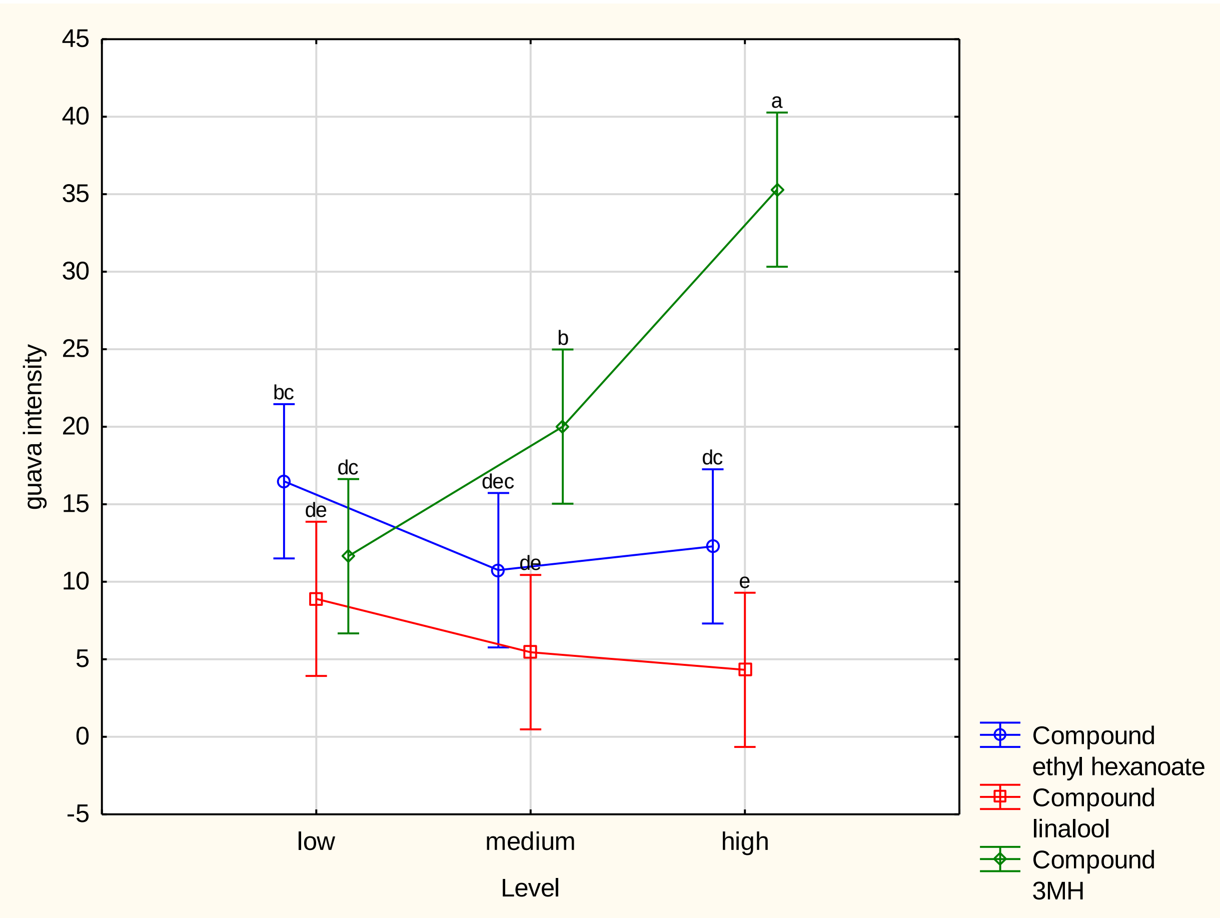

Figure 4.4 LS means plot illustrating the compound*level interaction effect on 'guava' aroma intensity for the single compounds with significant letters from LSD post-hoc test. Vertical bars denote 95% confidence intervals.

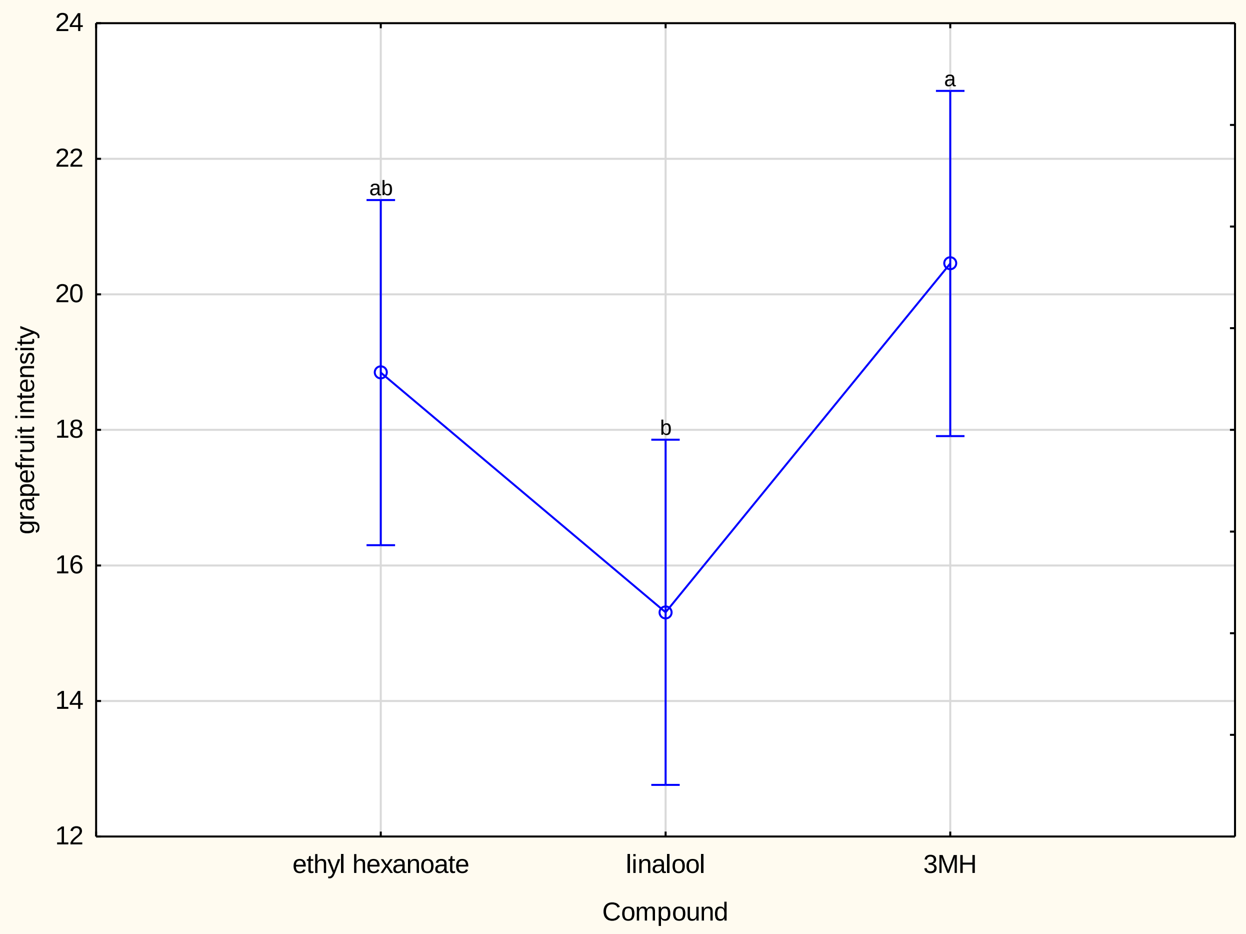

Figure 4.5 LS means plot illustrating the compound main effect on 'grapefruit' aroma intensity for the single compounds with significant letters from LSD post-hoc test. Vertical bars denote 95% confidence intervals.

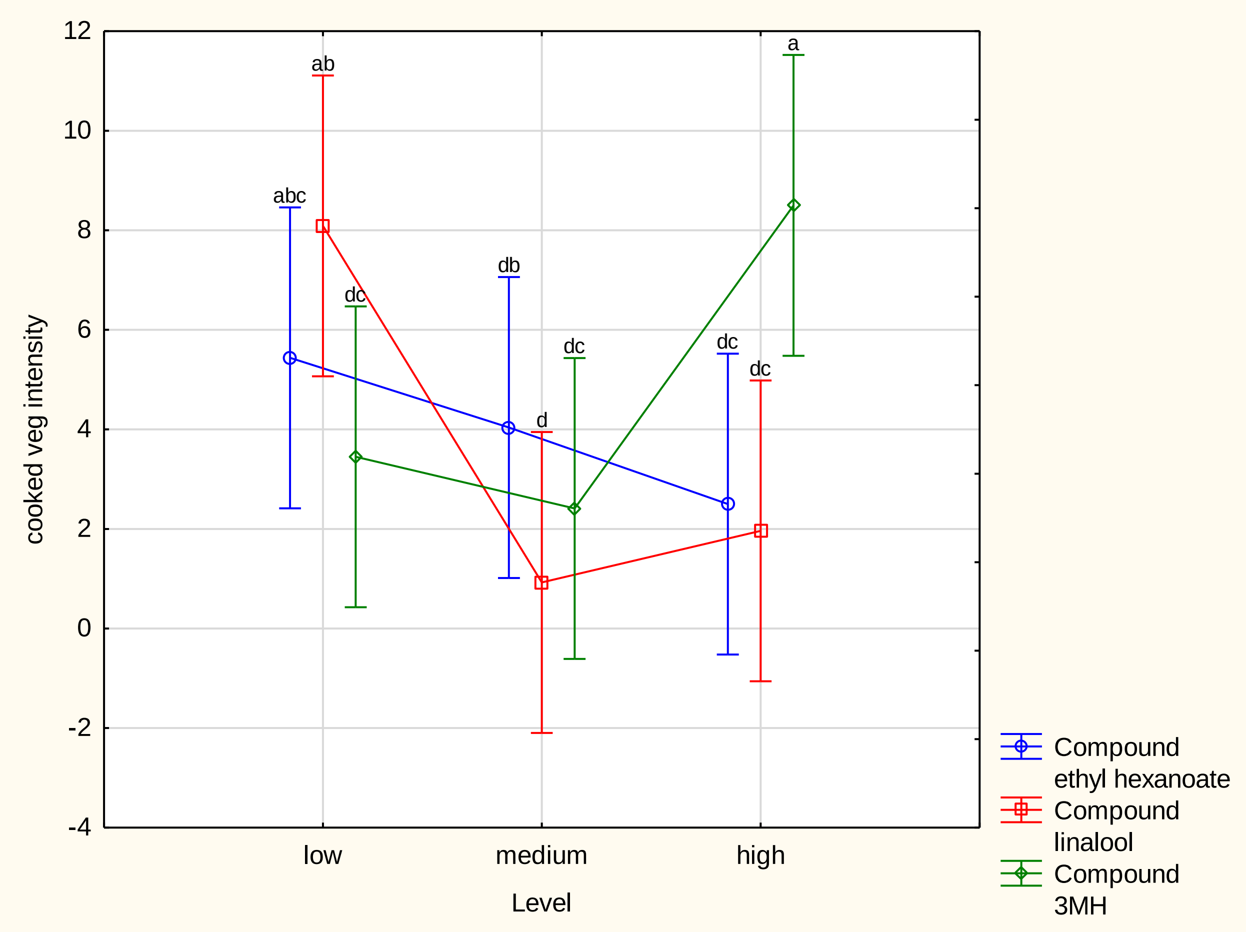

Figure 4.6 LS means plot illustrating the compound*level interaction effect on 'cooked veg' aroma intensity for the single compounds with significant letters from LSD post-hoc test. Vertical bars denote 95% confidence intervals.

The correlation of 'lemon' with the 3MH-spiked wines in the PCA (Figure 4.2) is misleading, as it was caused by a significant negative correlation of 'lemon' with the L_high, rather than a positive correlation with 3MH (Figure 4.7). The same is true for 'dusty/mineral' (Appendix B Figure B.1). Not well-explained by PC1 or PC2 in the PCA, but relevant to the 3MH-spiked samples was the descriptor 'apple'. The case of the descriptor 'apple' was particularly complex as it was affected by different concentrations of two compounds. For 3MH, it reached the highest intensity in the H_medium sample (Figure 4.8). Though ethyl hexanoate is described in the literature as 'apple peel' (Francis & Newton, 2005), 'apple' was higher in the E_low sample than the E_medium (Figure 4.8).

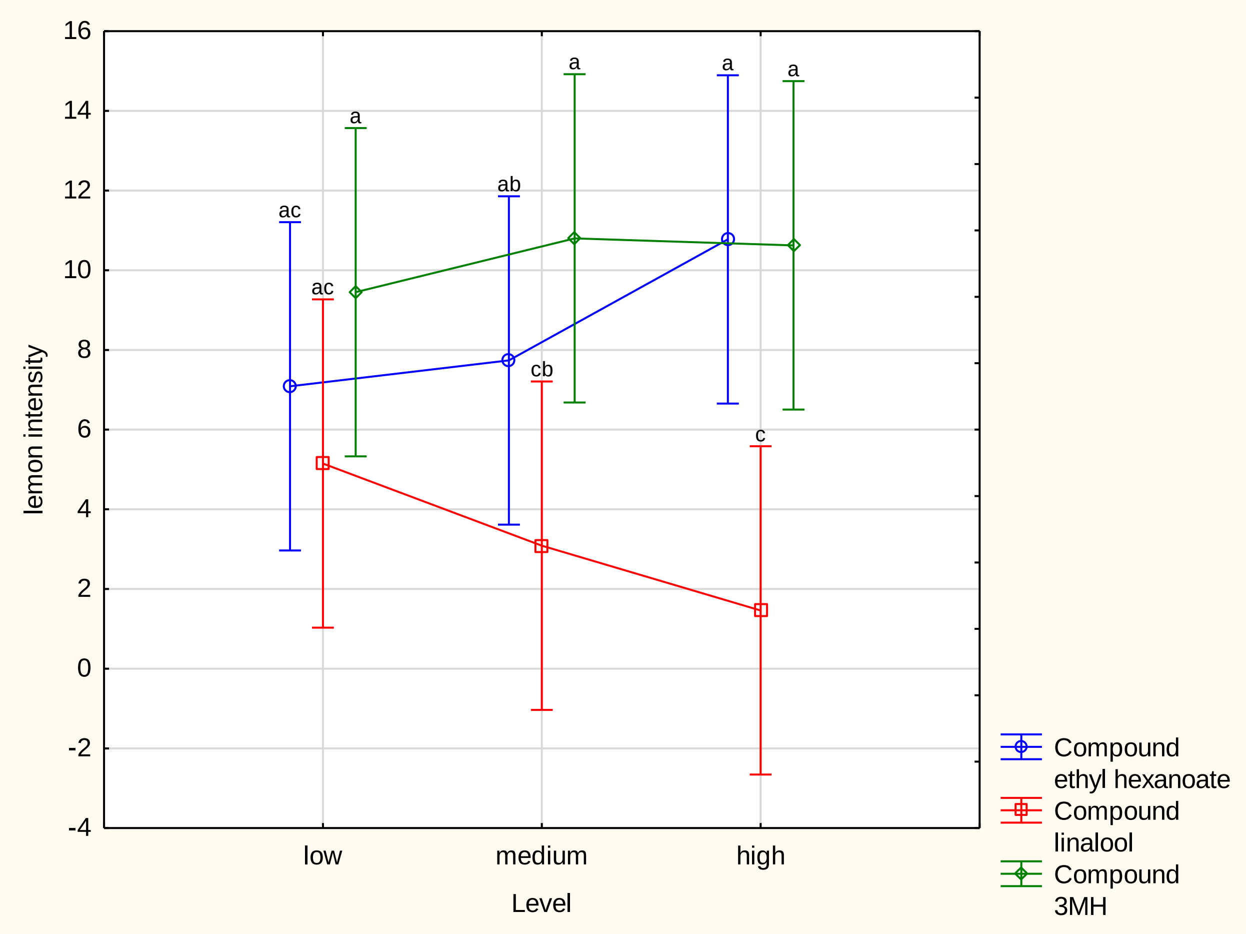

Figure 4.7 LS means plot illustrating the compound*level interaction effect on 'lemon' aroma intensity for the single compounds with significant letters from LSD post-hoc test. Vertical bars denote 95% confidence intervals.

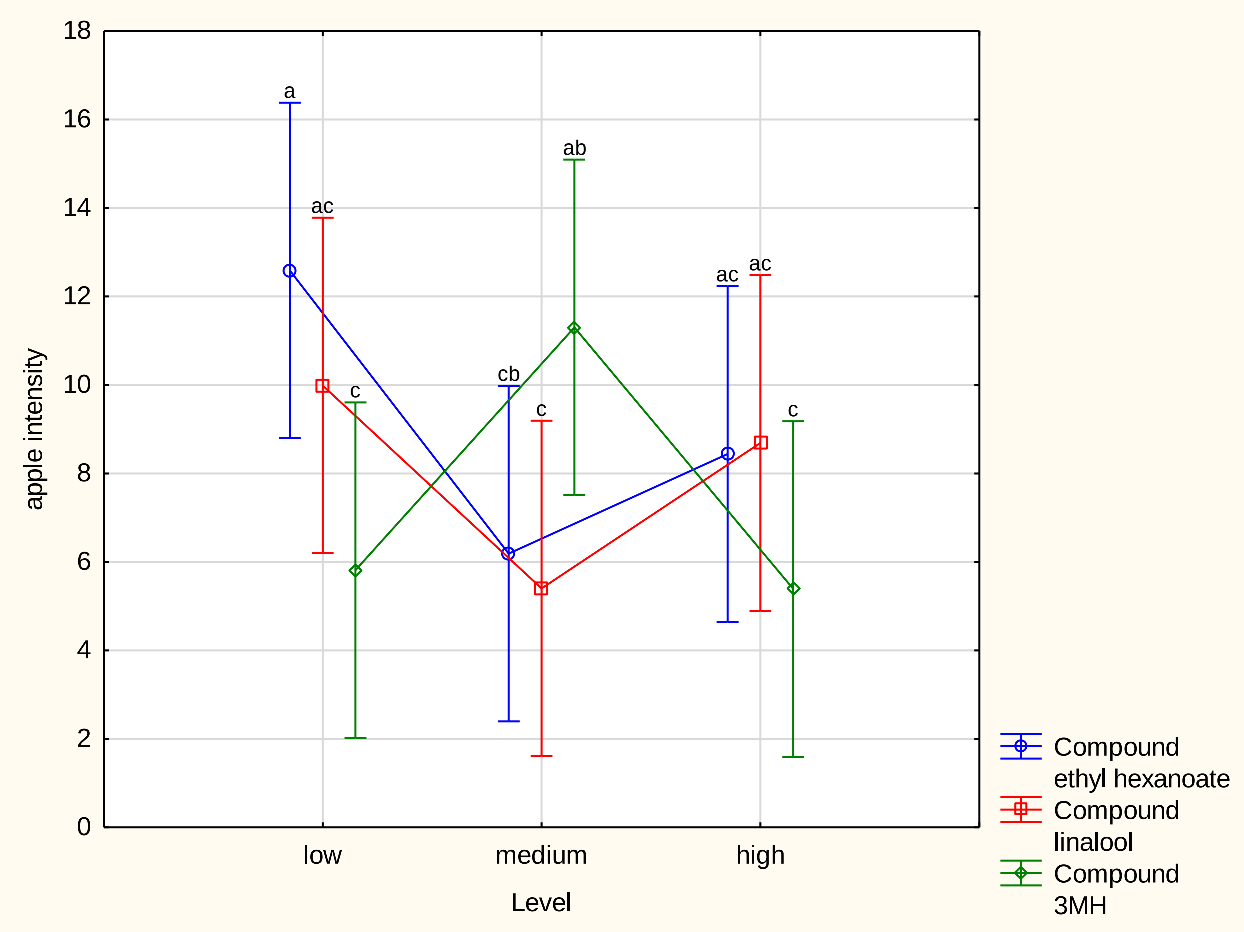

Figure 4.8 LS means plot illustrating the compound*level interaction effect on 'apple' aroma intensity for the single compounds with significant letters from LSD post-hoc test. Vertical bars denote 95% confidence intervals.

All three levels of linalool-spiked samples (L_high, L_medium, and L_low) in the PCA are positioned opposite H_high, H_medium and H_low, and are highly correlated with the descriptors 'bergamot/earl grey', 'orange blossom', 'orange', 'tea' 'floral', and 'peach' (Figure 4.2). In the ANOVA results for 'peach', 'tea', and 'orange' the compound main effect was significant (Table 4.4), showing that at all levels tested linalool increased the intensity of 'peach', 'tea', and 'orange' aroma in the samples, but the intensity did not change significantly between different linalool levels (Figure 4.9, Appendix B Figures B.2, B.3). A significant compound*level interaction (Table 4.4) for 'floral', 'orange blossom', and 'bergamot/earl grey' shows that the intensity of these descriptors increases with higher spiking levels (Figure 4.10, Appendix B Figures B.4, B.5). As monoterpenes are typically associated with 'floral' aromas, these results in agreement with existing literature (Marais, 1983). The descriptors correlated with linalool can be explained by the different aroma attributes of the two enantiomers in the racemic mixture of linalool. The (S)(+)-linalool enantiomer is 'citric', and is found in orange oils and the (R)-(-)-linalool has a 'woody lavender' attribute, and is found in lavender and bergamot oils (Padrayauttawat *et al.*, 1997). 'Floral' intensity is decreased in the H_medium and H_high samples, showing a potential suppressing effect of 3MH, but this is only seen for the overall 'floral' descriptor (Figure 4.10), not the specific floral attributes Linalool-spiked samples_also had a significantly lower 'lemon' intensity than samples spiked with the other compounds (Figure 4.11), as well as lower 'grapefruit' intensity than samples with 3MH (Figure 4.5) and lower 'dusty/mineral' intensity in L_high than in L_low (Appendix B Figure B.1) (Figure 4.3). In the case of 'cooked veg', it is highest in L_low, but L_medium and L_high have the lowest intensity (Figure 4.6). However, it should be kept in mind that some of these differences were small compared to differences in the intensity ratings of certain other descriptors.

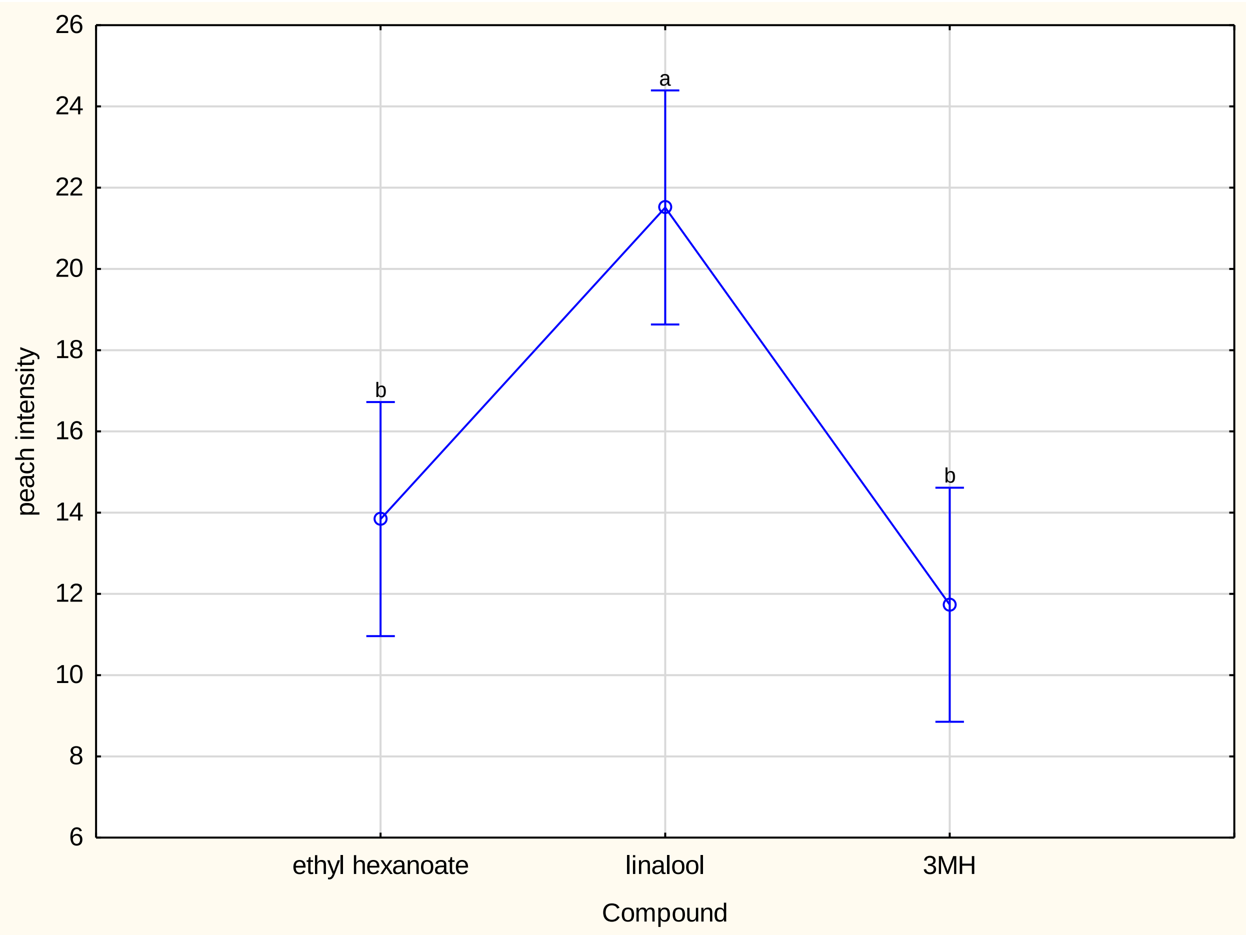

Figure 4.9 LS Means illustrating the compound main effect on 'peach' intensity for the single compounds with significant letters from LSD post-hoc test. Vertical bars denote 95% confidence intervals.

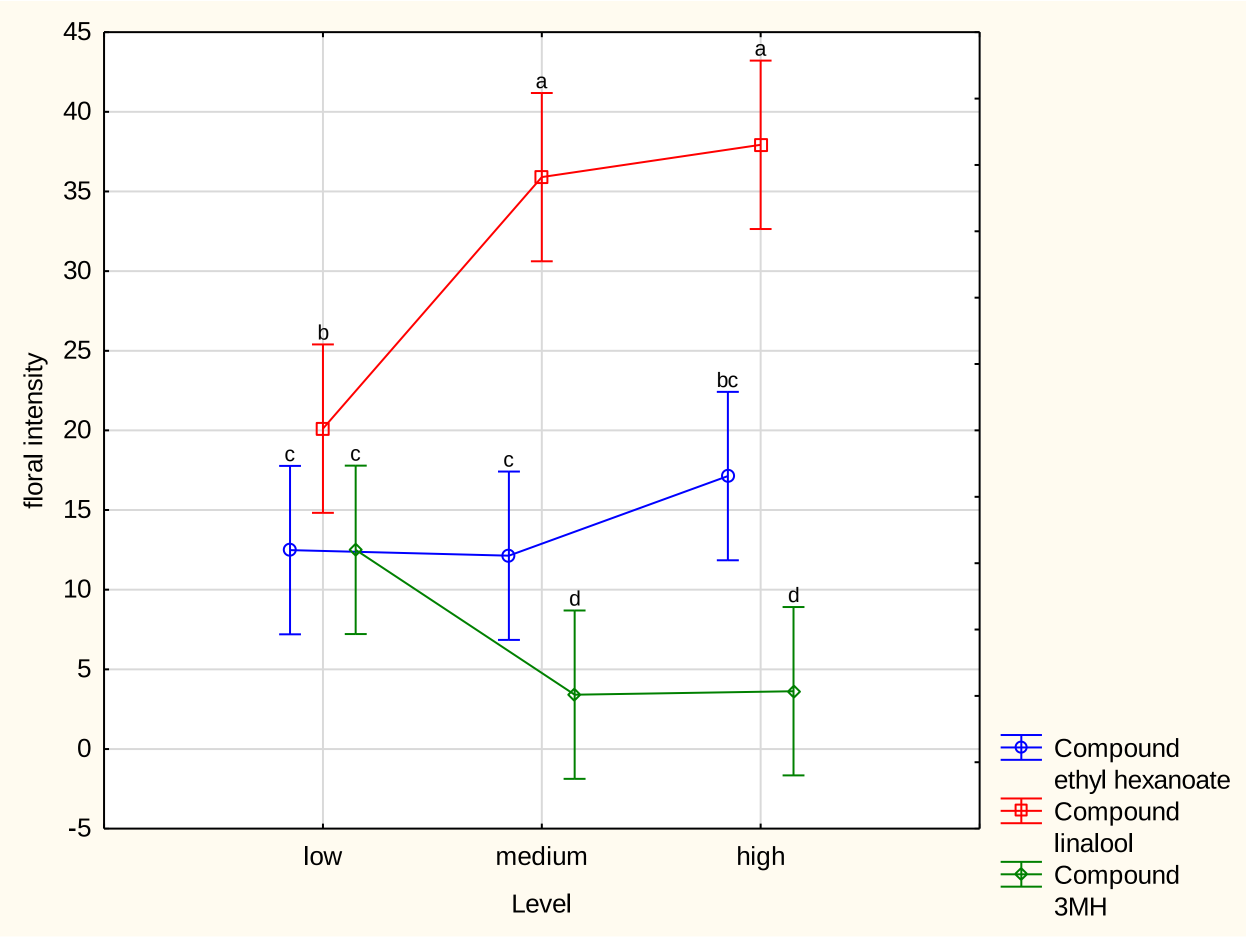

Figure 4.10 LS means plot illustrating the compound*level interaction effect on 'floral' aroma intensity for the single compounds with significant letters from LSD post-hoc test. Vertical bars denote 95% confidence intervals.

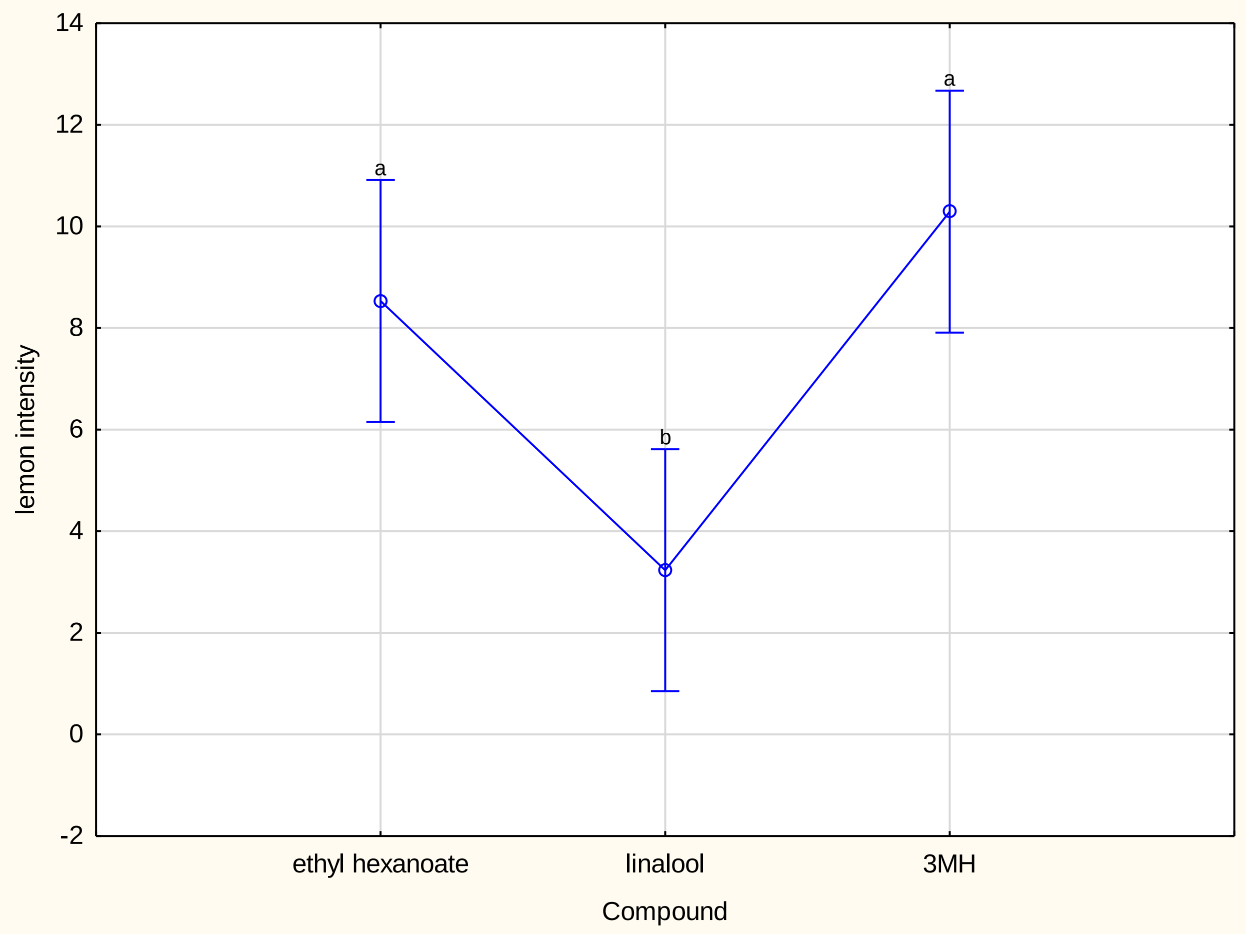

Figure 4.11 LS means plot illustrating the compound main effect on 'lemon' aroma intensity for the single compounds with significant letters from LSD post-hoc test. Vertical bars denote 95% confidence intervals.

The sensory contribution of ethyl hexanoate is subtle when compared to the other two compounds. While ethyl hexanoate is described in literature as ‘fruity’, and ‘green apple’, it was not described by ‘apple’ aroma in this study (Figure 4.8). In the case of ‘floral’/’orange blossom’ and ‘guava’/’tomato leaf’ where 3MH-spiked and linalool-spiked samples differ greatly, ethyl hexanoate-spiked samples have medium intensities of all attributes (Figure 4.3). The restrained effect of ethyl hexanoate on aroma could be similar to the behaviour of another ester, 2-phenylethyl acetate, found by Campo *et al.* (2005), where it had to be in combination with compounds of similar aroma character to have an impact.

In summary, higher 3MH levels increase the perception of ‘guava’, ‘tomato leaf’, and ‘cooked veg’, and may suppress ‘floral’. Samples spiked with linalool are described with ‘peach’, ‘tea’, ‘orange’, and ‘floral’ descriptors, including the specific floral attributes ‘bergamot/earl grey’ and ‘orange blossom’. Linalool decreases the intensity of ‘lemon’, and ‘grapefruit’, and at high concentration decreases ‘dusty/mineral’, and ‘cooked veg’. It is interesting that of the citrus descriptors, linalool increases ‘orange’, but decreases ‘lemon’, and ‘grapefruit’, so in this case rating only a general ‘citrus’ descriptor would have resulted in a loss of information. The highest mean intensities of all the descriptors are for ‘guava’ at high 3MH levels and ‘floral’ at high linalool levels. The sensory contribution of ethyl hexanoate is minimal compared to the aromatic power of the thiol and the terpene. These results will be compared to the combinations to see how the perception of these compounds change when in solution with one another.

4.5.2 Combinations

Sample codes used in the PCA and spider plot can be found in Table 4.1, and follow the format 1=low, 2=medium, 3=high level of each compound in the order: 3MH_ethyl hexanoate_linalool. The combinations were more difficult for panellists to evaluate. Not only were these samples more aromatically complex, but some panellists communicated that the aromas evolved quickly in the headspace of the glass, posing a challenge during evaluation. To address this, panellists were instructed to use their initial impression of the aroma to rate the samples. This change in difficulty and complexity is shown by a decrease in explained variance from 91% in the PCA of the singles (Figure 4.2) to 67.9% in the PCA of the combinations (Figure 4.12). It is further supported by the fact that the two centre samples, 2_2_2 are not very closely located on the PCA of the combinations (Figure 4.12). Considering that in the singles, the panel was able to differentiate between the samples, this can be attributed to complexity of the samples rather than panel performance. There were also fewer significant descriptors than in the singles evaluation (10 in the combinations *vs*. 13 in the singles). The non-significant descriptors for the combinations were ‘passion fruit’, ‘peach’, ‘apple’, ‘orange’, ‘tea’, ‘honey’, ‘dusty/mineral’, ‘cooked veg’ and ‘tomato leaf’ (Table 4.5). Descriptors with a significant main or interaction effect were ‘guava’, ‘pineapple’, ‘banana’, ‘lemon’, ‘grapefruit’, ‘floral’, ‘orange blossom’, and ‘bergamot/earl grey’, and ‘artificial sweet’ (Table 4.5).

Table 4.5 ANOVA table showing F-values for combinations. EH=ethyl hexanoate.

	3MH level	**EH level**	**linalool level**	**3MH*EH**	**3MH*linalool**	**EH*linalool**
	df=1	**df=1**	**df=2**	**df=1**	**df=1**	**df=1**
Sensory Attribute	**F-Value**					
guava	12.120*	0.019	0.754	0.497	3.361	0.876
pineapple	4.459*	0.220	1.410	0.246	0.004	1.187
passion fruit	3.913	0.058	1.265	1.176	0.493	3.638
banana	0.149	8.614*	1.494	0.906	0.019	1.761
peach	1.902	2.727	1.734	0.046	0.055	3.817
apple	0.084	2.332	0.119	2.147	1.203	0.442
lemon	1.975	0.108	0.673	0.699	4.546*	3.144
orange	0.937	0.091	0.188	0.206	0.212	0.052
grapefruit	0.130	1.575	8.069*	0.437	2.791	0.159
floral	0.862	0.008	16.498*	0.113	0.345	0.071
orange blossom	0.287	0.171	9.899*	0.420	0.136	0.660
bergamot/earl grey	0.592	0.150	4.969*	0.168	0.575	0.994
tea	1.023	0.720	2.646	0.555	0.382	0.562
artificial sweet	0.316	6.163*	1.796	0.005	1.190	0.882
honey	0.007	0.030	0.453	1.872	2.830	0.693
dusty/mineral	1.440	0.154	1.272	0.128	0.024	0.050
tomato leaf	1.499	0.279	2.102	0.505	0.969	0.164
cooked veg	0.562	0.209	2.961	0.012	1.117	0.332

* indicates significance at $\alpha=0.05$

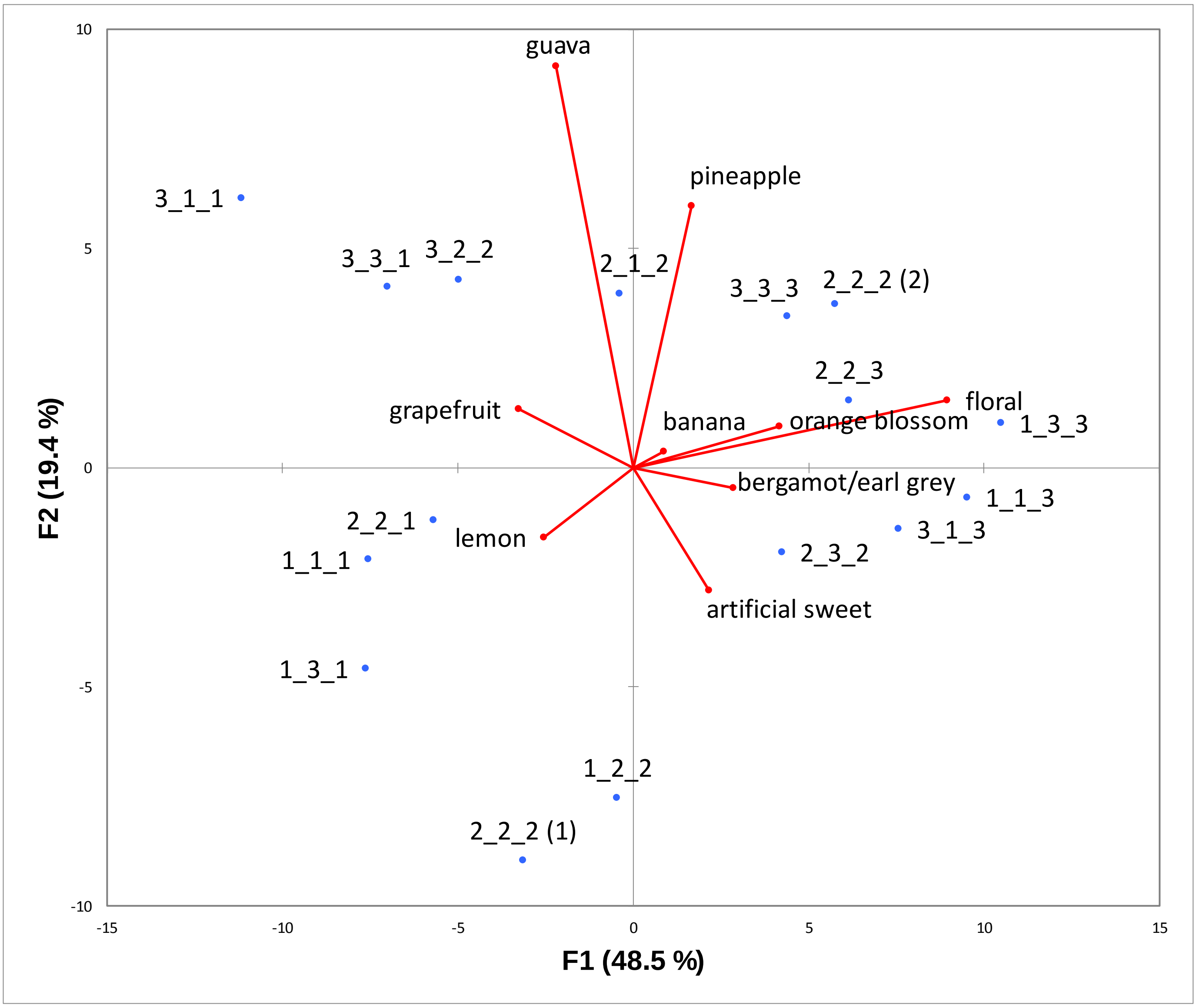

Figure 4.12 PCA of combinations data with significant attributes labelled. Sample codes represent the level of 3MH_ethyl hexanoate_linalool, where 1=low, 2=medium, 3=high. A full list of sample codes can be found in Table 4.1.

In the PCA, the high-3MH samples were spread along PC1. Three high-3MH samples (3_1_1, 3_3_1, and 3_2_2) were all associated with 'lemon', 'grapefruit', 'guava' and 'pineapple' (Figure 4.12). The two high-3MH samples not in this group also contained high linalool. One of them, 3_3_3 was associated both with 'guava' and 'floral', and the other, 3_1_3, was correlated best with 'floral' (Figure 4.12). From the ANOVA of the combinations, as in the singles, 3MH level had a significant effect on 'guava' (Table 4.5) and is increased at higher 3MH concentrations, which can be seen in the spider plot for 3_1_1 and 3_3_1 (Figure 4.13). In the singles, the potential enhancing of 'guava' by ethyl hexanoate or suppressing of 'guava' by linalool was hypothesized. In the combinations, it can be narrowed down to a suppressing effect by linalool (Figure 4.14), though the 3MH*linalool interaction is only significant at α=0.1 (Table 4.5). Berkwitz et al. (2012) also found that in aroma reconstitution and omission tests of Sauvignon Blanc wines that the omission of linalool led to higher intensities of sweat sweaty passion fruit descriptors, linked to 3MH and 3MHA. In the combinations, 'pineapple' became significant for the 3MH main effect, where in the singles it was not significant (Table 4.5).

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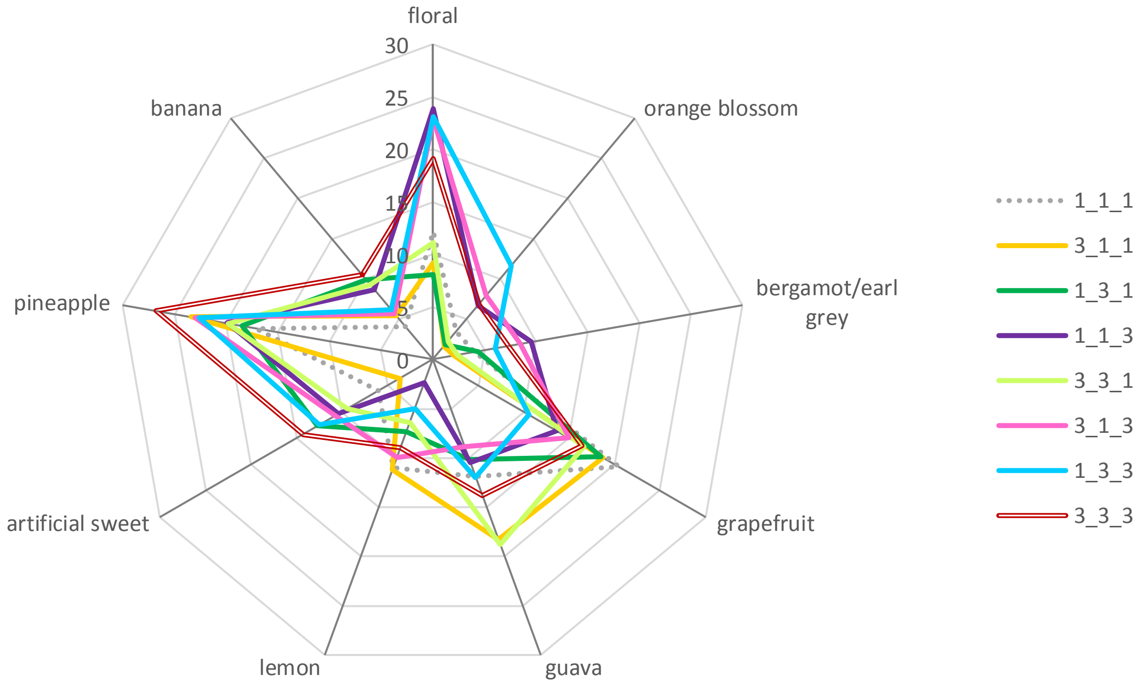

Figure 4.13 Spider web plot showing the aromatic profile of the combinations DA samples including only cube (more extreme) samples from the CCD for readability. Includes descriptors with a significant compound main effect or a significant two-compound interaction at α=0.05. Sample codes represent the level of 3MH_ethyl hexanoate_linalool, where 1=low, 2=medium, 3=high. A full list of sample codes can be found in Table 4.1.

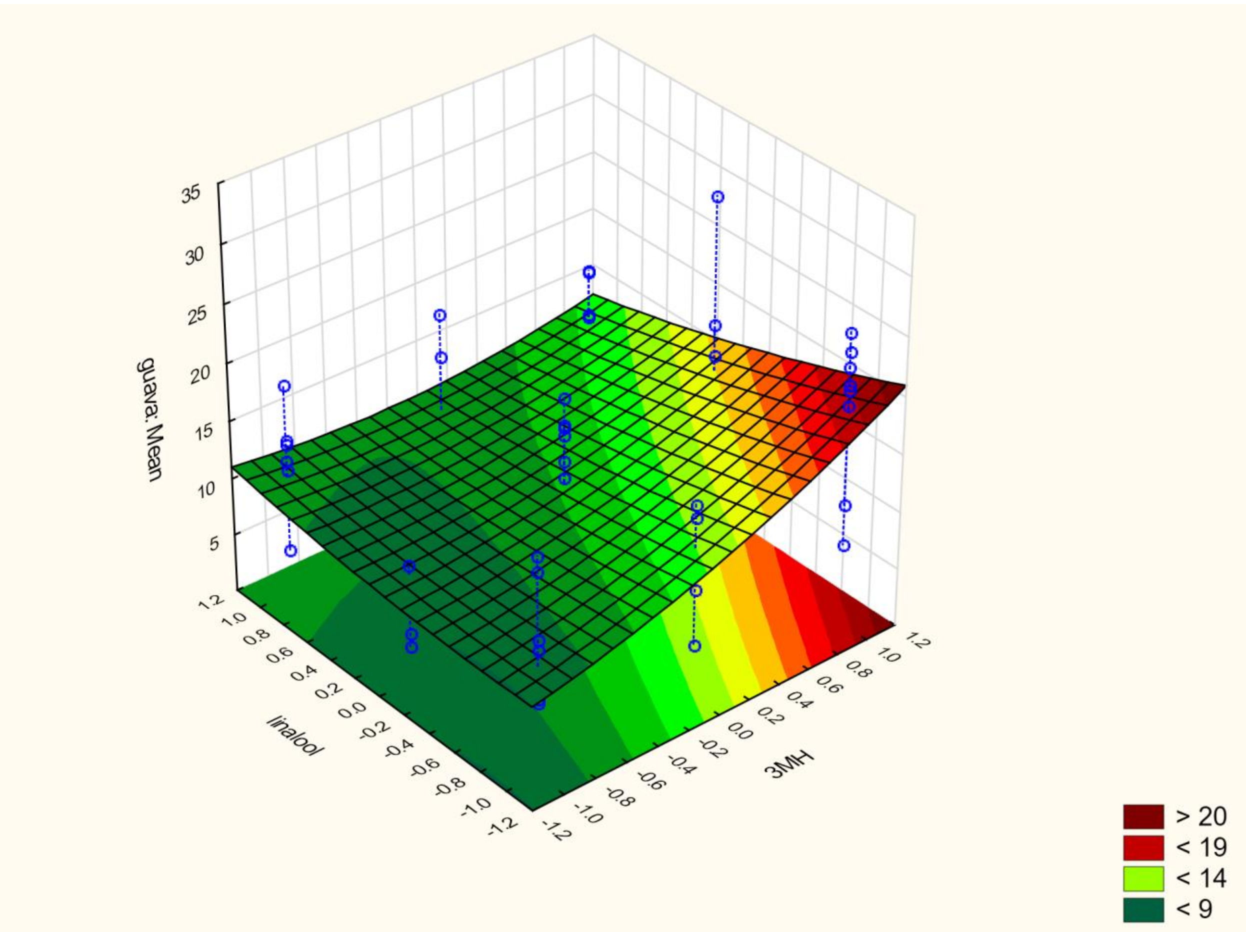

Figure 4.14 Response-surface plot for the 'guava' attribute intensity due to the interaction of 3MH and linalool.

Pineapple' intensity is highest for the sample 3_3_3 and lowest for 1_1_1 (Figure 4.13), so the three compounds seem to have an additive effect for 'pineapple', with 3MH having the strongest effect. For the descriptor 'tomato leaf', 3_1_1 does have higher 'tomato leaf' intensity than the other samples (raw data, not shown), which agrees with the significant positive correlation between 3MH and 'tomato leaf' in the singles. However, there are no significant compound or interaction effects for 'tomato leaf' according to the ANOVA (Table 4.5). This indicates that in the presence of other volatiles, this quality of 3MH is suppressed. Similarly, the increase of 'cooked veg' due to 3MH which was observed in the singes, is no longer present in the combinations.

'Passion fruit' was not significant in either the singles or combinations at $\alpha=0.05$, but in the combinations, there was a trend for 'passion fruit' intensity to increase at the medium 3MH concentration ($p=0.054$, Appendix B Figure B.6). There was also a trend ($p=0.063$) for linalool and ethyl hexanoate to interact with each other, increasing 'passion fruit' intensity when both compounds were at high or low concentration, and decreasing when both compounds were at medium concentration (Figure 4.15). The last descriptor affected by 3MH is 'lemon'. In the singles, 'lemon' was suppressed by linalool, but in the combinations, there was a significant 3MH*linalool interaction where the suppressing effect is only true when in combination with low 3MH (Figure 4.16). The presence of 3MH seems to counteract the suppressing effect of linalool on 'lemon'.

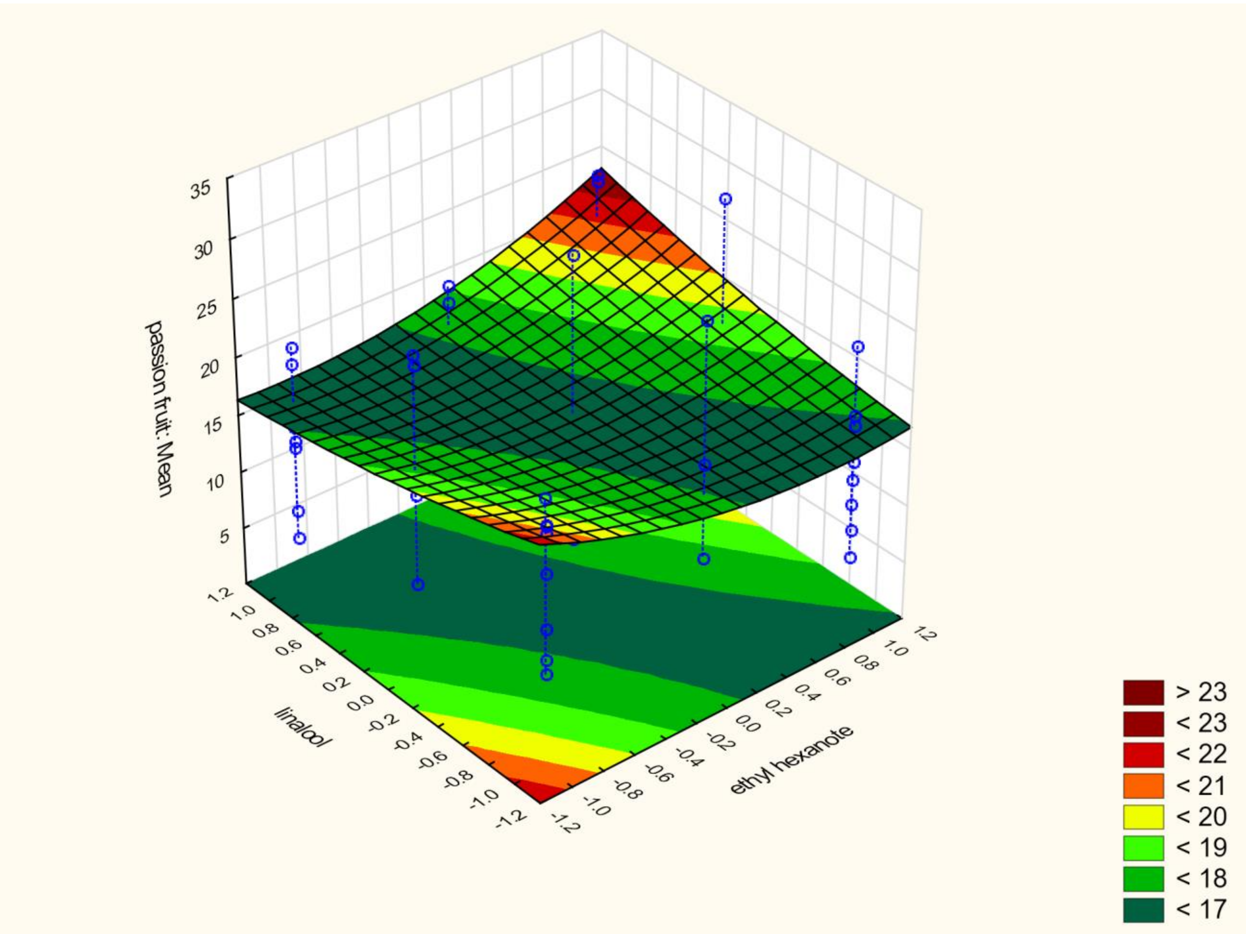

Figure 4.15 Response-surface plot for the 'passion fruit' attribute intensity due to the interaction of linalool and ethyl hexanoate.

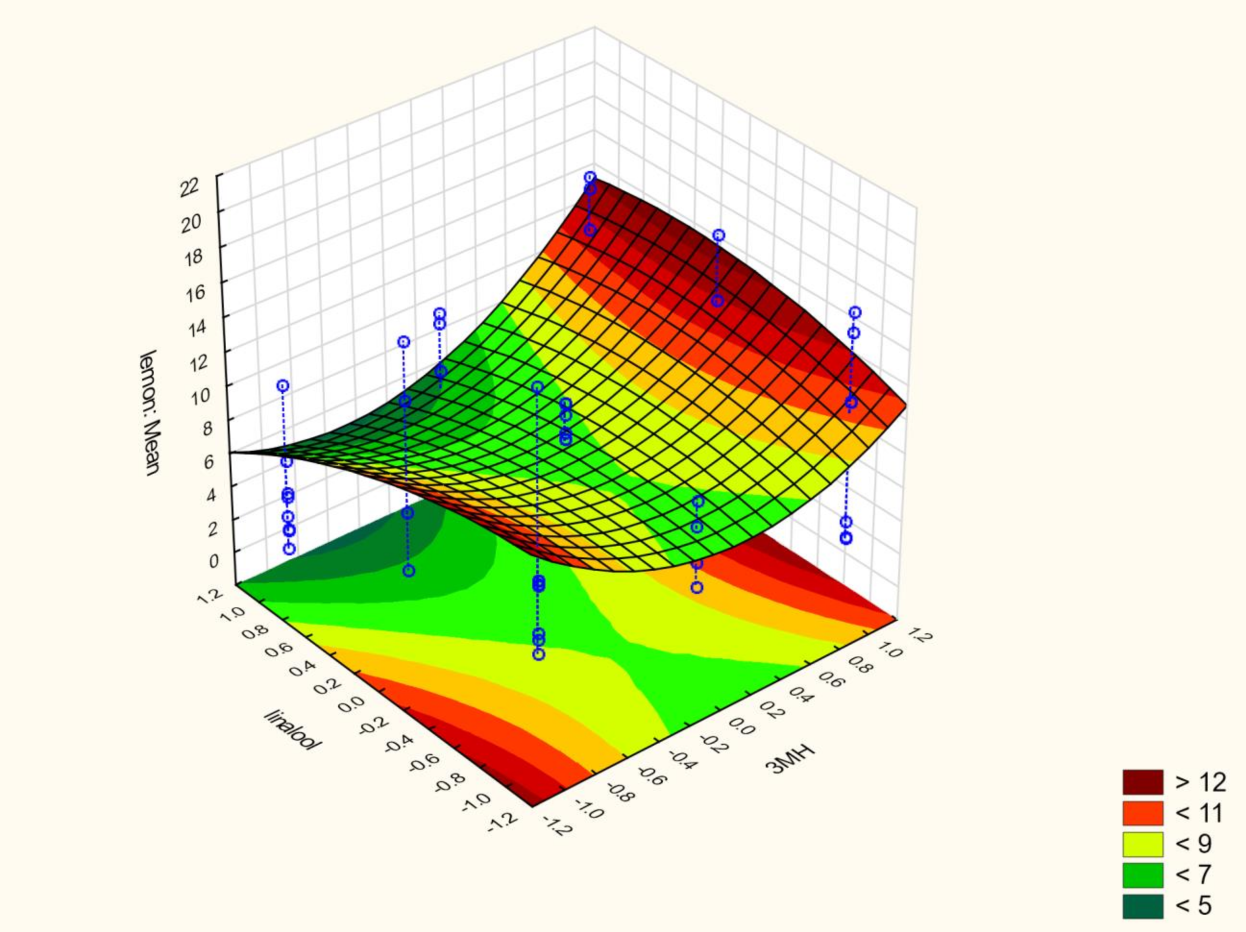

Figure 4.16 Response-surface plot for the 'lemon' attribute intensity due to the interaction of linalool and 3MH.

All the high-linalool samples were associated with the attributes ‘orange blossom’, ‘floral’, and ‘bergamot/earl grey’ attributes in the PCA (Figure 4.12). A large jump in ‘floral’ and ‘orange blossom’ intensities between the low and high-linalool samples is visible in the spider plot (Figure 4.13). From the ANOVA results, “floral”, “orange blossom”, and “bergamot/earl grey” had a significant main effect for linalool (Table 4.5), where samples with high linalool concentration were described by these attributes (Appendix B Figures B.7, B.8, B.9). There was a trend for the “tea” to behave the same way as these descriptors, but it was only significant at α=0.1, not α=0.05 (Appendix B Figure B.10). This group of descriptors showed the same behaviour in the singles, showing that that these descriptors are a result of linalool, and are not highly enhanced or suppressed by the thiol or the ester. ‘Grapefruit’ perception decreased significantly at medium and high linalool levels, showing the same suppressing effect which was apparent in the singles (Figure 4.17). The suppression of ‘cooked veg’ by linalool that was observed in the singles is not significant in the combinations at α=0.05 (Table 4.5), but the same behaviour is seen as a trend at α=0.1 (Appendix B Figure B.11).

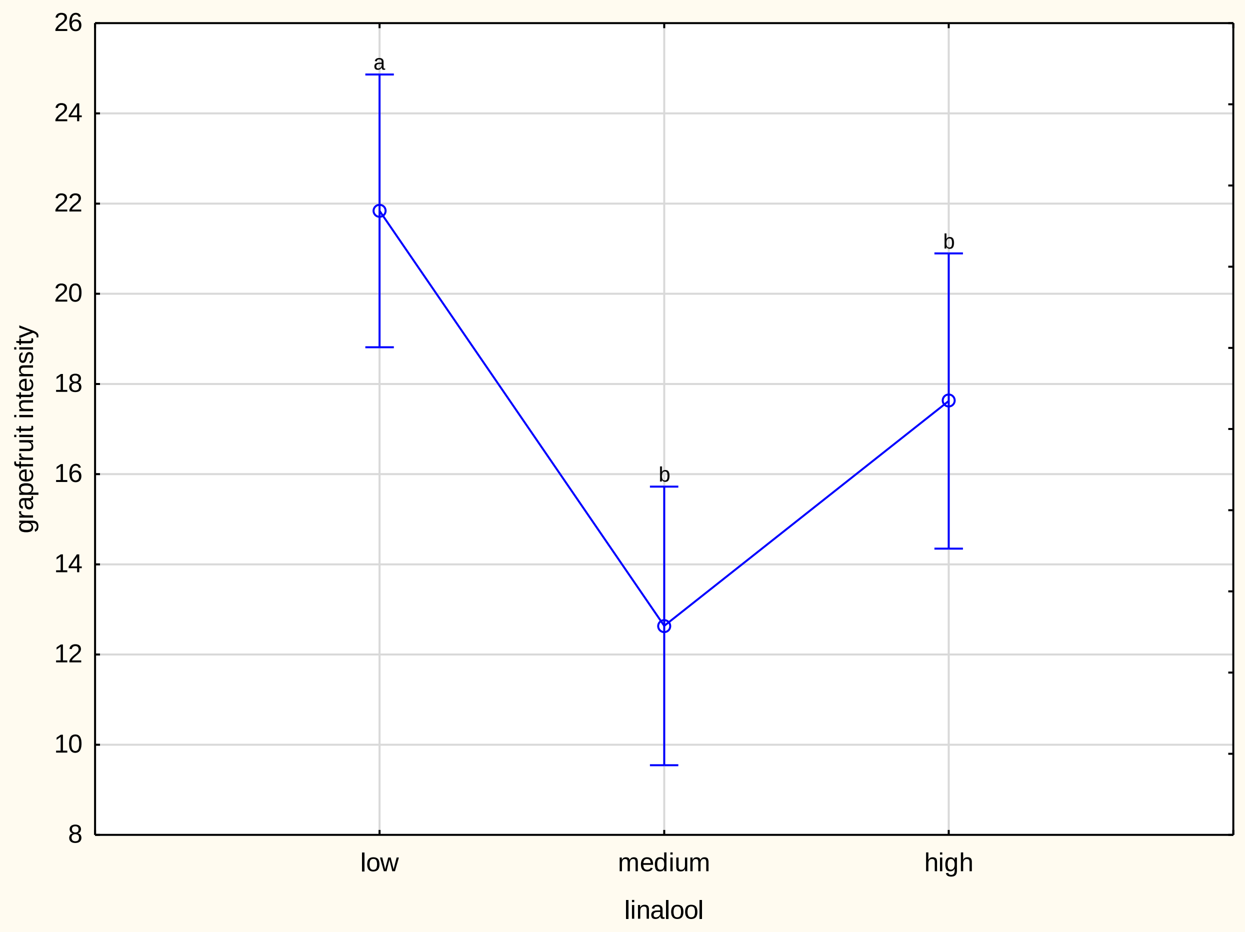

Figure 4.17 LS means plot illustrating the linalool level effect on 'grapefruit' aroma intensity for the combinations of compounds with significant letters from LSD post-hoc test. Vertical bars denote 95% confidence intervals.

The descriptors which behave differently in the combinations than in the singles in relation to linalool are ‘peach’, ‘lemon’, and ‘orange’. In the singles, linalool increased ‘peach’ intensity significantly, but in the combinations, there is an interaction which is nearly-significant (p=0.057) between ethyl hexanoate and linalool, where linalool still increases ‘peach’ intensity, but only when ethyl hexanoate levels are low. This means that ethyl hexanoate suppressed the ‘peach’ aroma which came from medium and high levels of linalool. ‘Lemon’ was also affected by an interaction, but between linalool and 3MH (Figure 4.16). In the singles, ‘lemon’ seemed to be a quality of the base wine which was suppressed by linalool. In the combinations, it was suppressed by linalool only when 3MH levels were low, as high 3MH levels enhanced ‘lemon’ intensity. In the combinations, ‘orange’ intensity is no longer increased by high levels of linalool, as it was in the singles.

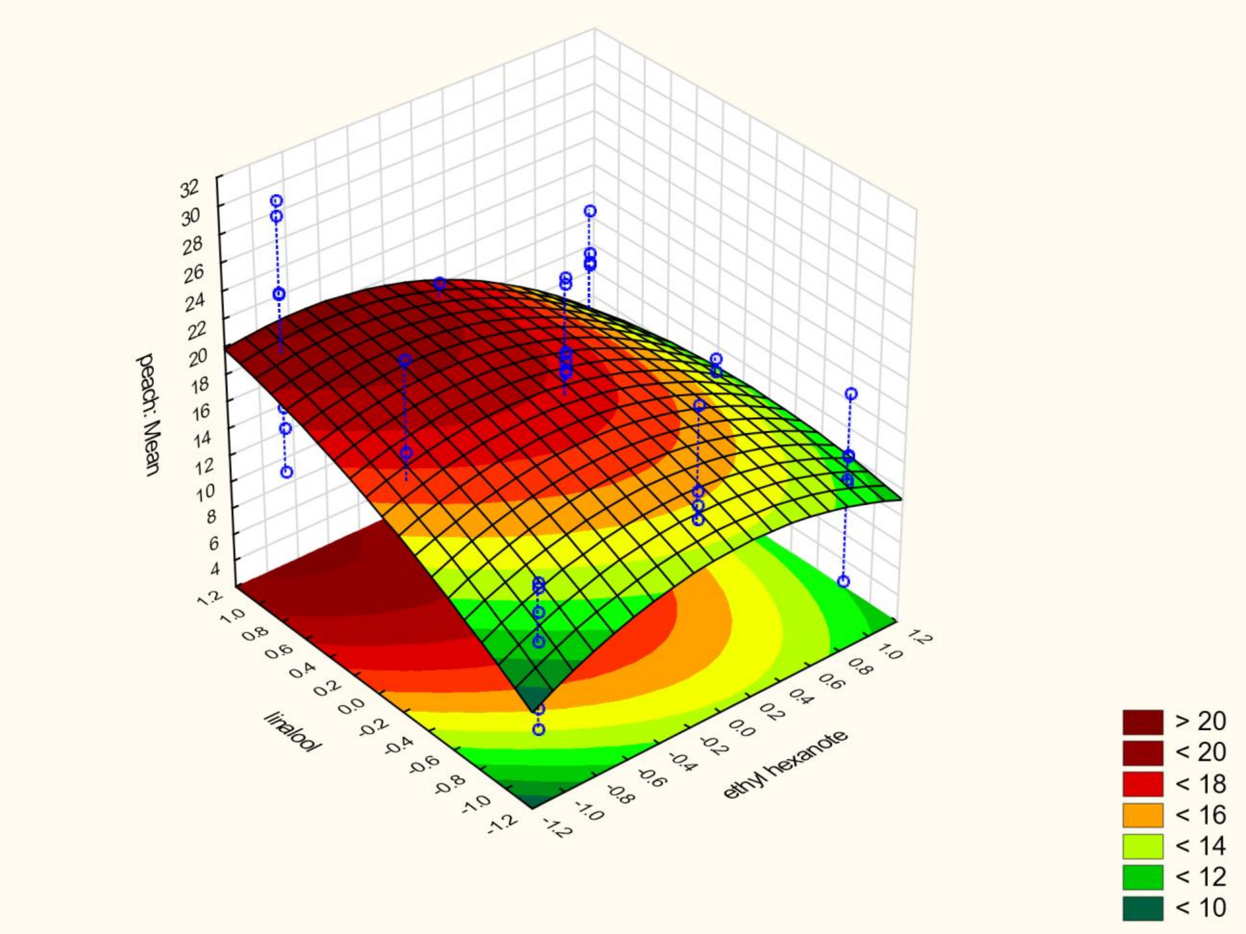

Figure 4.18 Response-surface plot for the 'peach' attribute intensity due to the interaction of linalool and ethyl hexanoate.

From the PCA of the combinations, it is clear that samples with high ethyl hexanoate are scattered around the PCA, which was expected considering the compound's subtle effect seen in the singles (Figure 4.12). In the combinations, ethyl hexanoate has a significant effect on 'banana' and 'artificial sweet, where it did not in the singles (Table 4.5). This 'artificial sweet' aroma could be similar to the 'confectionary' aroma given by a combination of esters including ethyl hexanoate, seen by King *et al.* (2011). The intensities of both 'artificial sweet' and 'banana' increased significantly with the higher levels of ethyl hexanoate (Figure 4.19, Appendix B Figure B.12). The fact that 'banana' and 'artificial sweet' were not significant in the singles coupled with the fact that intensity of both descriptors was highest for the sample 3_3_3 (Figure 4.13) suggests an additive effect, similar to that seen with 'pineapple'. These findings are in agreement other research in which esters are more likely to support the aromas of other volatiles, rather than contribute as impact compounds on their own (Campo *et al.*, 2005).

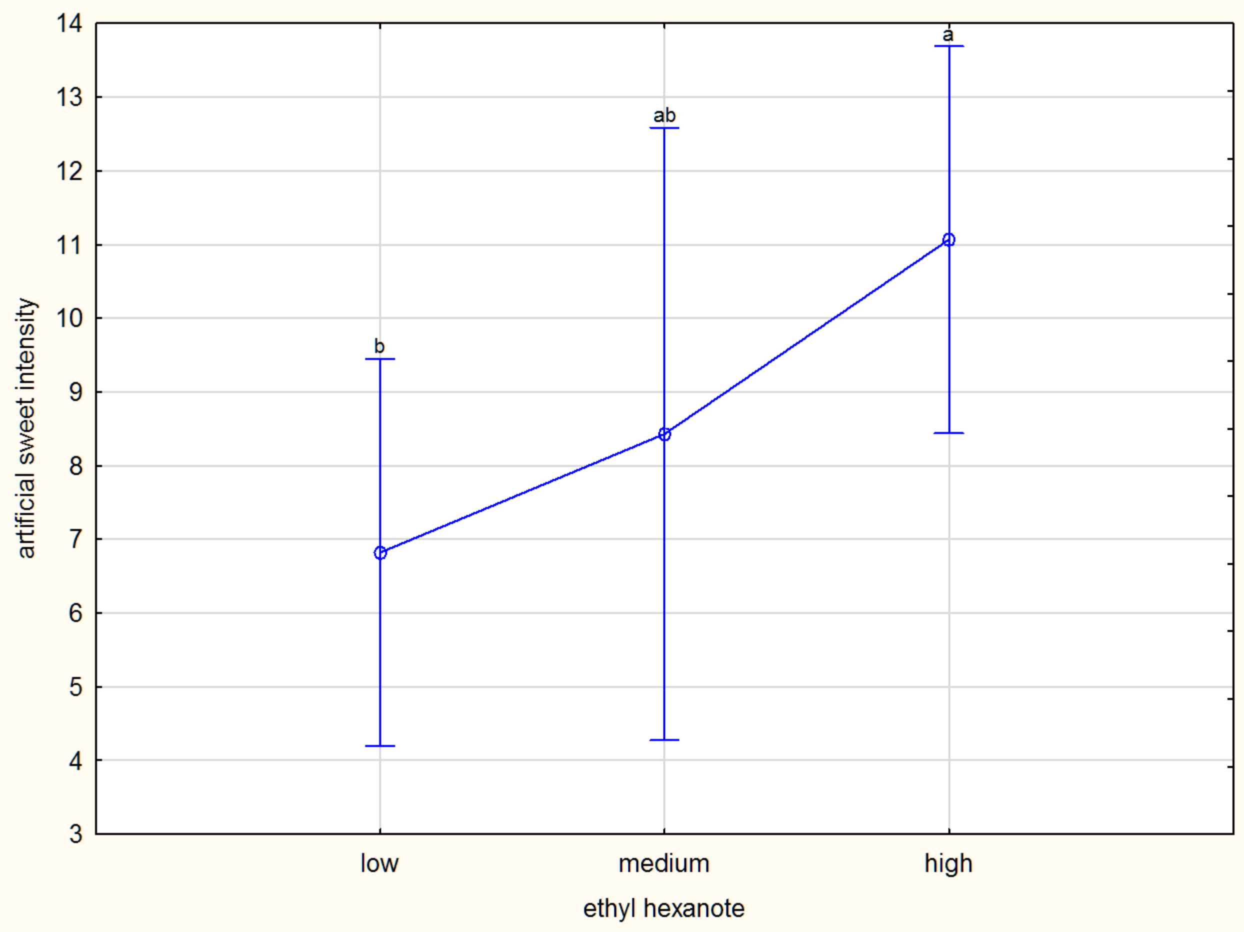

Figure 4.19 LS means plot of 'artificial sweet' aroma intensity at different ethyl hexanoate levels in the combinations of three compounds with significant letters from LSD post-hoc test. Vertical bars denote 95% confidence intervals.

4.6 Conclusions

In the context of the partially-dearomatized Chenin Blanc wine matrix, 3MH was described with attributes previously generated for thiols in Sauvignon Blanc wines (Dubourdieu *et al.*, 2006; King *et al.*, 2011; Van Wyngaard *et al.*, 2014; Coetzee *et al.*, 2015). However, unlike Sauvignon Blanc wines, ‘passion fruit’ and ‘grapefruit’ intensity did not change significantly at different 3MH levels. This could either be because the 3MH range used in this study was narrower than that used in studies on Sauvignon Blanc, or because matrix effects may cause 3MH to be perceived differently in Chenin Blanc than in Sauvignon Blanc. By following the approach suggested by Van Rooyen *et al.* (1982), it was found that the most intense aroma of 3MH was ‘guava’. This supports the hypothesis of du Plessis & Augustyn (1981) that a thiol was responsible for the ‘guava’ character of Chenin Blanc. Research on Sauvignon Blanc has established several ways that thiols can be manipulated by producers (Coetzee & Du Toit, 2012), and this information can be used to alter the typical ‘guava’ and other thiol-derived characters of Chenin Blanc wines.

In the singles, several enhancing and suppressing effects were hypothesized, which were confirmed by the combinations. Most notably, the ‘guava’ and ‘floral’ qualities of 3MH and linalool seem to be antagonistic, which was previously found by Benkwitz *et al.* (2012). This suggests that within the sensory characterization of Chenin Blanc wines, it may be difficult to have a wine which is perceived both as highly 'tropical' and highly 'floral'. This opposition may contribute to the different style categories of South African Chenin Blanc wines.

The strong influences of linalool and 3MH and weak influence of ethyl hexanoate on wine aroma shows that the relative sensory contribution of different compounds can not necessarily be predicted by their odour active values alone. The aromatic properties of linalool were dominant, while the influence of ethyl hexanoate was only apparent when in combination with other compounds. Whether these behaviours are unique to each compound, or whether trends within volatile compound classes exist warrants further investigation.

The goal of this study was to better understand some of the interactions which occur between volatiles in Chenin Blanc wines. It was shown that the perception of these compounds depends on their concentration and context. The interactions between these compounds are complex, but this type of knowledge can ultimately help researchers better understand the relationship between chemical composition and human sensory perception. Ideally, this study would be expanded to include other volatiles and replicated in other model solutions to confirm that the sensory response observed in this study are applicable to other matrices. Though the effect of the non-volatile matrix on aroma was not discussed, this concept will be explored in the following Chapter 5.

4.7 References

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Appendix B

Chapter 4 Additional figures

Appendix B. : Chapter 4 Additional figures

B.1 Singles

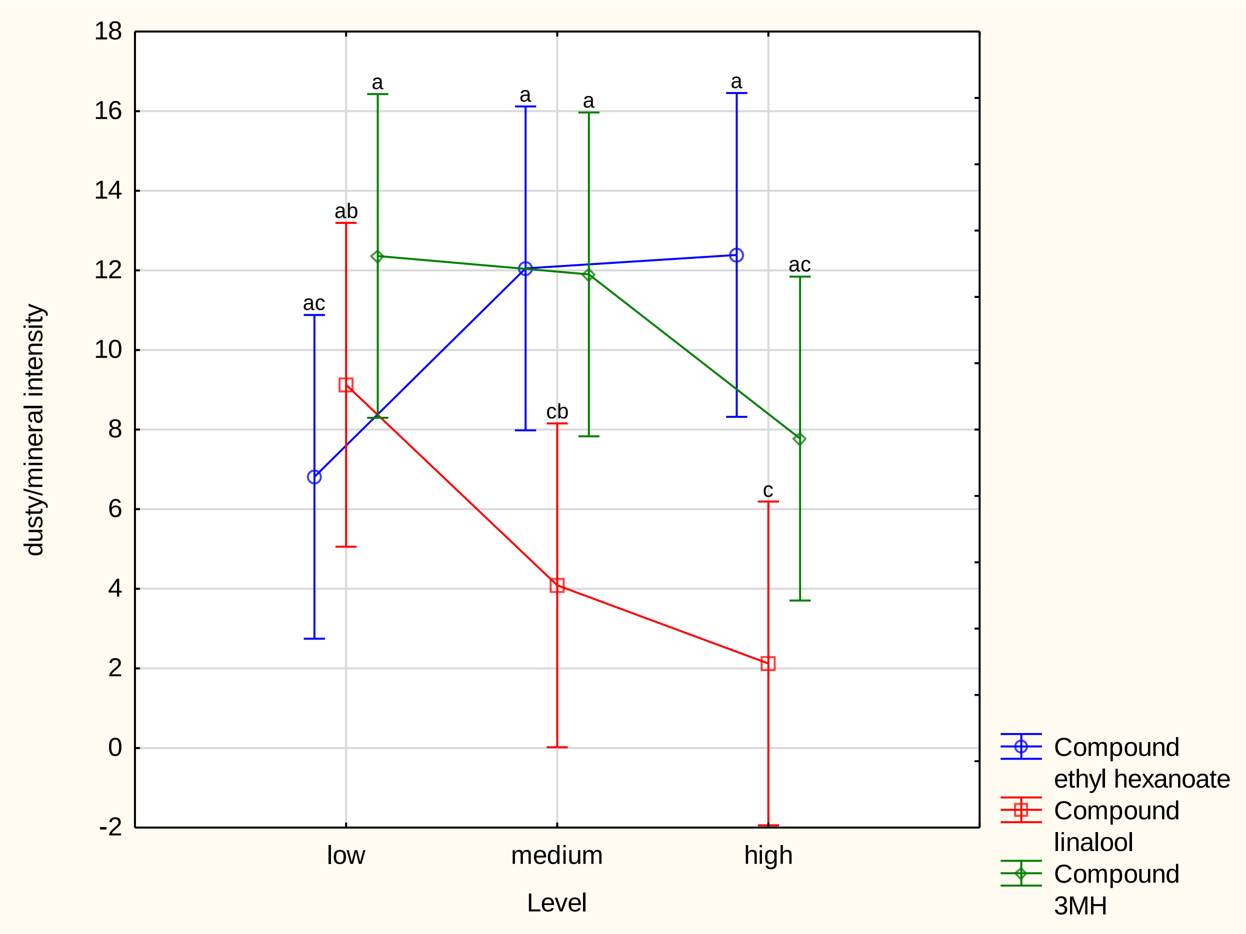

Figure B.1 LS means plot illustrating the compound*level interaction effect on 'dusty/mineral' aroma intensity for the single compounds with significant letters from LSD post-hoc test. Vertical bars denote 95% confidence intervals.

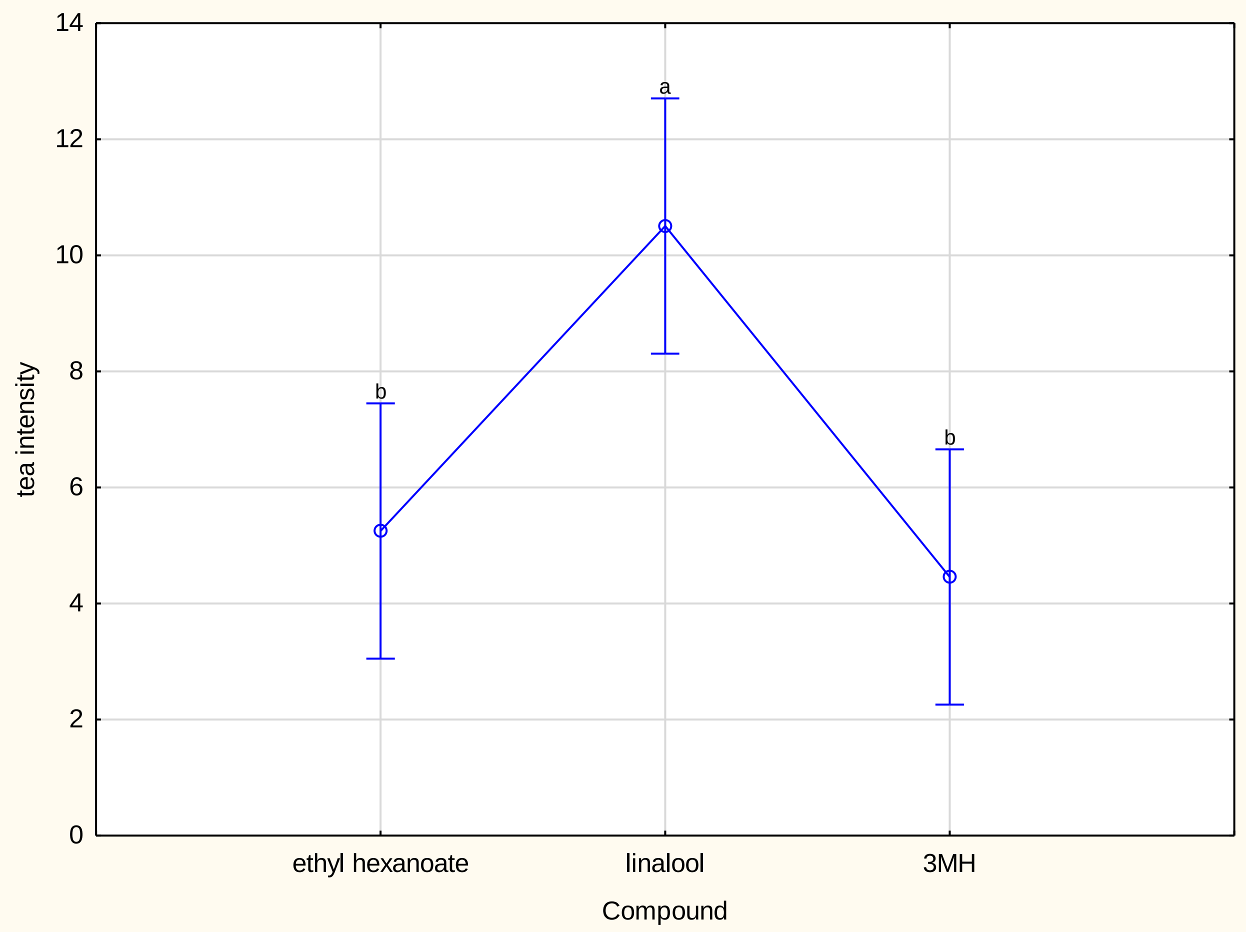

Figure B.2 LS means plot illustrating the compound main effect on 'tea' aroma intensity for the single compounds with significant letters from LSD post-hoc test. Vertical bars denote 95% confidence intervals.

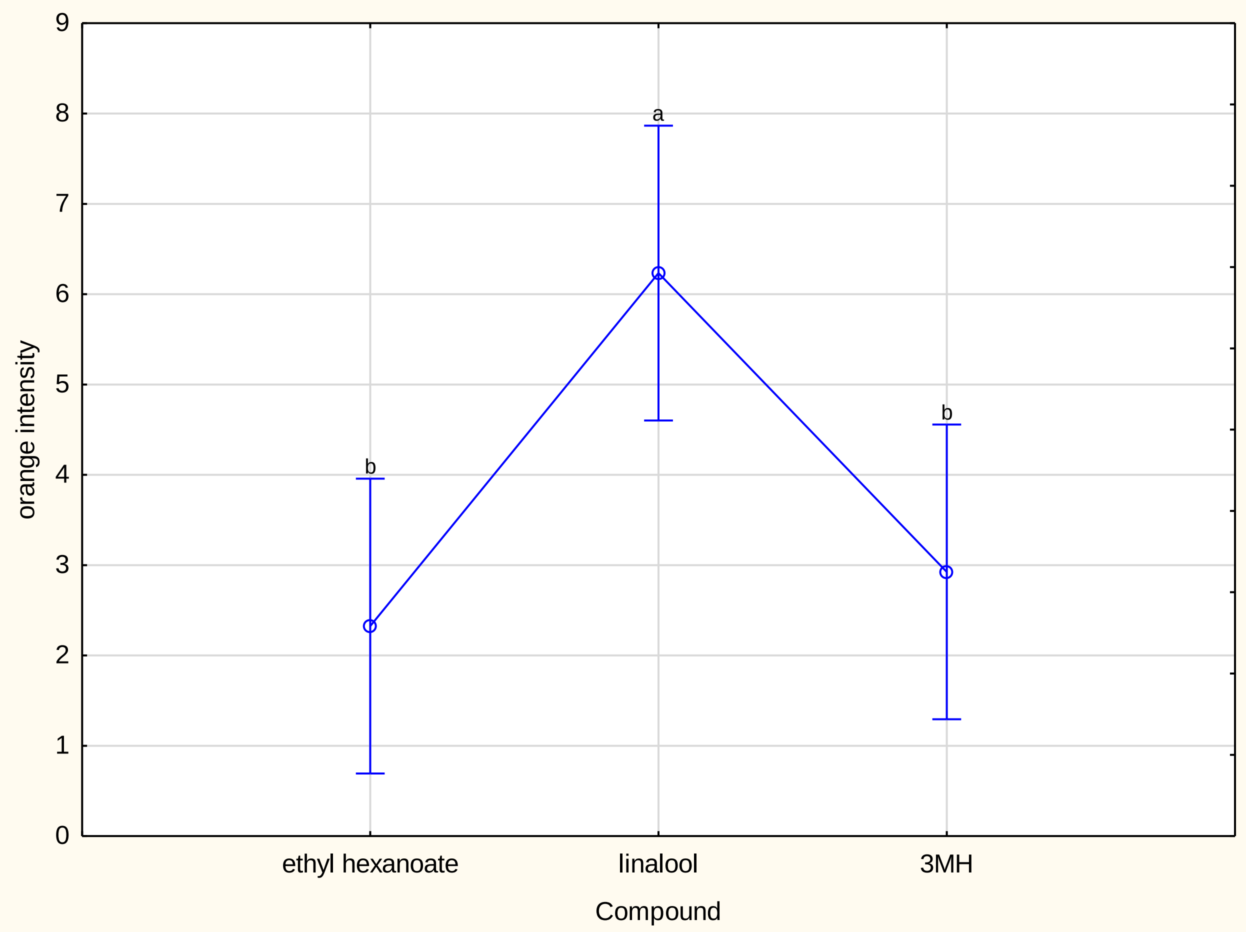

Figure B.3 LS means plot illustrating the compound main effect on 'orange' aroma intensity for the single compounds with significant letters from LSD post-hoc test. Vertical bars denote 95% confidence intervals.

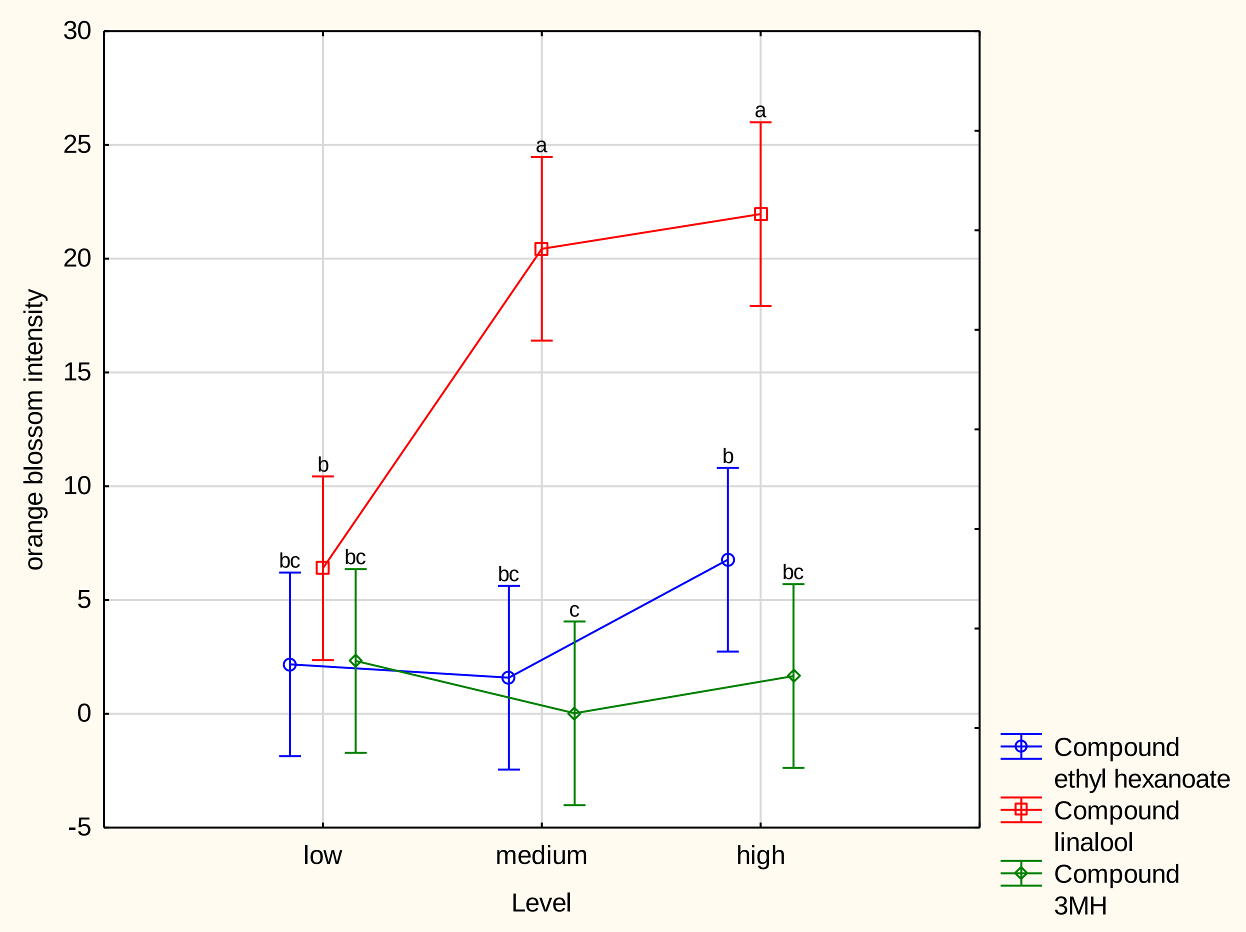

Figure B.4 LS means plot illustrating the compound*level interaction effect on 'orange blossom' aroma intensity for the single compounds with significant letters from LSD post-hoc test. Vertical bars denote 95% confidence intervals.

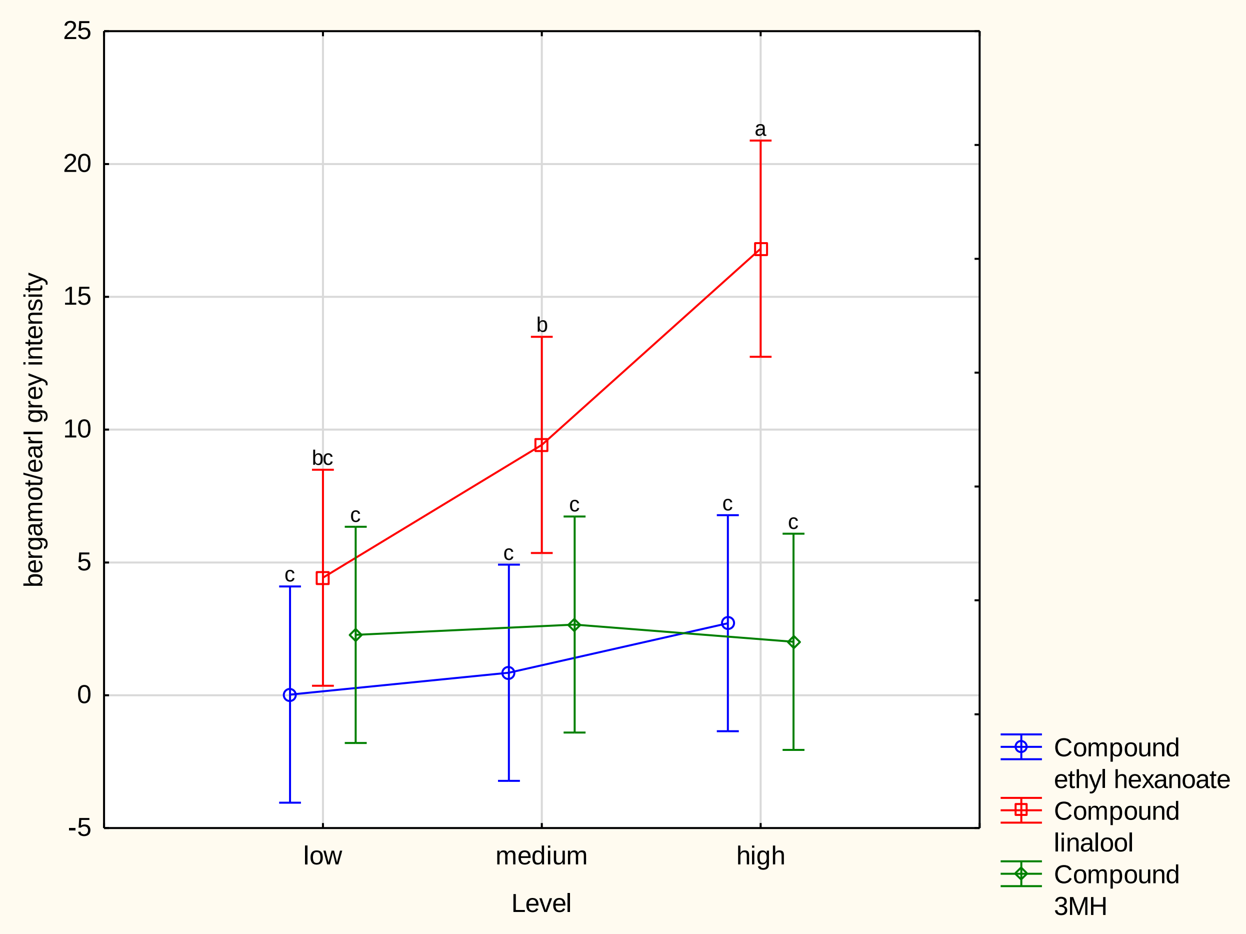

Figure B.5 LS means plot illustrating the compound*level interaction effect on 'bergamot/earl grey' aroma intensity for the single compounds with significant letters from LSD post-hoc test. Vertical bars denote 95% confidence intervals.

B.2 Combinations

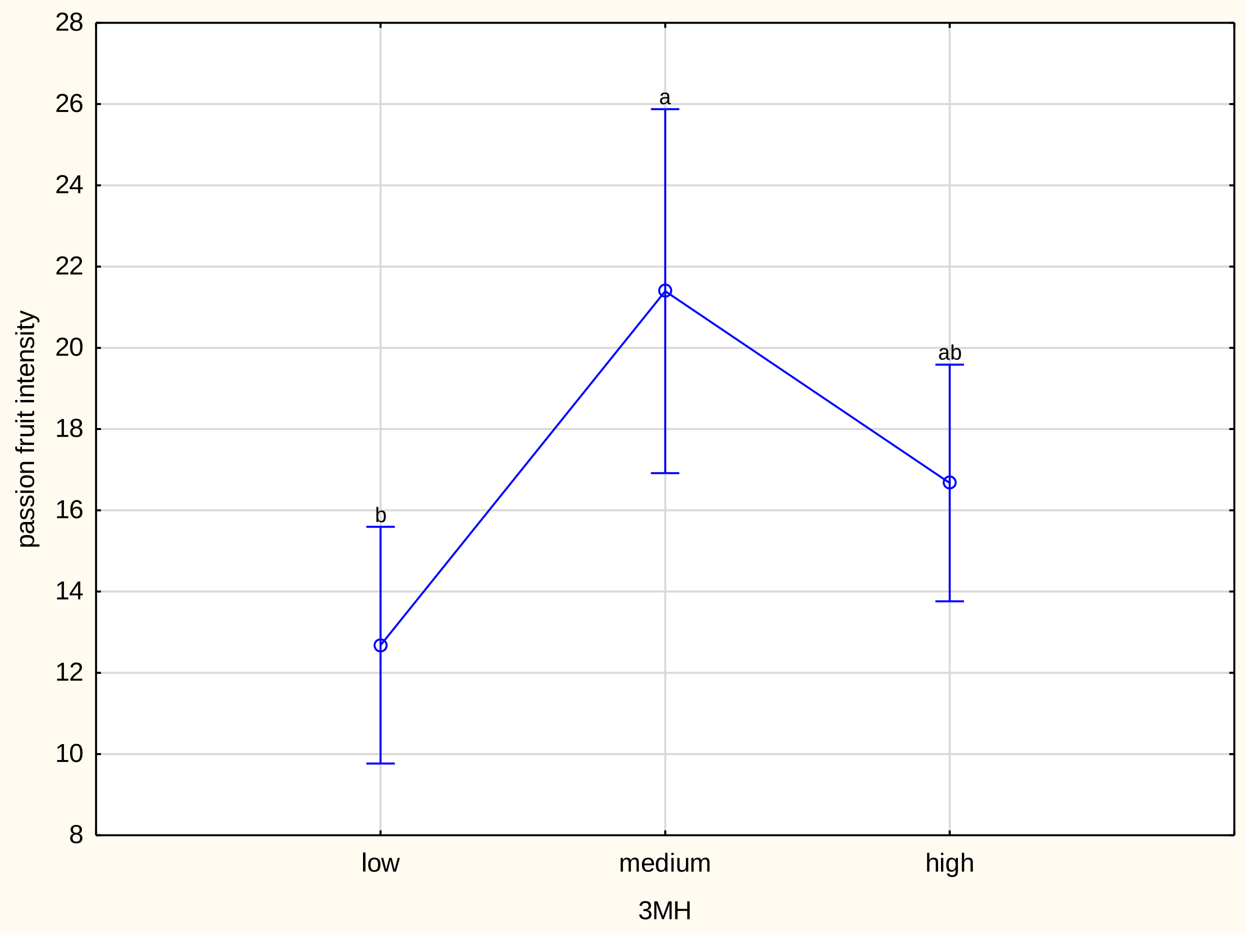

Figure B.6 LS means plot illustrating the 3MH level effect on 'passion fruit' aroma intensity for the combinations of compounds with significant letters from LSD post-hoc test. Vertical bars denote 95% confidence intervals.

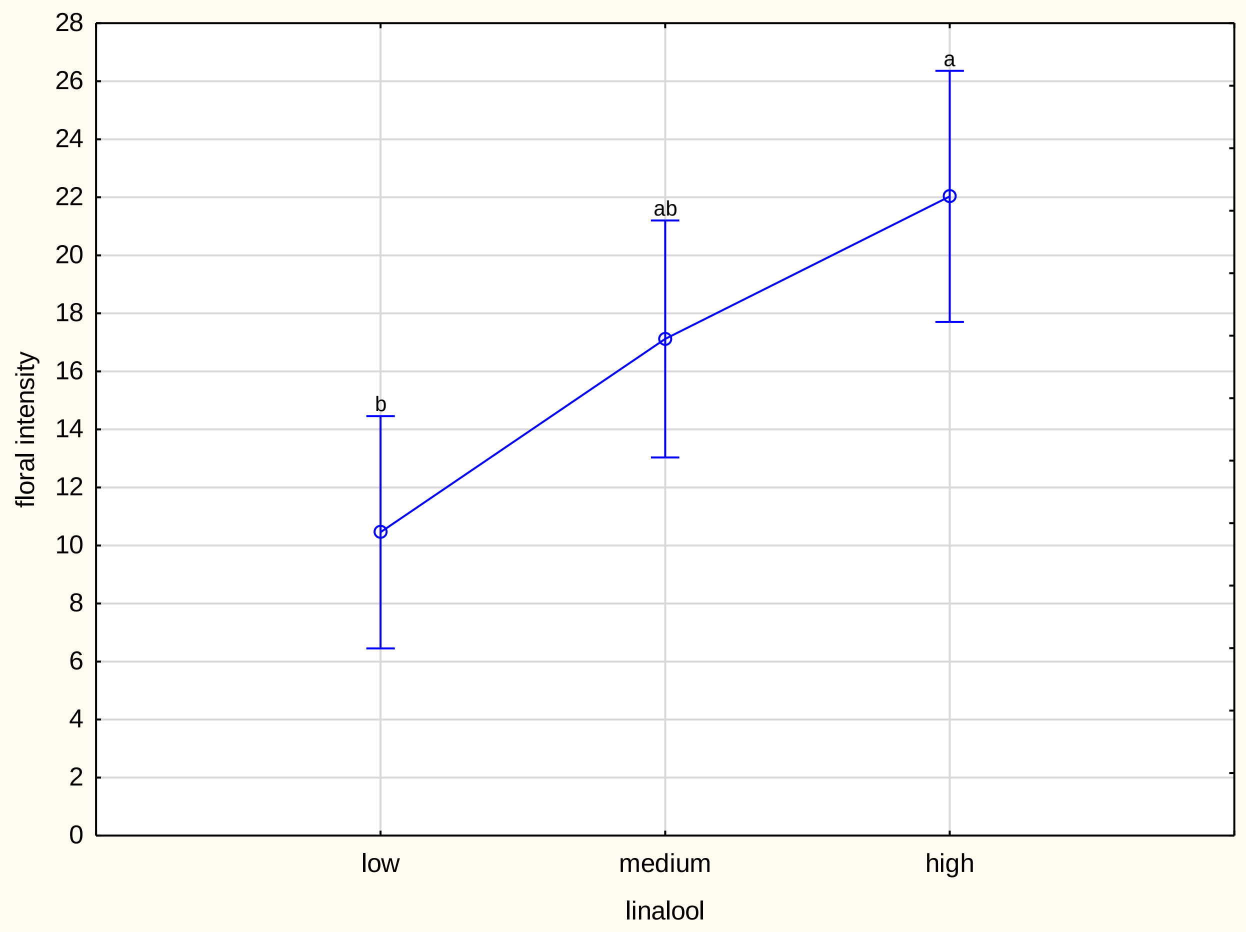

Figure B.7 LS means plot illustrating the linalool level effect on 'floral' aroma intensity for the combinations of compounds with significant letters from LSD post-hoc test. Vertical bars denote 95% confidence intervals.

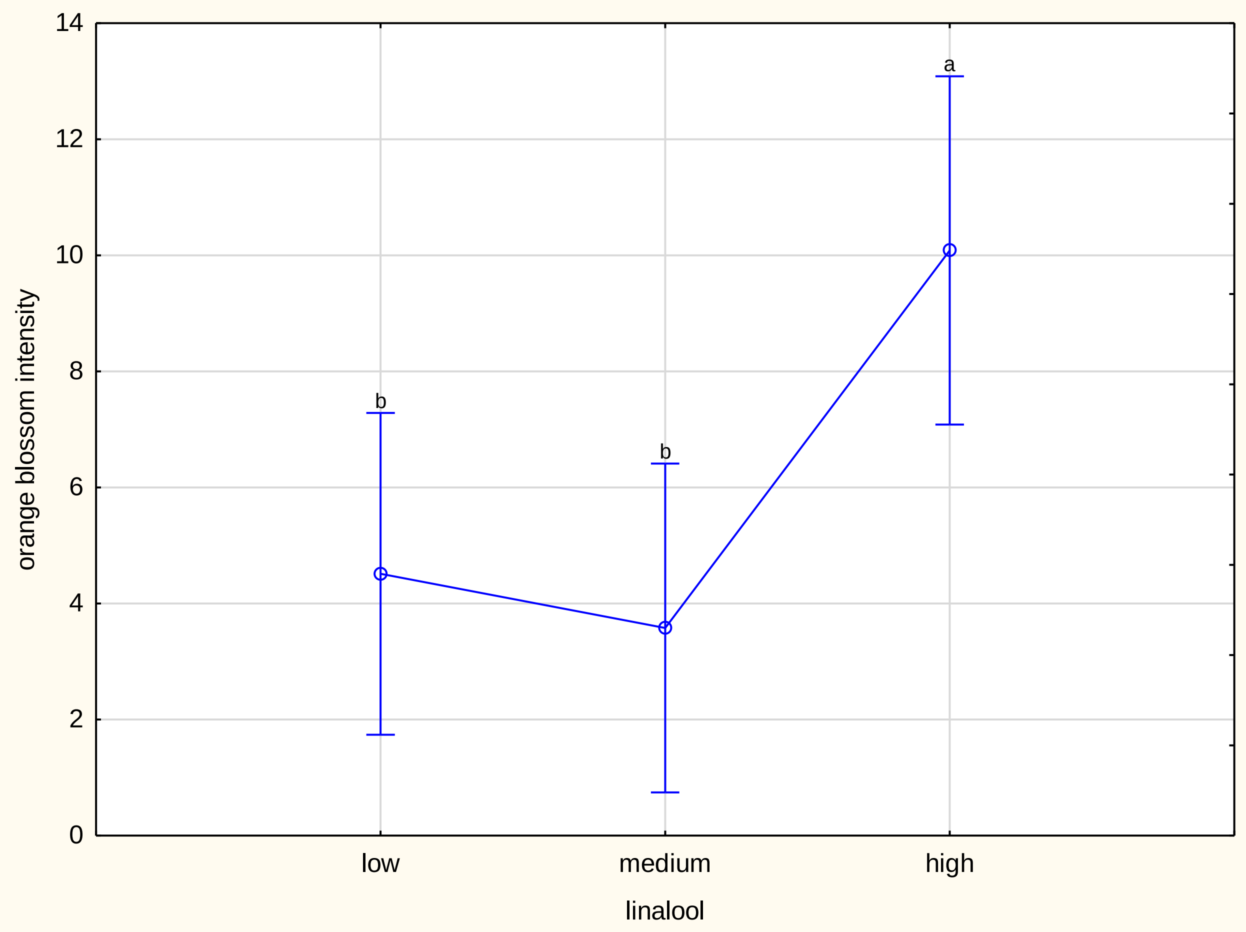

Figure B.8 LS means plot illustrating the linalool level effect on 'orange blossom' aroma intensity for the combinations of compounds with significant letters from LSD post-hoc test. Vertical bars denote 95% confidence intervals.

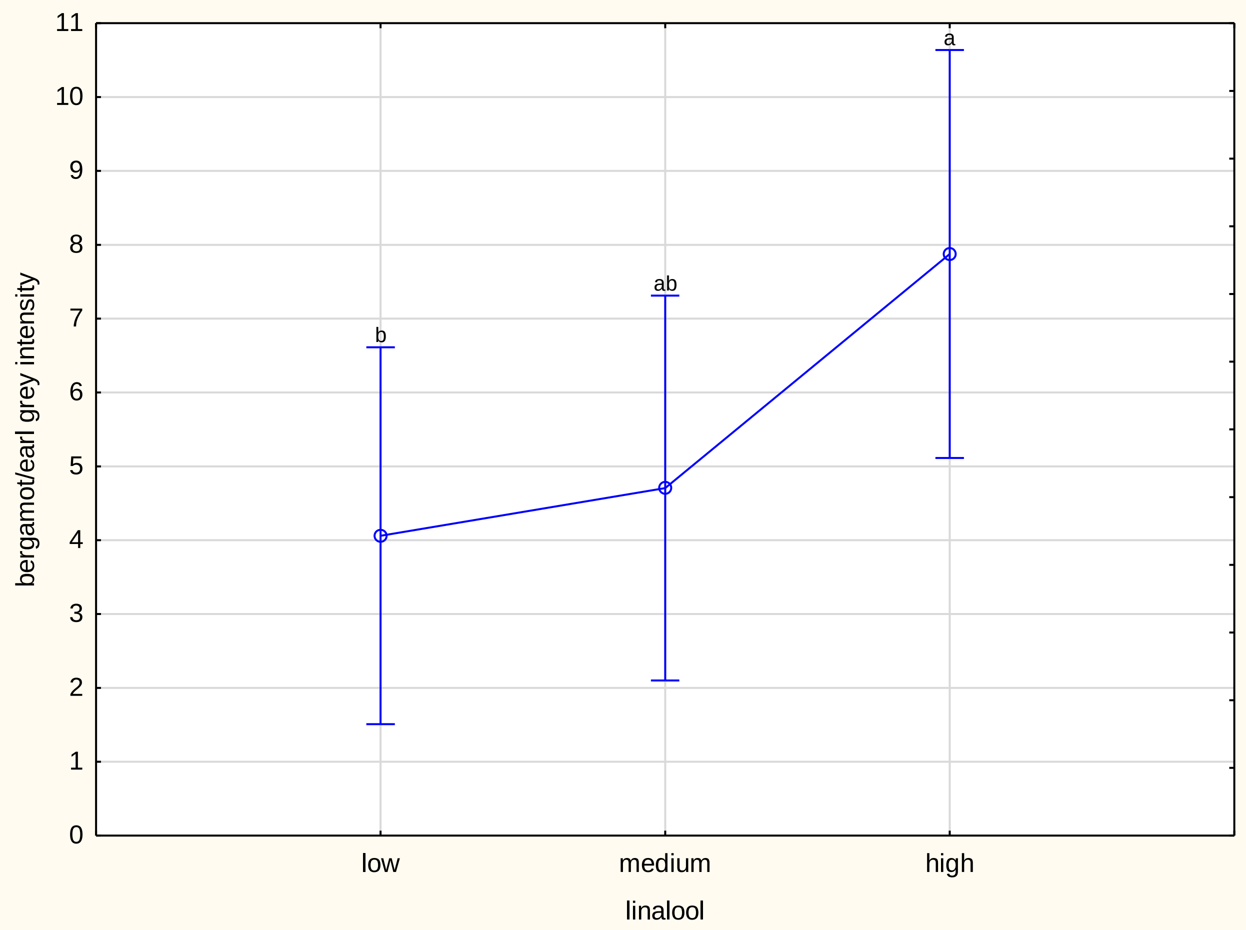

Figure B.9 LS means plot illustrating the linalool level effect on 'bergamot/earl grey' aroma intensity for the combinations of compounds with significant letters from LSD post-hoc test. Vertical bars denote 95% confidence intervals.

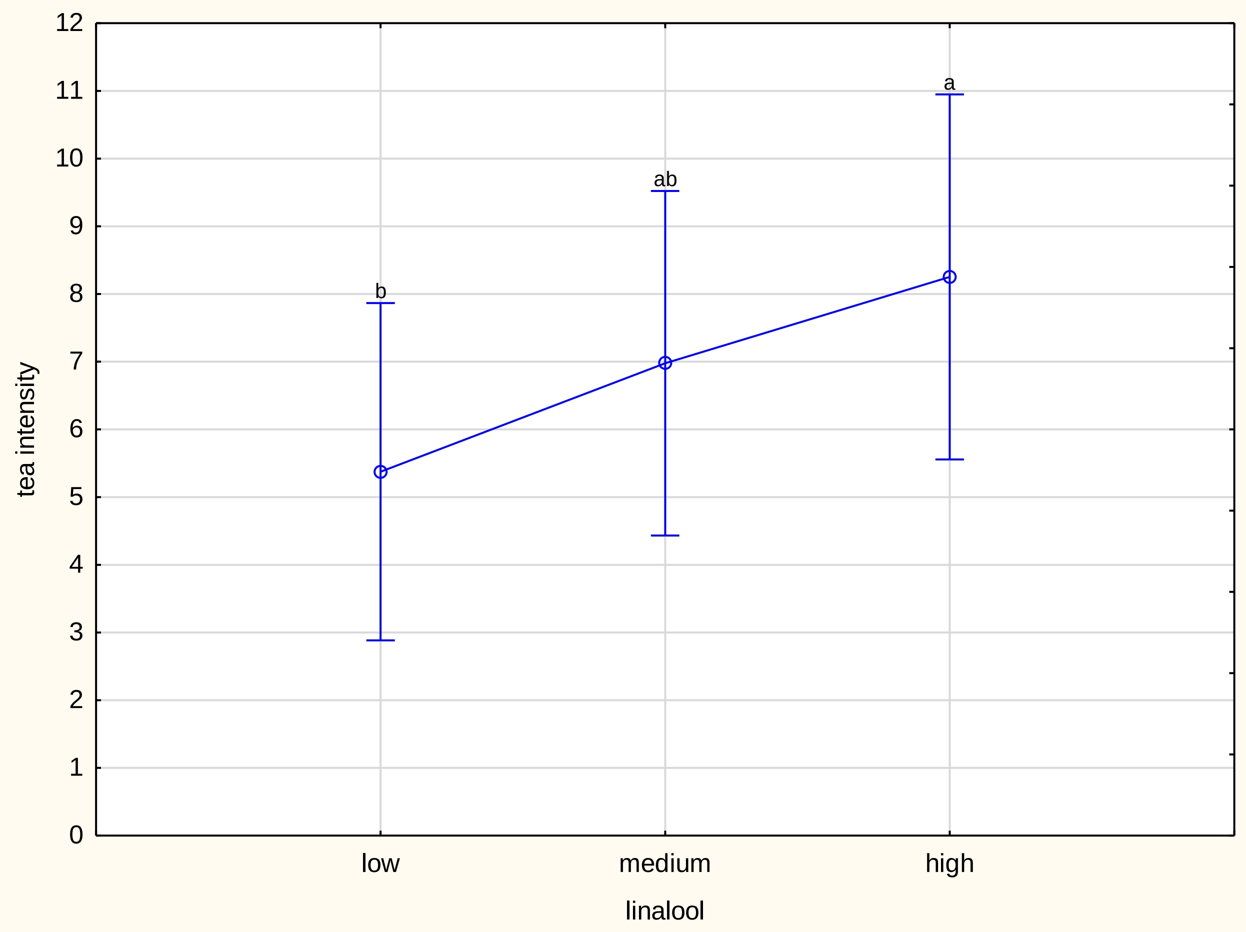

Figure B.10 LS means plot illustrating the linalool level effect on 'tea' aroma intensity for the combinations of compounds with significant letters from LSD post-hoc test. Vertical bars denote 95% confidence intervals.

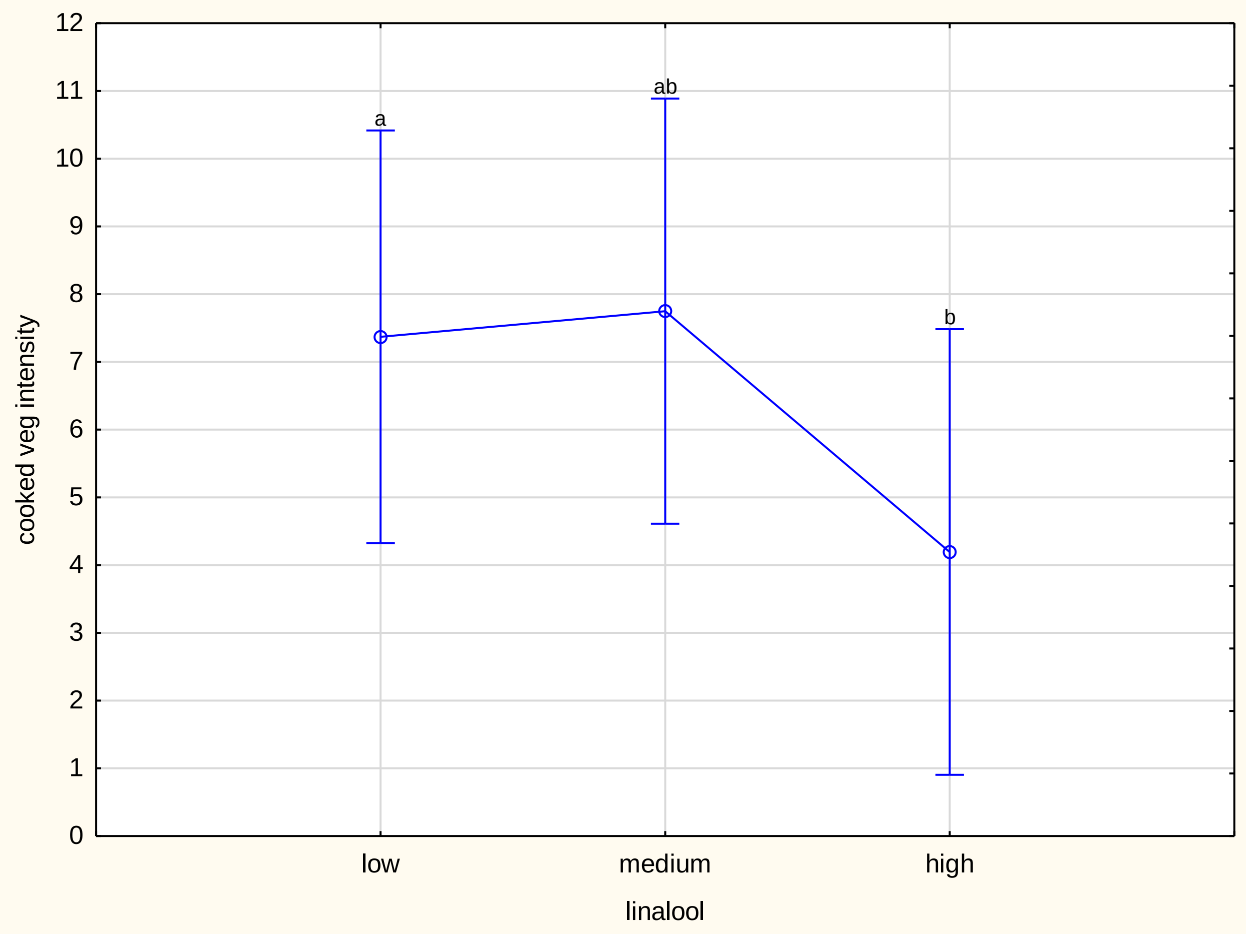

Figure B.11 LS means plot illustrating the linalool level effect on 'cooked veg' aroma intensity for the combinations of compounds with significant letters from LSD post-hoc test. Vertical bars denote 95% confidence intervals.

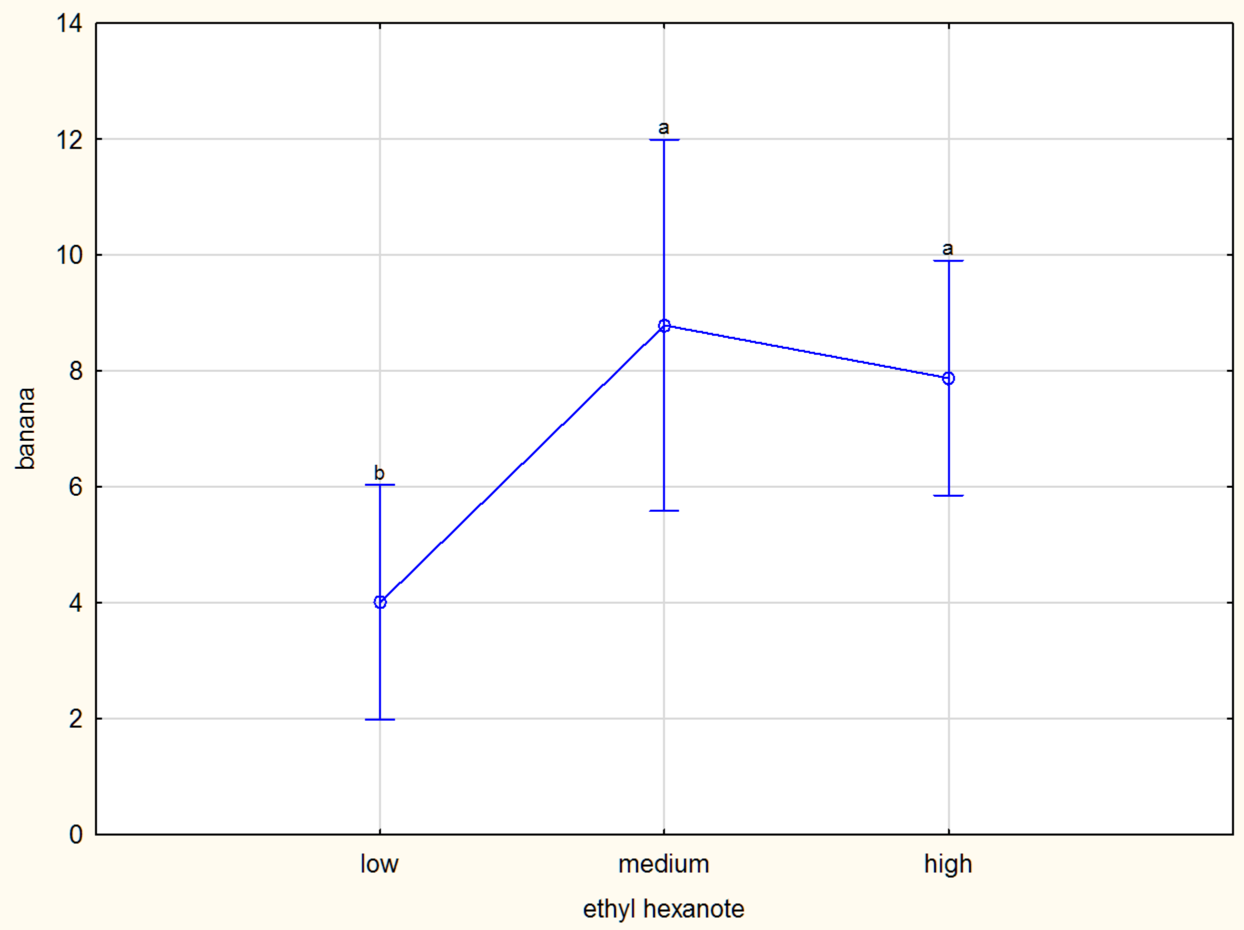

Figure B.12 LS means plot illustrating the ethyl hexanoate level effect on 'banana' aroma intensity for the combinations of compounds with significant letters from LSD post-hoc test. Vertical bars denote 95% confidence intervals.

Chapter 5

Research results

Interaction of 3-mercaptohexan-1-ol (3MH) and 3-mercaptohexyl acetate (3MHA) in different Chenin Blanc matrices by projective mapping (PM) with intensity

Chapter 5 : Interaction of 3-metcaptohexan-1-ol (3MH) and 3-mercaptohexyl acetate (3MHA) in different Chenin Blanc matrices by projective mapping (PM) with intensity

5.1 Introduction

Though both 3-mercaptohexan-1-ol (3MH) and 3-mercaptohexyl acetate (3MHA) are thiols which contribute to the fruity, tropical character of wines, the aroma descriptors used for each compound are not identical. While they both share the descriptions of ‘passion fruit’, ‘grapefruit’, and ‘box tree’ (Tominaga *et al.*, 1996, 1998; Dubourdieu *et al.*, 2006), higher concentrations of 3MHA correlate increasingly with ‘box tree’, while higher levels of 3MH correlate with a greater perceived intensity of ‘passion fruit’ and ‘grapefruit’ aroma (Dubourdieu *et al.*, 2006). Though these two compounds are simple to describe when on their own in model wine, their perceptions could be affected by interactions in real wine. For this reason, interaction studies evaluating the perception of volatile compounds in combination with one another and other wine constituents are important.

In real wine, these volatile thiols are present together with over 1000 other volatile compounds (Polášková *et al.*, 2008). It has been shown that both the non-volatile and volatile components of wine can interact to modify, enhance, or suppress the perception of other volatiles (Francis & Newton, 2005). These interaction effects can be studied by sensory analysis, and some examples of sensory interaction studies involving thiols have been published. Interactions of 3MH with 3-isobutyl-2-methoxypyrazine (IBMP) have been investigated (Van Wyngaard *et al.*, 2014) and extended to include methional and phenylacetaldehyde (Coetzee *et al.*, 2015). One sensory interaction study including more than one thiol was performed by King *et al.* (2011), which investigated combinations of different thiols, esters, and pyrazines. These studies all focussed on Sauvignon Blanc wines except for the interaction of 3MH with linalool and ethyl hexanoate which was presented In Chapter 4 of this thesis.

In wine, thiols are not only in solution with other aroma compounds, but they are also in contact with non-volatile components such as phenolic compounds and proteins (Pozo-Bayón & Reineccius, 2009). The combined interaction effects of volatile and non-volatile compounds on the overall perception of a product is referred to as the “matrix effect”. The matrix effect has been shown to be important to the perception of wine aroma, with glucose and ethanol (Robinson *et al.*, 2009) and phenolics (Lund *et al.*, 2009) affecting the volatility of aroma compounds, along with proteins and polysaccharides (Pozo-Bayón & Reineccius, 2009). Volatile compound interaction studies have been performed in a variety of matrices, including model wine (Coetzee *et al.*, 2015), dearomatized wine (Van Wyngaard *et al.*, 2014), and neutral base wine (King *et al.*, 2011). However, little research has compared the potentially important effect of using different matrices in such studies.

In Chapter 4, an interaction study was presented using descriptive analysis (DA). Because DA necessitated many costly training sessions, there was subsequently an interest to evaluate whether similar types of studies can be performed using rapid sensory methods, such as projective mapping (PM). In interaction studies, the important differences between samples are usually subtle variations in intensity of descriptors, and DA is the recommended sensory method for products with small differences (Lawless & Heymann, 2010). However, modifications to existing rapid methods to include intensity information could make these rapid methods more suited to interaction studies.

One of the drawbacks of the PM method is that, unless paired with an ultra flash profile (UFP), it only provides discriminatory information, not descriptive information. Even when PM is paired with UFP, the descriptive data generated is in the form of a contingency table, which shows the frequency of usage of each descriptor, but not their relative intensities. This intensity information is especially important for products which are all described with the same terms, but differences between products lie in the intensity of those descriptors. Typically, in order to obtain intensity information, DA must be performed. One modification that could be applied to rapid methods to address this issue is the addition of an intensity rating, as was done to create the rate-all-that-apply (RATA) method from the check-all-that-apply method (CATA) (Ares *et al.*, 2014). In this study, an intensity rating was added to the UFP portion of a PM experiment. This could allow for a more detailed understanding of the data than provided by a standard UFP.

In this study, three separate projective mapping experiments with combinations of different levels of 3MH and 3MHA in different matrices were performed. The three experiments used three different matrices to explore the effect of the matrix on the perception of the compounds. Model wine, partially dearomatized wine, and commercial wines were used. The aims of this study are threefold: 1) to study the interaction of 3MH and 3MHA at the levels found in South African Chenin Blanc wines, 2) to study the effect of spiking matrix on perception of the thiols, and 3) to assess whether the projective mapping method can be applied to aroma interaction studies by including descriptor intensity ratings.

5.2 Materials and methods

5.2.1 Experimental design

Three different partial projective mapping (PM) sessions were performed by the same judges following the same instructions, but on a varying sample set. Each evaluation was performed with a one- or two-day break between sessions. In the first two evaluations, the samples consisted of thiols spiked into different matrices, and in the third evaluation, a set of commercial wines with similar thiol content to the spiking levels was evaluated. In order of increasing matrix complexity, the judges evaluated spiked model wine, spiked partially-dearomatized Chenin Blanc wine, and non-spiked commercial Chenin Blanc wine.

In the first two evaluations where thiols were spiked into a model wine and a dearomatized wine, 3MH and 3MHA were added in combination at different concentrations using a full factorial design. Results of thiol analysis of 63 South African Chenin Blanc wines (Chapter 3) were used to select the 3MHA and 3MH levels used for spiking. A high, medium, and a low level of each compound was selected (Table 5.1). The concentration of the stock 3MH and 3MHA solutions used for spiking were quantified by Ellman's reagent prior to dilution (Ellman, 1959). Dilutions of these stock solutions were prepared in 99.8% v/v ethanol (Sigma-Aldrich, St. Louis, MO) and stored at -80°C for one week before spiking.

Table 5.1 Levels and coding of the full factorial design

Treatment Code	Factors		Levels (ng/L)	
	3MHA	3MH	3MHA	3MH
1A1H	1 (low)	1 (low)	0	600
1A2H	1 (low)	2 (med)	0	1200
1A3H	1 (low)	3 (high)	0	1800
2A1H	2 (med)	1 (low)	75	600
2A2H	2 (med)	2 (med)	75	1200
2A3H	2 (med)	3 (high)	75	1800
3A1H	3 (high)	1 (low)	150	600
3A2H	3 (high)	2 (med)	150	1200
3A3H	3 (high)	3 (high)	150	1800

5.2.2 Samples

Model wine

For the first evaluation, model wine (distilled water, 12% EtOH, 5 g/L tartaric acid (Everywine, Stellenbosch, South Africa), adjusted to pH 3.5 with sodium hydroxide (Merck, ≥ 97.0%) was used as the base matrix. This model wine was spiked with three levels of 3MH and 3MHA in a full factorial design, giving a total of 9 samples (Table 5.1). Three samples, 1A1H, 2A2H, and 3A3H, served as blind duplicates in the projective mapping exercise, so 12 samples were evaluated in the sensory analysis. All wines were spiked 12 hours prior to testing to allow for thorough mixing and integration of the volatiles into the wine matrix. The spiked model wine was stored at 4°C for 10 hours, and allowed to reach room temperature before sensory evaluation.

Partially-dearomatized wine

For the second evaluation, a partially-dearomatized Chenin Blanc was obtained through aroma dilution. An unwooded commercially-available Chenin Blanc wine was dearomatized with 5 g/L of activated charcoal powder (Merck, Darmstadt, Germany). It was allowed 8 hours of contact time without agitation, then filtered by diatomaceous earth filtration. The charcoal-treated wine was blended with untreated wine at a ratio of 4:1 to create a partially-dearomatized base wine. The blending ratio was chosen in a screening session by three experts to create a neutral wine with low aromatic intensity. Thiol levels of the charcoal-treated and commercial wines were measured using the method described in Chapter 3, Section 3.2.2. From this analysis, the base thiol levels of the partially-dearomatized wine were calculated: 612 ng/L 3MH and 0 ng/L 3MHA. Taking this base level of 612 ng/L of 3MH into account, the partially-dearomatized wines were spiked to the same final levels in the same design as in Table 5.1. The same spiking and storage procedure was used as with model wine.

Commercial wine

For the third evaluation, a subset of 10 commercial 100% Chenin Blanc samples was selected from the thiol analysis results (Chapter 3). These wines were chosen to fit a similar range of 3MH and 3MHA levels as the spiked wines. The selected wines were all 1-year old, unwooded wines.

Each bottle was confirmed as free of cork taint and *Brettanomyces*-related off-aromas sensorially by the researcher.

5.2.3 Sensory evaluation

Partial projective mapping with intensity

For all three evaluations, the same sensory methodology was followed. A partial PM exercise was performed where the panellists were restricted to evaluating a single modality, namely aroma. In order to include descriptive information, the PM method was paired with ultra flash profiling (UFP). The PM and UFP were performed simultaneously. The UFP procedure was modified to include intensity ratings of the descriptors, as is done in the rate-all-that-apply (RATA) rapid method (Ares *et al.*, 2014). A three-point scale ("low", "medium", and "high ") was used to rate each descriptor's intensity, making the UFP more of an "ultra flash *intensity* profiling".

Wines were poured in 20 ± 2 mL aliquots one hour before serving into black glasses (ISO 3591:1977) and covered with plastic Petri dish lids. Each glass was labelled with a unique, random 3-digit code. All evaluations took place in off-white individual sensory booths in a quiet, well-ventilated, odourless 20 ± 2 °C air-conditioned room (ISO 8589:2007). Three blind duplicates were included in each spiking experiment, and two were included in the evaluation of commercial wine, giving 12 total samples in each evaluation, as recommended in Pagès (2005). The 12 samples were presented in a different randomized order for each judge according to a Williams Latin Square design (Macfie *et al.*, 1989).

Panellists received visual and written instructions for the PM with intensity procedure (Appendix C). Judges were instructed to smell the samples from left to right and for each wine, generate 3-5 descriptors by free description, and record them on a Post-it® note along with the "low", "medium", or "high" descriptor intensity ratings. Panellists were unaided and unrestricted in their selection of sensory attributes. They arranged the samples on an A2 (40 cm x 60 cm) white sheet of paper according to their degree of similarity or dissimilarity, with similar samples being located close to one another, and dissimilar samples located far from one another. Each judge was encouraged to use their own criteria to arrange the samples. It was emphasized that there was no correct way to arrange the samples, as the exercise is free-form by nature. Two replications of each evaluation were performed with a 10-minute break between each replication to avoid sensory fatigue. The three separate tastings took place within one week with a one- or two-day break between tastings.

Panellists

The same panel of fifteen judges aged 22-41 years (6 males, 9 females) was used for all three tastings. The judges were students and staff of the Department of Viticulture and Oenology at Stellenbosch University, and were recruited based on their willingness to participate and previous experience evaluating South African Chenin Blanc. Eleven of the panellists were already familiar with the PM procedure.

Data capturing and treatment

The placement of each product was measured manually as an (X,Y) distance coordinate considering the bottom-left corner of the paper as the origin. The descriptors were recorded with their intensity ratings, and only linguistic synonyms were combined during the initial data collection. These descriptors were further condensed by the researchers. Condensing of the descriptors was performed separately for each experiment, with care taken to group terms as consistently as possible between the different experiments. For each tasting, descriptors used by fewer than 20% of the judges (citation frequency < 3) were combined with a similar descriptor, if available, or removed from the data set (Campo *et al.*, 2008). To improve the objectivity of decisions made, this consolidation was done in consultation with another experimenter, and when possible, the judges themselves.

5.2.4 Statistical analysis

To obtain a map of the product distribution, Multiple Factor Analysis (MFA) was performed on the results of each tasting. This analysis was run in the open-source statistical language R (R Core Team, 2015) using the function “MFA()” from the FactoMineR package (Lê *et al.*, 2008). In the construction of the MFA, descriptors were treated as quantitative supplementary variables, and projected onto the map of the MFA rather than contributing to the construction of the dimensions. The product coordinates were not scaled to unit variance, and the default of five dimensions were kept in the model. Each rep of each judge was treated as a separate table of variables in the MFA. Following the code published in Dehlholm *et al.* (2012) 95% confidence around the samples were constructed using the SensoMineR package in R (Lê & Husson, 2008). See Appendix C for an example of the R code used. RV coefficients between the model wine MFA and partially-dearomatized MFA scores plot coordinates were calculated using XLSTAT (Version 18.06, Addinsoft).

5.5 Results and discussion

5.5.1 Model wine

The effect of the volatile and non-volatile composition of the matrix can be assessed by changing the spiking base used. In the first tasting, model wine was used as the most compositionally simple base. This simplicity can be seen as an advantage or a disadvantage. On one hand, this matrix is reproducible and therefore easy to utilize in scientific experiments. Additionally, interaction with other compounds is eliminated, allowing for consistency and ease of interpretation. The disadvantage of using model wine is that it may be an over-simplification, as some interactions may be important to the perception of the volatiles in real wine.

In the MFA of the PM for the spiked model wine, the three pairs of blind duplicates (1A1H-1/-2, 2A2H-1/-2, and 3A3H-1/-2) grouped together within the confidence ellipses (Figure 5.1). Though the MFA explained 34.7% of the variance in the data, this relatively low percent explained variance was to be expected since the differences between samples were subtle (Figure 5.1). Though the explained variance was low, the groupings still followed a logical trend, which was supported by the descriptors. Along dimension 1, the low-3MHA wines were located in the positive direction, and the high-3MHA wines were grouped together in the negative direction. There was no visible trend for 3MH, indicating that 3MHA was a more important factor driving the differences in wine aroma among these samples. This was supported by the fact that the 1A1H and 1A3H samples grouped together, as well as the 3A1H and 3A3H samples.

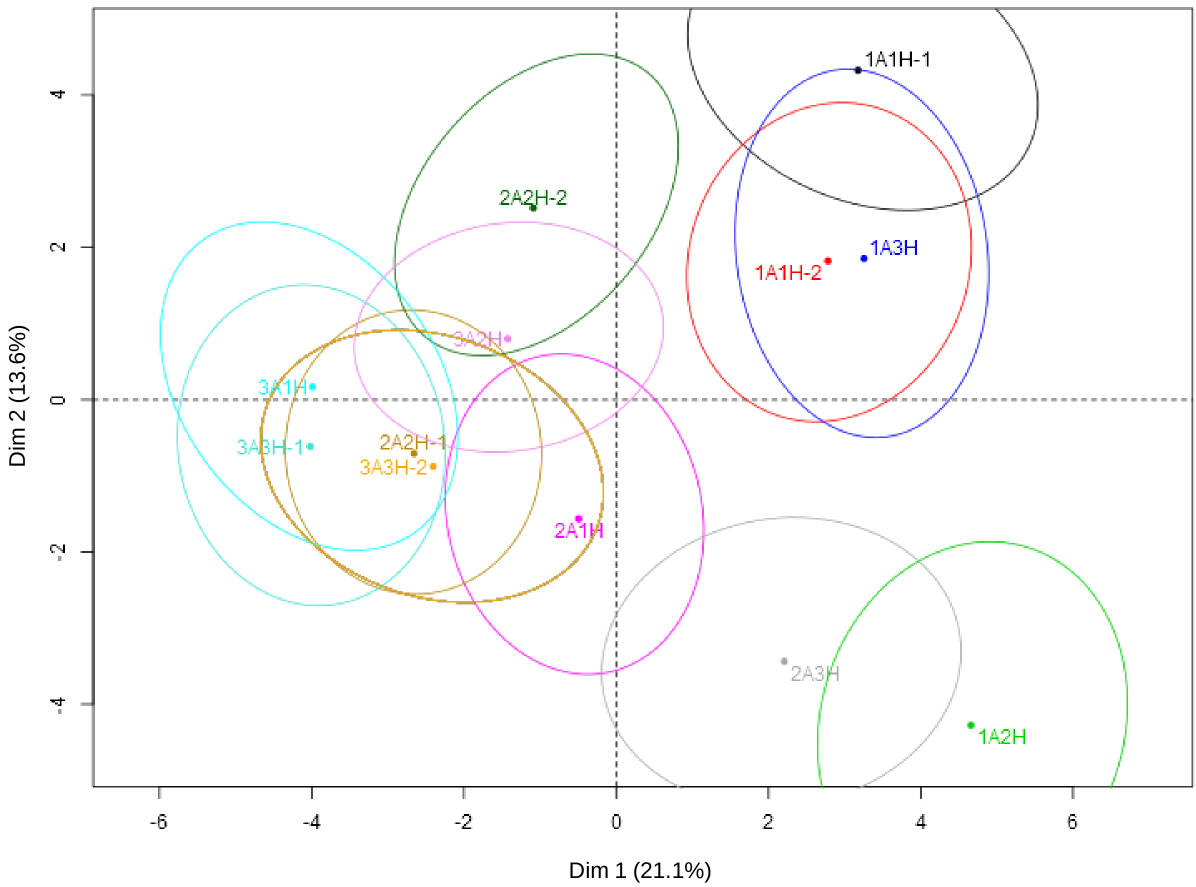

Figure 5.1 MFA scores plot of PM data for thiols spiked into model wine. Codes correspond to low (1), medium (2), and high (3) levels of 3MHA (A) and 3MH (H). Codes ending in -1 or -2 are blind duplicates. The 95% confidence ellipses were created according to Dehlholm *et al.* (2012).

A total of 111 descriptors were consolidated down to 28 following the rules previously described. In the loadings plot of the MFA, the right side of the plot correlates with ‘alcohol’, and ‘solvent’, (Figure 5.2) which describe the low-3MHA wines in the scores plot (Figure 5.1) In these low-3MHA wines, high intensity of the solvent-like character of the model wine was the dominating character. On the other hand, the attributes associated with the high-3MHA wines were ‘fresh green/herbaceous’, and ‘tomato leaf’, as well as ‘guava’, ‘passion fruit’, ‘pineapple’, ‘sweaty’, and ‘tropical’ (Figure 5.2). Except for ‘pineapple’, these descriptors are a mixture of more classical thiol-related terms, as well the green and ‘guava’ characters published more recently (King *et al.*, 2011; Van Wyngaard *et al.*, 2014; Coetzee *et al.*, 2015), as noted in Chapter 4. The descriptor ‘grapefruit’ it not as highly-associated with the high-3MHA wines as the other descriptors mentioned. Unlike other studies where ‘box tree’ is associated with 3MHA, this descriptor was not cited in this experiment, most likely due to cultural unfamiliarity.

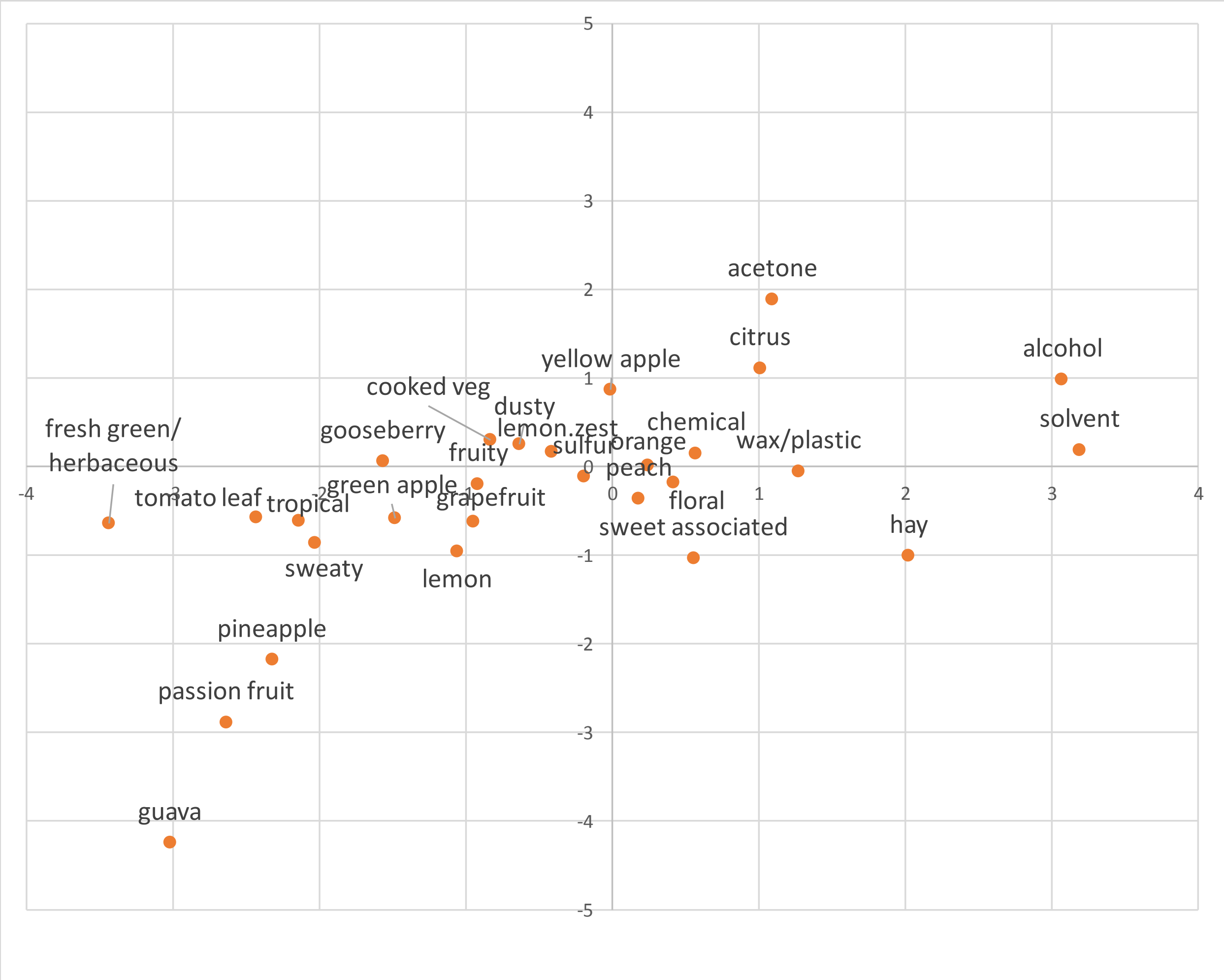

Figure 5.2 MFA loadings plot of PM data for thiols spiked into model wine. Descriptors are weighted by intensity.

5.5.2 Partially-dearomatized wine

The second matrix, partially-dearomatized wine, has the disadvantage of not being reproducible, but it is more representative of a real wine matrix. Though the complexity of this base means it is impossible to identify and explain all the reactions and interactions which take place, when trying to model a real-wine situation allowing these reactions to take place is important. The charcoal treatment was used to create a wine with neutral aroma, and low levels of thiols. Considering the thiol levels of the base wine, the thiols were spiked to match the same levels used in the previous section.

In the MFA of the PM on spiked partially-dearomatized wine, 32.1% explained variance was represented (Figure 5.3), which was similar to the previous PM on the spiked model wine. The low and the medium-level blind duplicates (1A1H-1/-2 and 2A2H-1/-2) grouped together, but the high-level blind duplicates (3A3H-1/-2) did not. In fact, one of the 3A3H samples grouped with one of the 1A1H samples. There was a general trend from low-thiol wines in the bottom-left corner of the graph to higher-thiol wines in the upper-left quadrant of the MFA. However, it seemed that these samples were more difficult for assessors to differentiate, as the groupings in partially-dearomatized wine were less clear than in model wine. This was to be expected since it was a more aromatically complex system. The RV coefficient calculated between the coordinates of the model wine and partially-dearomatized wine MFA scores plots also showed disagreement between the two different matrices with a very low RV coefficient of 0.243. This poor agreement illustrates the importance of the matrix to sensory perception. The presence of other aroma compounds may have obscured some of the differences in 3MHA levels, which were more apparent in model wine. On the other hand, differences in 3MH levels may have been more important in this partially-dearomatized solution, as both thiols appeared to drive the groupings and the low-3MH samples grouped more closely than in the previous experiment.

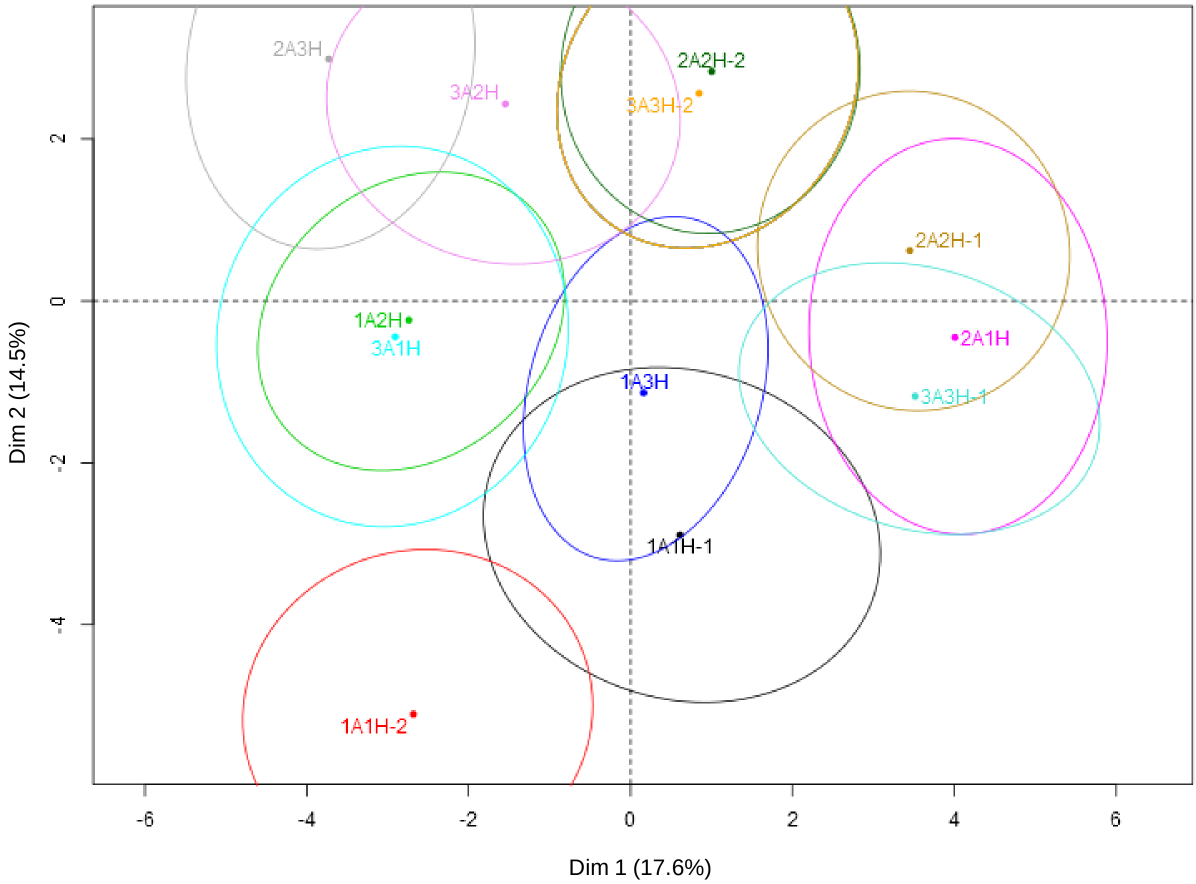

Figure 5.3 MFA scores plot of PM data for thiols spiked into partially-dearomatized wine. Codes correspond to low (1), medium (2), and high (3) levels of 3MHA (A) and 3MH (H). Codes ending in -1 or -2 are blind duplicates. The 95% confidence ellipses were created according to Dehlholm *et al.* (2012).

A larger number of discrete descriptors were generated for this more complex matrix, with 102 condensed down to 40. Though the sample grouping was less defined than in the model wine, the general trend seen in the product arrangement (Figure 5.3) was supported by the descriptors (Figure 5.4). The descriptors which best described the higher-thiol wines were ‘gooseberry’, ‘tropical’, ‘guava’, ‘green apple’, and ‘passion fruit’. In this matrix, the green attributes ‘tomato leaf’ and ‘fresh green/herbaceous’ were less discriminating than in the model wine. Additionally, rather than opposing *solvent-like* terms, the thiol-related descriptors opposed generally *sweet* terms, such as ‘floral’, ‘peach’, ‘banana’, ‘marmalade’, and ‘banana’. Since these attributes were not present in the spiked model wine, they came from other classes of the compounds present in the partially-dearomatized base wine. The opposition of ‘floral’ and ‘peach’ with the thiol-related descriptors agrees with the results of the interaction study in Chapter 4.

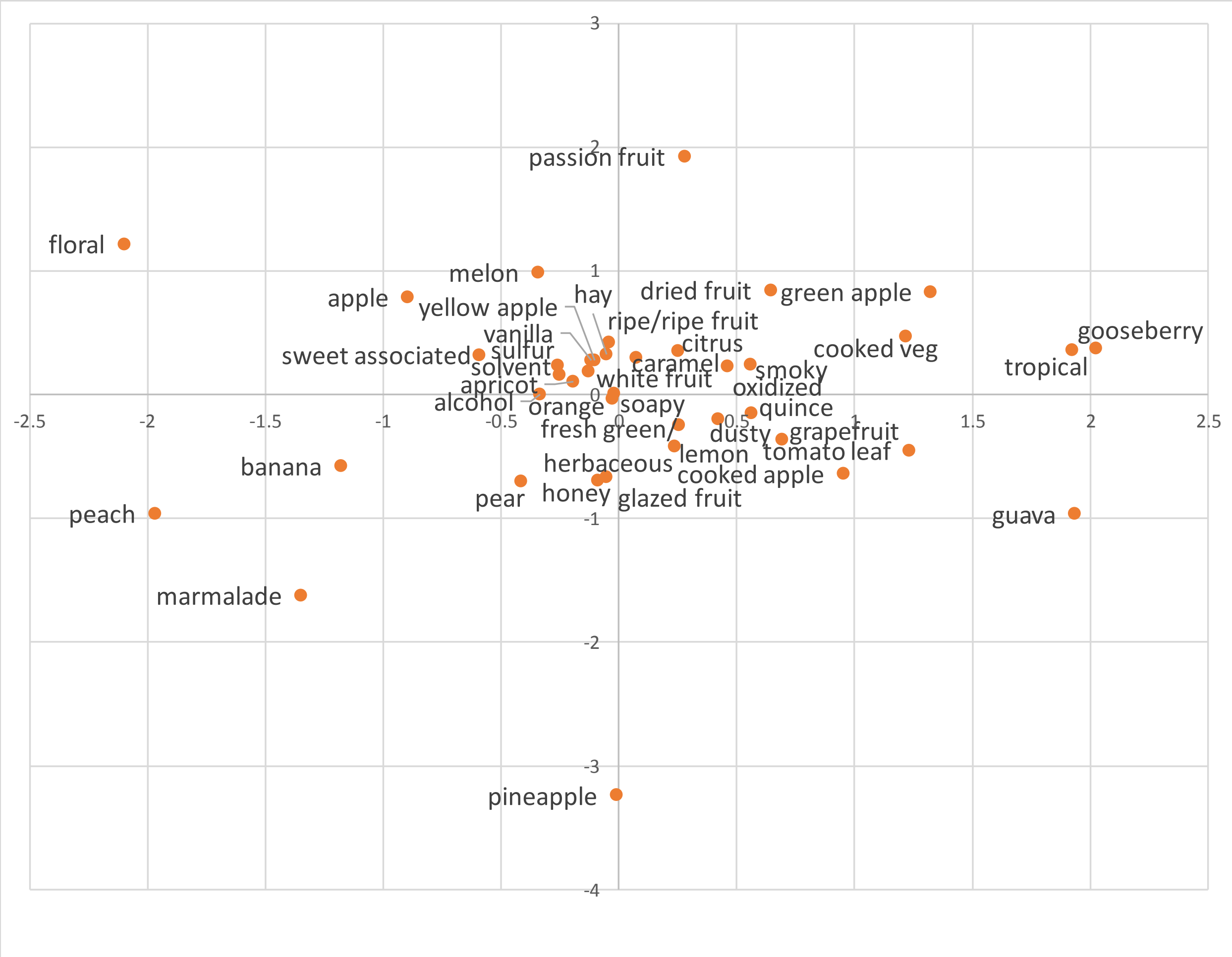

Figure 5.4 MFA loadings plot of PM data for thiols spiked into partially-dearomatized wine. Descriptors are weighted by intensity.

5.5.3 Commercial wine

In the third experiment, commercial wines with 3MHA and 3MH levels within the ranges used in the spiking experiments were assessed. All the wines were unwooded to avoid the dominant wood-derived aromas driving the sensory discrimination. As a matrix, commercial wines have the advantage of being a real-life model. The difficulty in using this matrix is due to the unique non-volatile and volatile composition of each wine. It is not a controlled solution, and the levels of thiols are not the only variables which differ from sample to sample.

The MFA of these commercial wines explained 36.2% of the variation in the data set (Figure 5.5). This percentage, though slightly higher that found in the other two evaluations, was still relatively low. The two blind duplicates (SP and DT) grouped well, showing that the panel was able to differentiate between the wines. Thus, the low explained variance showed that differences in aroma between unwooded Chenin Blanc wines were relatively subtle. A few more descriptors after condensing (44 condensed from 132) were obtained for the commercial wine than the partially dearomatized matrix.

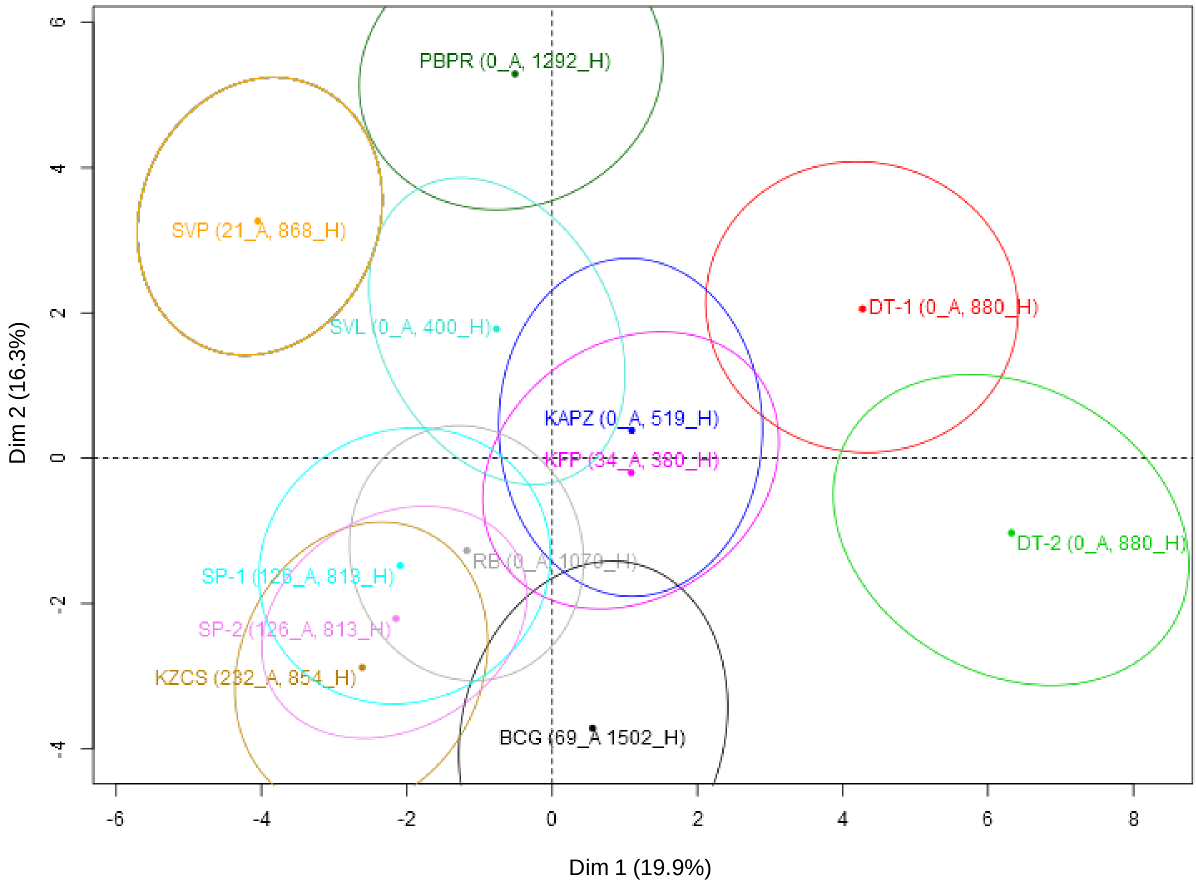

Figure 5.5 MFA scores plot of PM data for commercial Chenin Blanc wines with 3MH and 3MHA levels within the range used in the spiking experiments. Codes include 3MHA (_A) and 3MH (_H) levels, as listed in Table 5.2. Codes ending in -1 or -2 are blind duplicates. The 95% confidence ellipses were created according to Dehlholm *et al.* (2012).

Referring to the thiol results of the wines, they ranged from 0 ng/L – 232 ng/L 3MHA, and 380 ng/L – 1502 ng/L 3MH. (Table 5.2), RB, BCG, and PBPR had the highest 3MH levels, while KZCS, SP, and BCG had the highest 3MHA levels. Other than PBPR, these wines were all clustered together in the negative quadrant of dimensions 1 and 2 in the MFA (Figure 5.5). As in the partially-dearomatized matrix, these high-thiol wines were not separated into high-3MHA *vs*. high-3MH groups, but rather were all grouped together. The wines in this group were most frequently described as 'pineapple', as well as the thiol-related attributes 'passion fruit' and 'guava' (Figure 5.6). PBPR did not group with the rest of the high-thiol wines. Looking at the raw frequency of descriptor citations for this wine, it was described with the attributes 'high passion fruit' and 'high grapefruit', similar to the high-thiol group. However, it was also described as 'high floral', which may have driven its separation from the other high-thiol wines (Figure 5.6). The wines with the lowest thiol levels (SVL, KAPZ, and KFP) were clustered in the middle of the MFA, though their confidence ellipses overlapped slightly with some of the high-thiol wines. The wine which was most opposed to the high-thiol cluster, DT, was described as 'oaky/wooded', 'honey', and 'sweet associated', though interestingly this wine was vinified in stainless steel. Again, the thiol-related descriptors oppose *sweet* descriptors like 'honey' and 'marmalade', as in the partially-dearomatized wine. Additionally, for the commercial wine the *wood-related* terms like 'toasted wood' and 'vanilla' also oppose the thiol-related terms.

Table 5.2 3MHA and 3MH levels of commercial wine samples used in the third tasting

Wine Code	3MHA (ng/L)	3MH (ng/L)
KZCS	232	854
KFP	34	380
SP*	126	813
RB	0	1070
BCG	69	1502
DT*	0	880
PBPR	0	1292
SVL	0	400
SVP	21	868
KAPZ	0	519

* Were used as blind duplicates

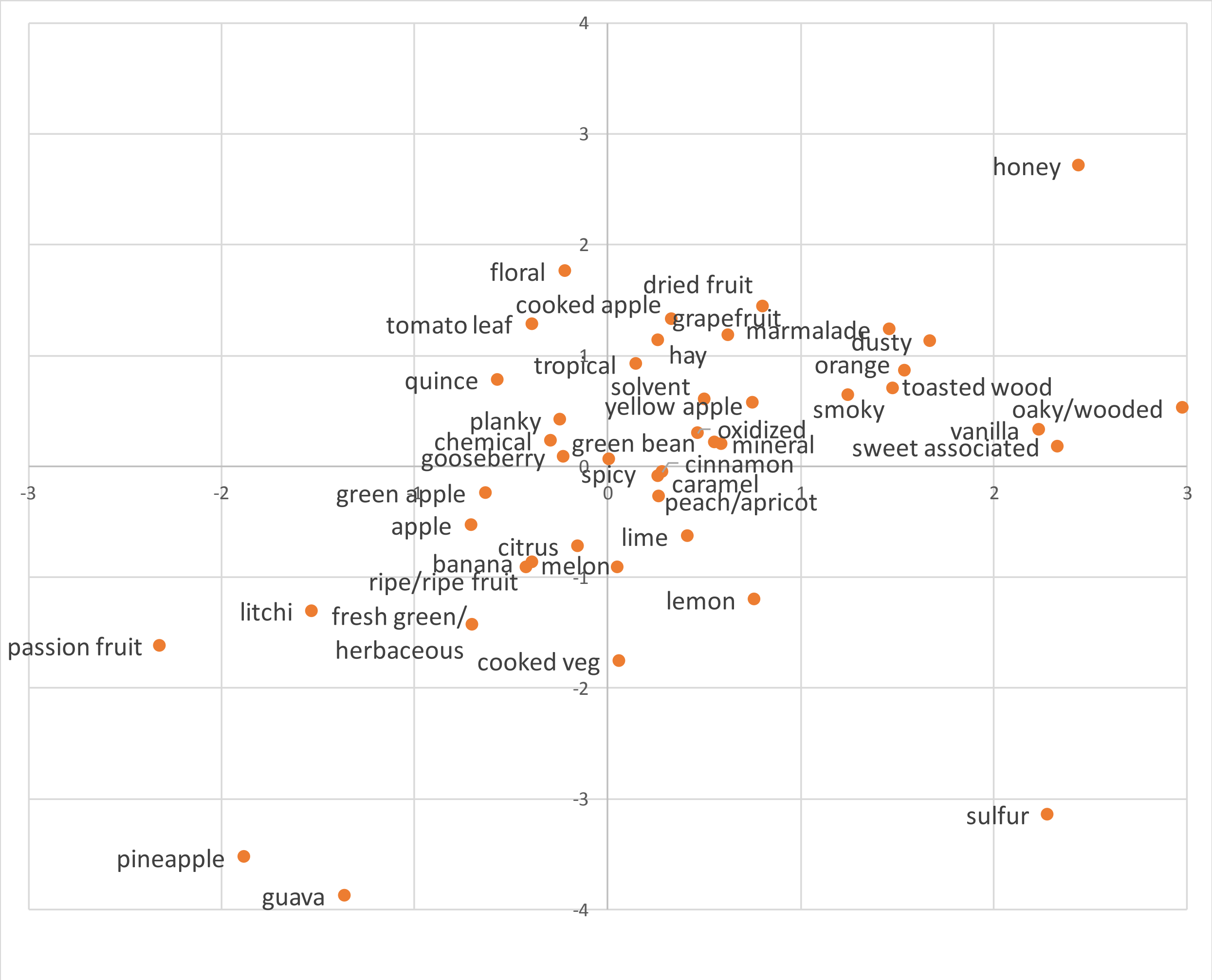

Figure 5.6 MFA loadings plot of PM data for commercial Chenin Blanc wines with 3MH and 3MHA levels within the range used in the spiking experiments. Descriptors are weighted by intensity.

Overall, the groupings in the MFA corresponded with a high-thiol cluster, opposed by ‘floral’ wines on dimension 2 and an ‘oaky/wooded’, ‘sweet associated’ wine on dimension 1. It appeared that within this group of unwooded Chenin Blanc wines, thiols were an important driver of one of the groupings, but other aroma compounds were responsible for causing PBPR and DT to be perceived differently.

5.5.4 Loadings plot methodology

As intensity ratings had not been previously used with the PM method, a discussion of different ways to represent the descriptive data is warranted. One option explored was representing intensities qualitatively with the words “low”, “medium”, or “high” attached to each descriptor. In this way, each intensity of each descriptor was a separate variable and the frequency of each intensity citation was used to project the descriptors onto the loadings plot. Using the results from the spiked model wine, this method resulted in an over-crowded loadings plot (Figure 5.7). The spiked model wine experiment generated fewer descriptors than the other two experiments, yet the plot is not easily readable and only the most discriminating descriptors are visible. The detail in this version of the loadings plot was still useful when interpreting the data, as it was possible to observe the intensities associated with each position. For example, in Figure 5.7 the ‘passion fruit high’ descriptor opposed the ‘passion fruit low’ descriptor, which confirmed that the products span the range of ‘passion fruit’ intensities. On the other hand, the three intensities of ‘fresh grass/herbaceous’ were more closely located and all associated with the high-thiol wines. This representation could work better for a less-complex product with fewer descriptors, but for this data set this method was not useful for a clear presentation of the data.

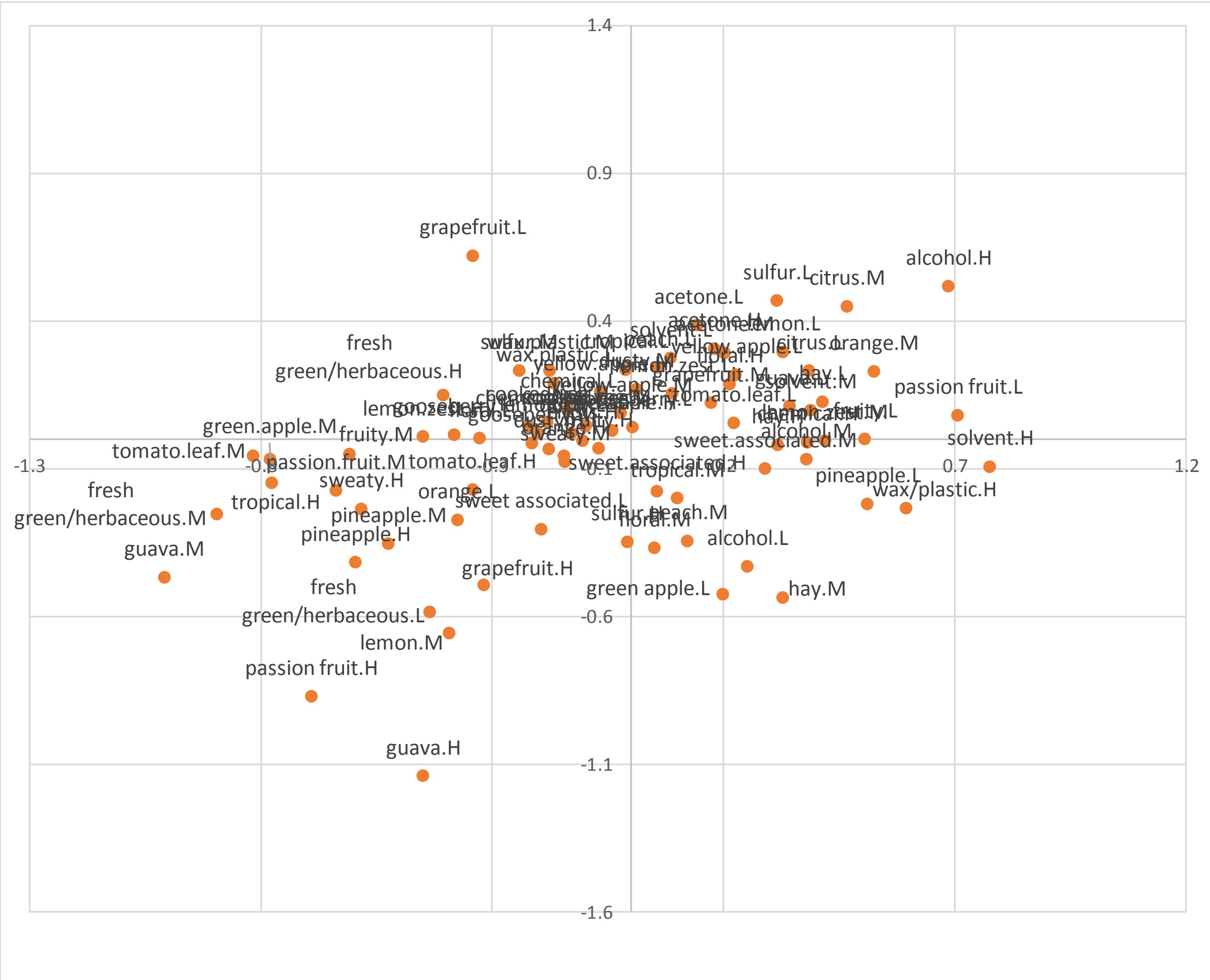

Figure 5.7 Loadings plot of the spiked model wine data with each the low (L), medium (M), and high (H) intensities of each descriptor treated as a separate variable. This plot illustrates the overcrowded result of this method.

Another option was translating the qualitative data into quantitative data by assigning a value to each intensity. In this way, each descriptor was weighted according to the intensity rating. In order to assign weights to the data, the procedure used in the RATA method by Ares *et al.* (2014) was followed, where "low"=1, "medium"=2, and "high"=3. This second method yields a much more readable plot (Figure 5.8). Compared to the locations of the descriptors in Figure 5.7, the terms in Figure 5.8 are drawn toward the position of the 'high' intensity citation due to the weighting effect. This method does simplify the data, but did not greatly change the descriptor placements. This method also represents the intensity information in an easily-interpretable way. Overall, this method yields a more illustrative plot, but since combines the intensity data some information is lost. For example, whether the weights for a certain descriptor are driven more by 'medium' or 'high' intensity citations would not be visible from the plot. This limitation can be addressed by consulting the raw data for more in-depth analysis of intensities.

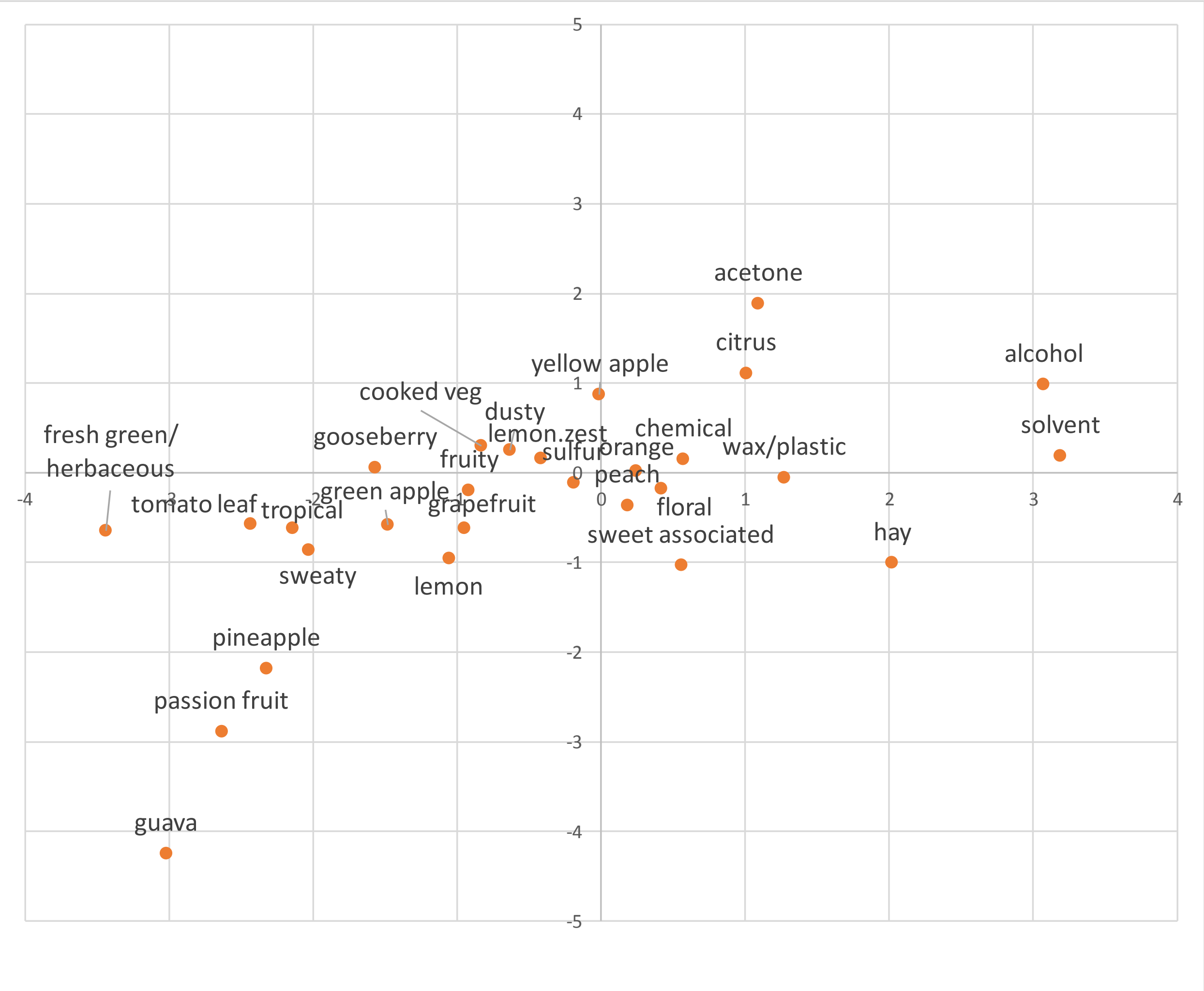

Figure 5.8 Loadings plot of the spiked model wine data with each descriptor weighted by intensity.

5.6 Conclusions

When performing interaction studies, it is important to consider the goal of the experiment when selecting a base matrix If the goal is simply to explain the fundamental sensory interaction between the compounds, then model wine is appropriate. If the goal is to explain the behaviour of these compounds together in wine, then perhaps a dearomatized wine is a more suitable matrix.

Unfortunately, the ideal matrix which would be both controlled yet realistic does not exist. The closest method that exists is aroma reconstitution, where representative levels of aroma compounds are added to a dearomatized wine (Mateo-Vivaracho *et al.*, 2010; Benkwitz *et al.*, 2012; Lytra *et al.*, 2016) or wine extract (Ferreira *et al.*, 2016). However, this technique needs further development in terms of base matrix preparation method. Additionally, the selection of the compounds used for reconstitution and their concentrations requires a large body of available data, which may not be practical in all situations.

In this case, though the model wine is not the most realistic matrix it did allow for easier differentiation between samples by the assessors. This experiment allowed us to see that at the selected levels, when analysed in a simple model wine solution, the strong solvent-like aroma of this matrix which was apparent in the low-3MHA samples may have actually dominated some of the more subtle differences due to 3MH levels. In this matrix, 3MHA level was a more powerful driver of sensory difference than 3MH level, which agrees with the results of King *et al.* (2011). In combination with other volatile and non-volatile components, however, this trend did not hold true. In the partially-dearomatized wine, there was a general continuum from low- to high-thiol wines, with both 3MHA and 3MH driving the differences between wines. A similar differentiation was seen for the commercial wines, with high-thiol and low-thiol wines forming clusters, and other compounds likely driving additional separation. These different conclusions illustrate the importance of considering the effect of matrix in interaction studies, as trends that are clear in simplistic base solutions may or may not be relevant to a real wine. One result which was the same in all three matrices was the description of high-thiol wines as ‘guava’ and ‘passion fruit’, which serves as further evidence that thiols could be responsible for the ‘guava’ aroma of South African Chenin Blanc wines.

Though PM is not the ideal technique for sample sets with subtle differences, some useful conclusions could be drawn from this work. The addition of an intensity rating allowed the MFA loadings plots to not only associate descriptors with the groupings, but better represent the importance of each descriptor in discriminating between products. This allowed for a more in-depth analysis of the data than is possible with traditional UFP.

Additionally, the data was not detailed enough to illustrate specific enhancing or suppressing effects of the two thiols, other than a suppressing effect on the perception of ethanol in model wine. As a suggested improvement, the intensities could have been of greater use if fewer attributes had been assessed. In the future, a list of suggested descriptors could be generated from previous experiments or a screening session as performed in free choice profile. With a full DA, detailed information about descriptor intensities could have allowed a more in-depth analysis and included significant differences between the samples. For the case of this experiment where two thiols which have relatively similar ‘tropical’ aromas were used, the differences between samples was particularly subtle and DA would have been a better choice. Though PM cannot replace DA for interaction studies, it did prove to be of use, especially with the addition of an intensity rating. It could be a helpful accompaniment to descriptive analysis, or substituted for DA in situations where a more general analysis of the samples is sufficient.

5.7 References

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Appendix C

Projective mapping (PM) instructions and R script

Appendix C. : Projective mapping (PM) Instructions and R script

Napping Instructions

You will be evaluating the wines in front of you according to the **similarities** and **dissimilarities** in their **AROMA only**.

You will be asked to mark the positions of the wines on the sheet in front of you so that wines you perceive as **similar** wines are **near (close to each other)**, and wines you perceive as **different** are **distant (far from each other).**

- Write the 3-digit code of the wine on a post-it. **Smell** the wine and write down **3-5 descriptors per wine** on the post-it.
    - **PLEASE categorize each descriptor as either LOW, MEDIUM, or HIGH intensity**
- Place the wine glass and post-it on the provided paper.
- Write the next wine's code on a new post-it, smell the wine, record your descriptors and place the glass/post-it on the paper according to how similar or dissimilar it is to the other wine.
- You can move the wines/post-its around as you smell each sample and relate them to one another. You can smell the wines and revise your descriptors as many times as you wish.
- Please use the **whole sheet** to express your opinion in differences among the wines, but stay within the borders of the paper.
- When you are finished, underneath each glass mark an **X** on the paper, write down the 3-digit code of the wine, and stick the post-it next to it.
- Use as much time as you need.
- There are no right or wrong answers. It is important that you use your own criteria to arrange the samples.
- Please do not comment on the wines or discuss them with other panellists.
- Please take a 10-minute break before evaluating the second set of wines.

Have fun, and thank you for your participation!

```
#Projective Mapping R Script

library(FactoMineR)

res.Napping<-
MFA(Napping,group=c(rep(2,30),53),num.group.sup=31,type=c(rep("c",31)),axes=c(1,
2))

library(SensoMineR)

#For Dehlholm(2012) ellipses

MFAresob<-res.Napping

# The number of samples (n)
n = dim(MFAresob$ind$coord)[1]
# The number of groups of variables (m)
m = dim(MFAresob$group$coord)[1]
# Creating a new data frame with one row for each sample's assiociated MFA group
of variables.
CATnames <- vector(mode="character", length = n*m)
for (j in 1:n){CATnames[(((j-1)*m)+1):(j*m)] <-
dimnames(MFAresob$ind$coord[order(row.names(MFAresob$ind$coord)),])[[1]][j]}
PartielDim <- cbind.data.frame(names = CATnames, MFAresob$ind$coord.partiel)
# Bootstrapping the new data frame
Boot <- simule(PartielDim, nb.simul = 500)
# Creating ellipses around 95% of the bootstrapped means
EllipCoord <- coord.ellipse(Boot$simul, level.conf = 0.95, bary = FALSE)
# Plotting the ellipses

plot.MFA(MFAresob, choix = "ind",ellipse=EllipCoord, ellipse.par = NULL,
habillage="ind", title="Projective Mapping Dehlholm Confidence Ellipses")
```

Chapter 6

Research results

Polarized projective mapping (PPM) as a rapid sensory analysis method applied to South African Chenin Blanc wines

Chapter 6 : Polarized projective mapping (PPM) as a rapid sensory analysis method applied to South African Chenin Blanc wines

6.1 Introduction

When selecting a sensory methodology, the primary considerations are whether the method is appropriate for the type and number of samples, as well as the cost and time involved. Researchers must take into account the value and detail of information gained versus the costs of gaining such knowledge. Time-effective "rapid methods" have been developed and popularized within sensory research to address the issues of lengthy, costly training sessions involved in descriptive analysis (DA). Though projective mapping/Napping® (PM) was originally suggested as a companion method to DA (Pagès, 2003, 2005), several studies have compared the accuracy and usefulness of information gained in PM to results from DA and have found rapid methods to have the potential to stand alone, especially when differences between products are large or do not need to be quantitatively described (Cartier *et al.*, 2006; Perrin *et al.*, 2008; Hopfer & Heymann, 2013; Varela & Ares, 2014). However, one important limitation of both PM and DA is the number of samples that can be tested. The suggested sample size is around 12 for PM and for each product included in DA, the cost and time needed for training and testing increase. This limitation of sample size is not a problem for studies which test the differences between a control sample and a few treatments, or compare samples within a small or well-defined category of products. Larger sample sets are required, though, in the case of categorizing the sensory space of a complex and diverse category, such as a certain style or cultivar of wine. This type of categorization using the current sensory methods is very challenging and expensive.

Variants of the rapid methods are being developed and validated in order to improve the quality of information gained and address certain disadvantages of the above-mentioned methods. One such variant of the PM method (utilized in Chapter 5) is polarized projective mapping (PPM), which provides a solution to the issue of limited sample size. PPM is a form of PM which integrates the concept of reference samples, termed "poles", from polarized sensory positioning (PSP) (Ares *et al.*, 2013). In PPM, the poles have a fixed, pre-determined location on the panellist's map. Panellists are presented with "free-moving" products to arrange around the poles to create a two-dimensional product map. This use of poles, which serve as consistent references, allows direct comparison of data from multiple sessions where new "free-moving" samples can be introduced. This effectively increases the maximum sample size, thus giving PPM the ability to analyse large sample sets.

This method is an exciting addition to the field of sensory science, but has only been applied to orange-flavoured powdered drinks, which are relatively simple products with large differences between them (Ares *et al.*, 2013; De Saldamando *et al.*, 2015a, 2015b). Complex products with small differences between them, such as wine, have not been analysed by this method. Additionally, only one study has looked at the possibility of combining data from separate sessions as theorized, by comparing results when sample sets were evaluated as a whole, and the aggregated data when sample sets were split and evaluated separately (De Saldamando *et al.*, 2015b).

In order to be able to analyse the perception of thiols in a wide variety of Chenin Blanc wines using PPM, the first step was to validate the use of this method in the sensory evaluation of wine. To test the applicability of PPM to dry South African Chenin Blanc wines, one PM and four PPM experiments were performed. As it is the established method, PM was used to create an initial product map for comparison with PPM results. This product map was also used for selection of the three poles for PPM. Four PPM experiments were performed with varying sets of wines. The poles were kept constant among the PPM experiments, while different combinations of “free-moving” wines were evaluated to test the consistency of product groupings. In all tasks, sensory descriptors were generated with ultra flash profiling (UFP), and results were analysed by multiple factor analysis (MFA) with 95% confidence ellipses (Pagès, 2005; Dehlholm *et al.*, 2012). As the use of poles as stable references between evaluations allowed, the PPM results from all four experiments were combined in a single statistical analysis. This gave a single figure which compared samples from all evaluations in a global “Super MFA”. The sensory results were related by PCA to extensive chemical volatile analysis, including major acids, esters, monoterpenes, and the volatile thiols 3-mercapto-hexan-1-ol (3MH) and 3-mercapto-hexyl acetate (3MHA).

This experimental design was conceived to validate the use of PPM in wine in two ways: 1) When using the same product set, whether MFA groupings resulting from PPM are similar to those found in PM, and 2) Following the natural progression of the method, if the product configuration and explained variance remain similar when new samples are evaluated against the same poles.

6.2 Materials and methods

6.2.1 Samples

Seventeen commercially available South African dry 100% Chenin Blanc wines from the Western Cape were selected for this study. The product set was selected to cover a range of price-points and vinification styles, and span the entire sensory space of South African dry Chenin Blanc wines. Label information (descriptors and oenological procedures *i.e.* wood contact) and previous experience in the Department of Viticulture and Oenology at the University of Stellenbosch were used to select wines of varying styles. Prices of the wines ranged from R35 - R150 per bottle and included 10 one-year-old, 6 two-year old, and 1 three-year old wines. Of the 17 wines, ten received oak contact, three were made from bush vines, and nine were made from vines 35 years or older.

6.2.2 Sensory evaluation

Experimental design

Five separate sensory evaluation tasks were performed with a one-week break between each evaluation to mitigate the effect of product familiarity. Initially, a projective mapping with ultra flash profiling (UFP) was performed. The results of this PM were used to select three wines which spanned the sensory space to serve as poles for PPM. These poles were included in four separate PPM (with UFP) experiments with varying product sets. The same set of wines were evaluated in PM and PPM1, a different set was evaluated in PPM 2, and mixtures of the two sets were evaluated in PPM3 and PPM4 (Table 6.1).

Table 6.1 Experimental design detailing which wines were evaluated in each experiment.

Wine	PM	PPM1	PPM2	PPM3	PPM4
PETIT	✓	†*	†*	†*	†*
MH	✓	†	†	†	†
RB	✓	†	†	†	†
KZFR	✓*	✓			✓
KZCS	✓*	✓			✓
KZVS	✓	✓			✓
CG	✓	✓		✓	
SPIER	✓	✓			✓*
BOO	✓	✓*		✓	
BBS	✓	✓		✓	
56H			✓*		✓
MB			✓	✓	
DG			✓	✓	
HB			✓		✓
SIM			✓	✓	
SR			✓	✓*	
RH			✓		✓

✓=included, †=included as pole, *=blind duplicate

Procedure

In all experiments, evaluations took place in off-white individual sensory booths in a well-ventilated, odourless 20 ± 2 °C air-conditioned room (ISO 8589:2007). Samples were served in black glasses (ISO 3591:1977) labelled with random 3-digit codes unique to each judge and rep. Wines were stored at 20 ± 2 °C for no more than three weeks prior to testing. Samples at ambient temperature were poured 30 minutes before testing in 20 ± 2 mL aliquots, and immediately covered with plastic Petri dish lids. Absence of TCA and *Brettanomyces*-related spoilage in the samples was confirmed sensorially by the researcher. Products were presented in a different randomized order for each panellist according to a Williams Latin Square design (Macfie *et al.*, 1989). Two replications were performed with a 10-minute break between reps. Panellists evaluated aroma only of the wines.

Panellists

Fifteen panellists participated in each experiment. All judges were students or staff members from the Department of Viticulture and Oenology at Stellenbosch University with previous experience in sensory analysis of South African Chenin Blanc wines. While it was not possible to use the same judges for each test, care was taken to keep the panel as consistent as possible by recruiting judges with similar levels of experience. Eight judges participated in all five evaluations, and a total of thirteen judges were present in at least four out of five experiments. The entire group of 21 panellists consisted of 6 males and 15 females, aged 22-41.

Projective mapping

A PM experiment was conducted where a single sensory modality, namely aroma, was evaluated. This PM experiment was performed to create a consensus map by multiple factor analysis (MFA) which was used to select the three poles used in the following PPM experiments. The MFA was also used to compared the sample configurations between the PM and PPM results, following the precedent of Ares *et al.*, (2013).

A total of 12 samples were presented to the panellists, as suggested by Pagès, (2005). The 12 samples consisted of 10 unique wines and 2 blind duplicates to establish reliability of the results. Three of the ten wines evaluated were vinified with wood contact (barrel fermentation or barrel maturation). One wooded and one unwooded wine were chosen as the blind duplicates.

Panellists were instructed to smell each of the wines from left to right, record 3-5 descriptors for each one by free description, and arrange them on an A2 (40 cm x 60 cm) white sheet of paper according to their similarities and differences. Due to the complexity and number of the wines, the UFP step was performed at the same time as the arranging step to assist with panellists' memory of each wine's main aroma attributes. It was explained that wines with similar aroma should be located close to one another, while wines with dissimilar aroma profiles should be located far from one another. Panellists were allowed to return to each wine as many times as they liked. It was emphasized that the exercise was intended to be free-form with no correct answer, and each judge was to use their own criteria to create their unique map. Panellists were provided with verbal and written instructions (Appendix D).

Polarized projective mapping

All four PPM experiments were conducted following the procedure set out in Ares *et al.* (2013). Considering the bottom-left corner as the origin (0,0), the three poles selected from the PM experiment were pre-located on the A2 (40 cm x 60 cm) white sheets of paper at the (X,Y) coordinates (15 cm,13 cm), (30 cm, 30 cm), and (45 cm,13 cm). This placement allowed space for samples to be placed between and outside of each pole. As in PM, 12 samples were presented, 10 of which were unique wines and 2 were blind duplicates. Of the two blind duplicates, one was a pole and one a "free-moving" blind duplicate.

All panellists were again asked to evaluate aroma only, and freely choose 3-5 descriptors for each wine. They were asked to smell the poles first, and then smell the "free-moving" samples from left to right. Panellists were instructed to place the "free-moving" wines in relation to both the poles, and to one another with similar wines being closely located, and different wines being far from one another. Repeat smelling of samples was allowed. Verbal directions, as well as written directions were provided (Appendix D).

Capturing and treatment of the data

Product locations were measured manually as (X,Y) distance coordinates from the bottom-left corner of each assessor's map. As the use of free description generated many synonymous attributes and attributes used by one or two judges, the list of attributes generated was condensed. The aroma descriptors were treated in a systematic manner to minimize the subjectivity inherent to the condensing process. Condensing was done separately for each experiment, with care taken to group terms as consistently as possible between the different experiments. In the initial step of descriptor processing, linguistic synonyms were combined under a common synonym, while semantic synonyms were kept separate. In the second step, descriptor condensing was undertaken in consultation with two other experimenters using a strict set of rules. If the descriptor was cited by fewer than 20% of the judges, it had to be combined with a similar term (as agreed upon by the three experimenters) (Campo *et al.*, 2008). If no similar term was available, the descriptors were eliminated. When possible, judges were consulted on how they would like their terms to be combined.

6.2.3 Chemical analysis

Chemical analysis was performed on the wines in order to characterize the wines and explore correlations between descriptors from the sensory analysis and levels of aromatic compounds. Four methods were used to measure a total of 54 compounds of various classes.

Thiols (3-mercapto-hexan-1-ol and 3-mercapto-hexyl acetate) were measured by UPLC-MS/MS according to Piano *et al.* (2015), as described in Chapter 3.

Major volatiles (alcohols, esters, and acids) were analysed by GC-FID using a high-throughput in-house method, which consists of a direct extraction of 5 mL sample (with 100 µL of 0.5 mg/L 4-methyl-2-pentanol as internal standard) in 1 mL diethyl ether under sonication during 5 minutes. Extracts were centrifuged at 4000 rpm for 3 minutes, and the extract was dried under Na_2SO_4 (Merck, 99%) before injection in duplicate. Details of the method validation were previously described in Louw, 2007.

Monoterpenes were extracted using solid phase extraction (SPE) according to Piñeiro *et al.* (2004) with the following modifications: the last phase of the conditioning step where the cartridges are rinsed with an ethanol-water solution, wine simulant was used (12% ethanol, 2.5 g/L tartaric acid adjusted to pH 3.5 with NaOH). Extraction was performed in HF Bond ElutLRC-C18 OH, 500 mg SPE cartridges (Agilent Technologies, Santa Clara, CA). Drying time was increased from 10 min to 15 min, and the extract was dried with Na_2SO_4 (Merck, 99%) before injection in duplicate.

Wood-derived compounds were analysed by headspace (HS) solid phase microextraction (SPME) gas chromatography tandem mass spectrometry (GCMS/MS). Samples were prepared by vortexing 1 mL of sample with 20 µL of 100 µg/L anisole-d8 internal standard and 9 mL of 20% NaCl solution (w/v% in Milli-Q-Water®). Samples were held in an autosampler with the agitator temperature set to 55 °C. They were extracted onto SPME (DVB/CAR/PDMS, 30/50 µm)-coated fibres for 30 minutes and the fibres were then desorbed in the GC injector for 2 minutes at 220 °C. The injector was run in 'splitless' mode with a helium carrier gas flow rate of 1 mL/min. Separation was done using on a polar free fatty acid phase (Zebron FFAP) column fitted to a TRACE™ 1300 GC (Thermo Scientific, Waltham, MA). Analytes eluting from the column were detected using a TSQ™ 8000 Triple Quadrupole Mass Spectrometer (Thermo Scientific, Waltham, MA). The oven temperature program started at 50 °C held for 5 min and then ramped to 250 °C at a rate of 10 °C/min. The total run time was 30 min.

6.2.4 Statistical analysis

Sensory results of the PM and experiment was analysed by multiple factor analysis (MFA), (Pagès, 2005). The analysis was run in the open-source statistical language R (R Core Team, 2015) using the function "MFA()" provided in the FactoMineR package (Lê *et al.*, 2008). Each repetition of each judge's product coordinates was considered as a table of variables in the MFA. The frequency of use of the condensed list of descriptors in the form of a contingency table, was treated as a single table of supplementary variables and did not contribute to the construction of the dimensions of the MFA. Quantitative variables were not scaled to unit variance, and the default number of five dimensions were kept in the analysis. The MFA coordinates of the wines and descriptors were extracted and plotted in Microsoft Excel (2013) to create an MFA biplot. Only descriptors with cos^2 values > 0.6 were included due to cluttering. To strengthen the interpretation of the results, confidence ellipses around the products were constructed in the SensoMineR package (Lê & Husson, 2008) in R by parametric bootstrapping. These ellipses are run on the principle of sampling with replacement, and the code is published (Dehlholm *et al.*, 2012). RV coefficients between reps and panellists were used to judge panel performance.

For the PPM experiments, the same procedure was followed with one modification: as the coordinates of the poles were pre-determined by the experimenter rather than generated by the panellists, they were treated in the analysis as supplementary individuals. As such they did not contribute to the construction of the dimensions but were projected onto the space.

Due to the feature of the PPM method where poles are kept consistent from one experiment to the next, the wines from all PPM experiments could be analysed in one single MFA, termed “Super MFA”. Again, the poles were projected onto the final map as supplementary individuals. Only 10 out of 15 judges participated in all four PPM tasks, so only the results of the 10 judges who participated in all PPM experiments were used.

Two Pearson’s (n-1) Principal Component Analyses (PCA’s) were performed on the chemistry results to chemically characterize the different wines. These were done firstly to relate the wines to the compounds, and then to relate the compounds to the descriptors generated by the PPM experiments.

6.3 Results and discussion

6.3.1. Sensory evaluation

Projective mapping

The MFA of the PM data (Figure 6.1) wines explains 54.1% (Dim 1=43.5%, Dim 2=10.6%) of the variation between the products in the first two dimensions, which is acceptable for this type of data. Both sets of blind duplicates (KZCS-1 and -2 and KZFR-1 and -2) are paired well, indicating good reliability of the configurations. Wines located along the positive axis of Dimension 1 (BBS, BOO, MH, KZFR-1, and KZFR-2) are characterized as ‘oaky/wooded’, ‘caramel’, ‘vanilla’, ‘toasted’, ‘honey,’ and ‘marmalade’ (Figure 6.2). Wines located along the negative axis of Dimension 1 are all described as ‘passion fruit’ ‘pineapple’, and ‘guava’. Wooded and unwooded wines are opposed along Dimension 1. Due to the separation and percentage of explained variance along Dimension 1, it can be said that there was consensus among the panellists on wooded *vs.* unwooded wine aromas.

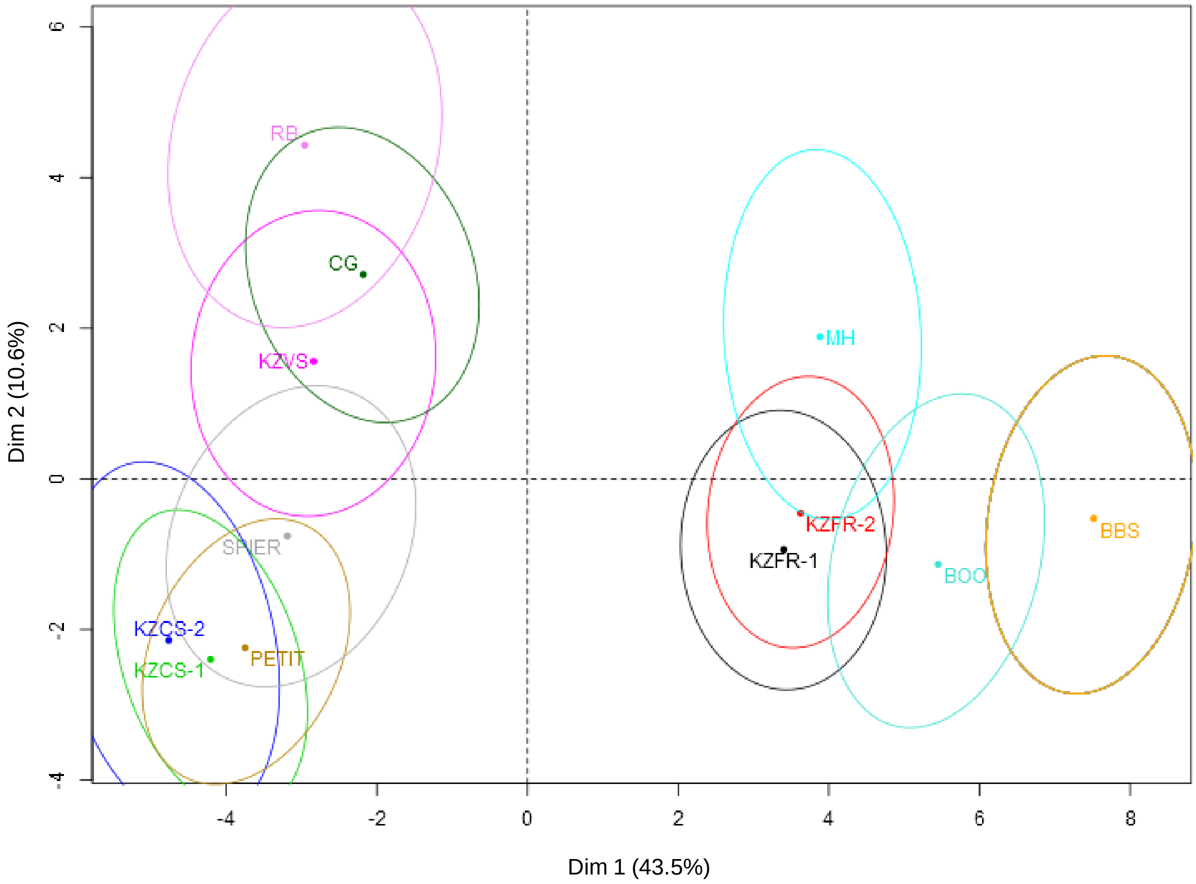

Figure 6.1 MFA scores plot of the Projective Mapping data. Codes ending in -1 or -2 are blind duplicates. The 95% confidence ellipses were created according to Dehlholm *et al.* (2012).

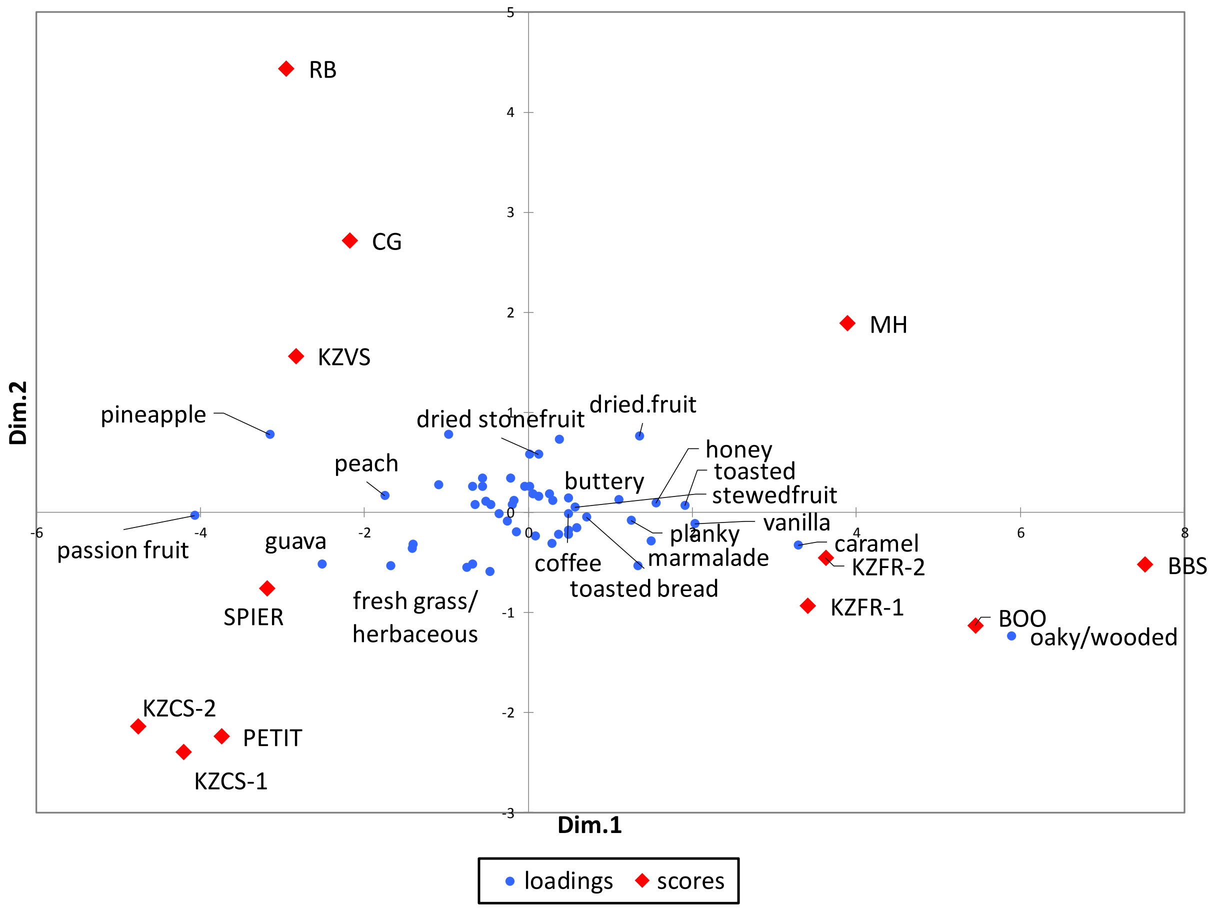

Figure 6.2 MFA biplot of the Projective Mapping data. Descriptors which are well-represented on the MFA ($cos^2 > 0.6$) are included.

Illustrated by the overlapping confidence ellipses, the unwooded wines form a continuum along Dimension 2 (Figure 6.1), with wines in the positive direction (RB, CG, KZVS) more correlated with 'pineapple' and 'dried stonefruit', and wines in the negative direction (SPIER, KZCS-2, KZCS-1, PETIT) described as 'guava' and 'fresh grass/herbaceous' (Figure 6.2). These wines spanning Dimension 2 only explain 10.6% of the variance and appear to have been difficult to describe, as none of the descriptors are located far from the origin along Dimension 2.

These results support previous work on the style classifications of South African Chenin Blanc. According to the Chenin Blanc Association of South Africa, there are 6 recognized styles of South African Chenin Blanc wines. Of these, there are three dry, still styles: Fresh & Fruity (FF), Rich & Ripe – Unwooded (RRUW), and Rich & Ripe – Wooded (RRW) (CBA, 2016). Bester, (2011) argues that RRW South African Chenin Blanc wines are easily characterized, but unwooded wines form a continuum from FF to RRuW styles, rather than separating distinctly into the two groups. While the wines in this experiment were not evaluated in terms of style, it appears that wines in the positive direction of Dimension 2 could be more RRuW, as they are described with richer terms ('peach', 'dried fruit'), and wines in the negative direction could be of the FF style, as they are described as 'fresh grass/herbaceous'.

MH, RB, and Petit were selected as the poles for PPM, as they span the sensory space represented in the MFA. MH is a wooded wine, and was described as 'buttery', 'caramel', 'oaky/wooded', and 'toasted'. RB was described as 'apricot', 'dusty', 'floral', 'pineapple', and 'passion fruit'. Petit was described as 'pineapple', 'passion fruit', 'lemon', and 'guava'.

Polarized projective mapping 1

Explained Variance increased from 54.1% in PM to 62.8% in PPM1 (Dim 1=51.5%, Dim 2=11.3%) (Figure 6.3). Though the same set of wines were evaluated as in PM, different wines were chosen as blind duplicates in PM vs. PPM1 (Table 6.1). Potential sources of this increase in explained variance could be that the panellists found the blind duplicates in PPM1 easier to pair, or that the poles gave guidance to the panellists and led to better agreement. Also, in the construction of the MFA, the poles did not contribute to the model but rather were projected onto the MFA. This was because the poles were pre-located by the researcher, not placed by the panellists. This exclusion of the poles from the model means that the MFA was constructed from fewer individuals, and it may have been easier to construct a better consensus map.

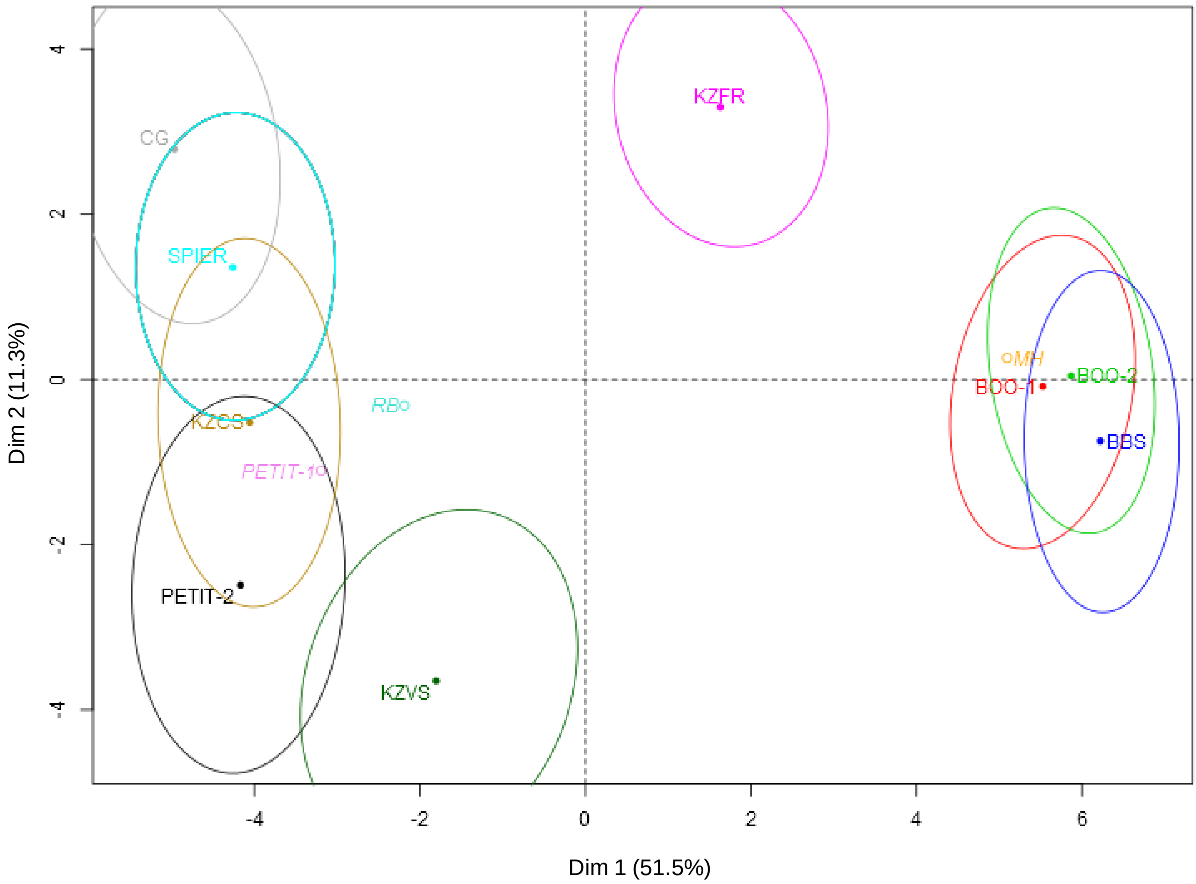

Figure 6.3 MFA scores plot of the PPM1 data. Codes ending in -1 or -2 are blind duplicates. The 95% confidence ellipses were created according to Dehlholm *et al.* (2012).

The same overall configuration of groups was observed in PPM1 as in PM. The confidence ellipses of both pairs of blind duplicates overlap, indicating they were placed together by the panellists (Figure 6.3). There was better agreement for the wooded pole (BOO) since the wooded wines were easier for panellists to differentiate as in PM. The group of wines described with wood-related terms in PPM1 still grouped together along the positive axis of Dimension 1 (Figure 6.4), with the exception of KZFR, which stands on its own. KZFR was the only wine described with high frequency as both 'oaky/wooded', and 'passion fruit' (raw data, not shown). Both KZFR and KZVS, which lie closer to the middle of Dimension 1, were fermented in a blend of old and new barrels, with KZFR having a higher proportion of new barrels and longer aging on the lees.

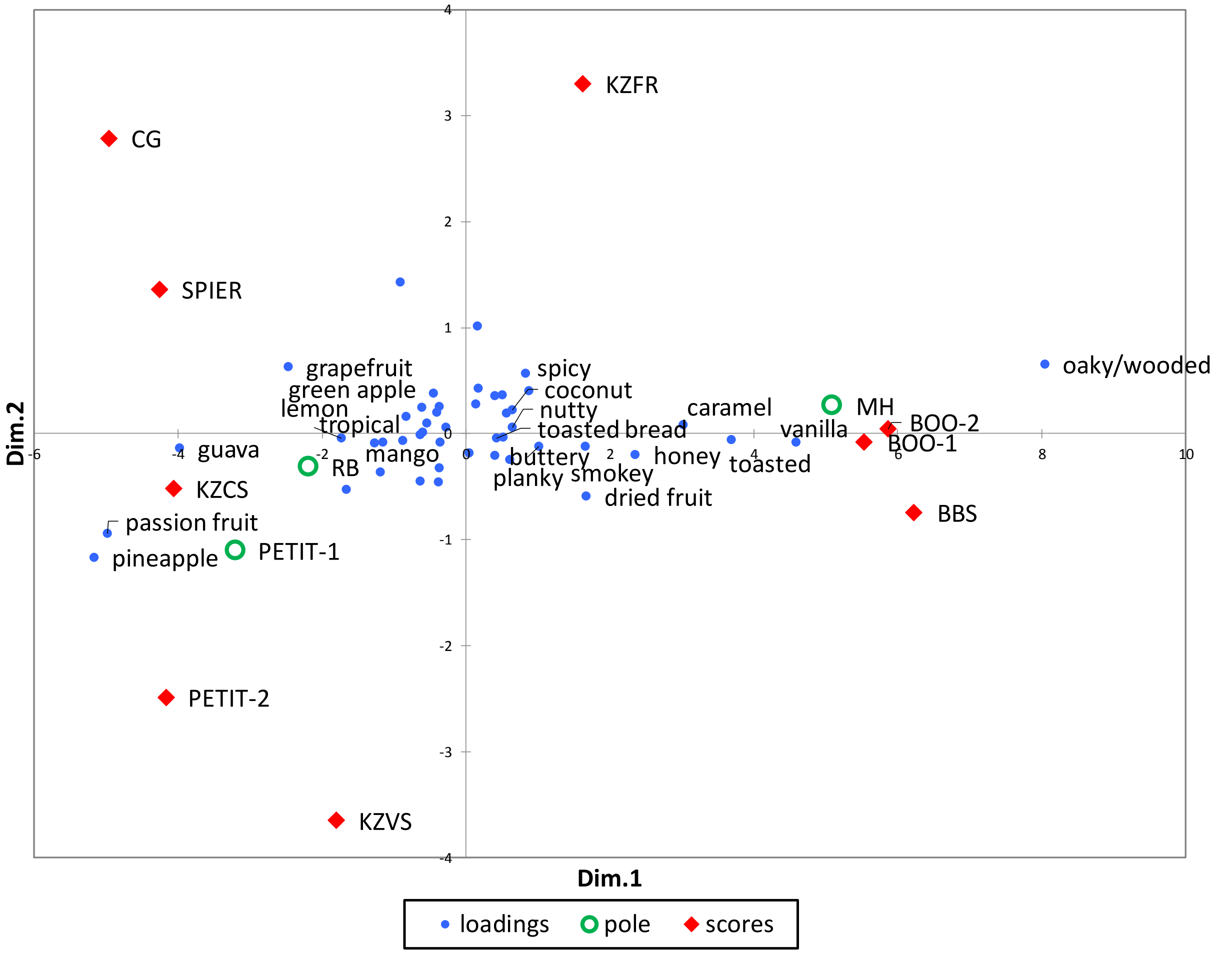

Figure 6.4 MFA biplot of the PPM1 data. Descriptors which are well-represented on the MFA ($cos^2 > 0.6$) are included.

As in PM, the unwooded wines form a continuum along Dimension 2 and are all described as 'pineapple', 'passion fruit', and 'guava' (Figure 6.3), though the arrangement of wines within this continuum is not the same as in PM. In PPM1, the wines in the positive direction of Dimension 2 were described as 'grapefruit', 'green apple', and 'lemon', while those in the negative direction were more frequently described as 'pineapple' and 'passion fruit'. Thus, it seems that between-group configurations of these wines are stable, but within-group configurations wines may differ between PM and PPM. Interestingly, in the PPM1 exercise, the Petit and RB poles are no longer at the extremes of Dimension 2, which may be because the poles were considered as supplementary individuals in the MFA of PPM1, as described above.

Polarized projective mapping 2

When a new set of free-moving wines (Table 6.1) was arranged around the same poles in PPM2, explained variance decreased to 53.3% (Dim 1=37.2%, Dim 2=16.1%) (Figure 6.5), which was comparable to that of the PM. The explained variance along Dimension 2 is higher than in PM or PPM1. The two pairs of blind duplicates once again were located with one another.

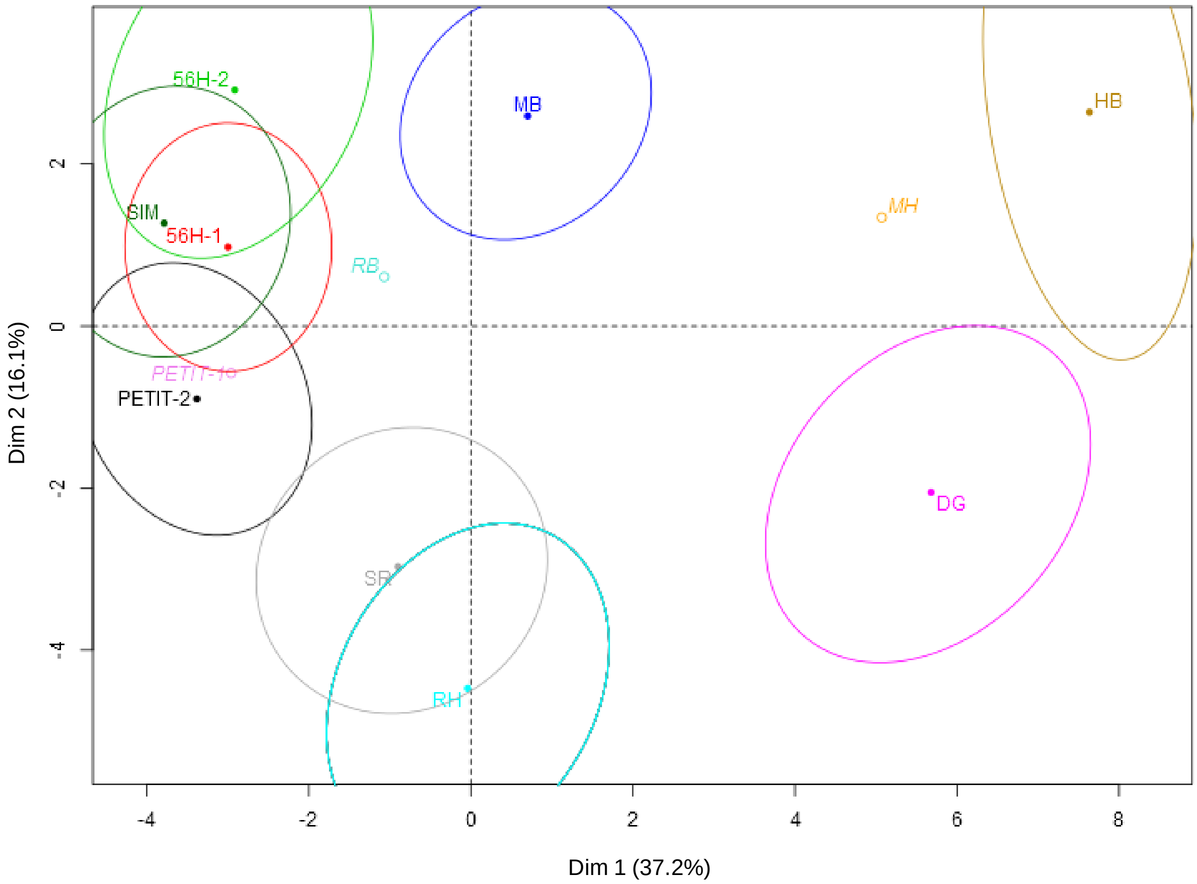

Figure 6.5 MFA scores plot of the PPM2 data. Codes ending in -1 or -2 are blind duplicates. The 95% confidence ellipses were created according to Dehlholm *et al.* (2012).

A similar configuration to PPM1 was observed with a continuum of oak contact along Dim 1. The wines cited most frequently as ‘oaky/wooded’ (MH, DG, and HB) group along the positive axis of Dimension 1, though not as closely as the wooded wines in PM and PPM1. MB, SR and RH group along the centre of Dimension 1 and were also described as wooded, but with lower frequencies than MH, DG, and HB. This trend corresponds with their vinification, as they are all partial barrel fermentations with 10-20% fermented in barrel. Along Dimension 2, SR and RH correlate along the negative axis with more ‘honey’, ‘baked apple’, and ‘dried fruit’ citations, and fewer ‘pineapple’ and ‘fresh grass/herbaceous’ citations (Figure 6.6). These two wines are described more with classically “ripe” terms than the wines used in PM and PPM1, and this greater differentiation between unwooded wines explain the higher explained variance along Dimension 2. The unwooded wines PETIT-1 and -2, SIM, and 56H-1 and -2 form one group and are associated with ‘fresh grass/herbaceous’, ‘pineapple’, ‘guava’, ‘grapefruit’, and ‘passion fruit’ (Figure 6.6).

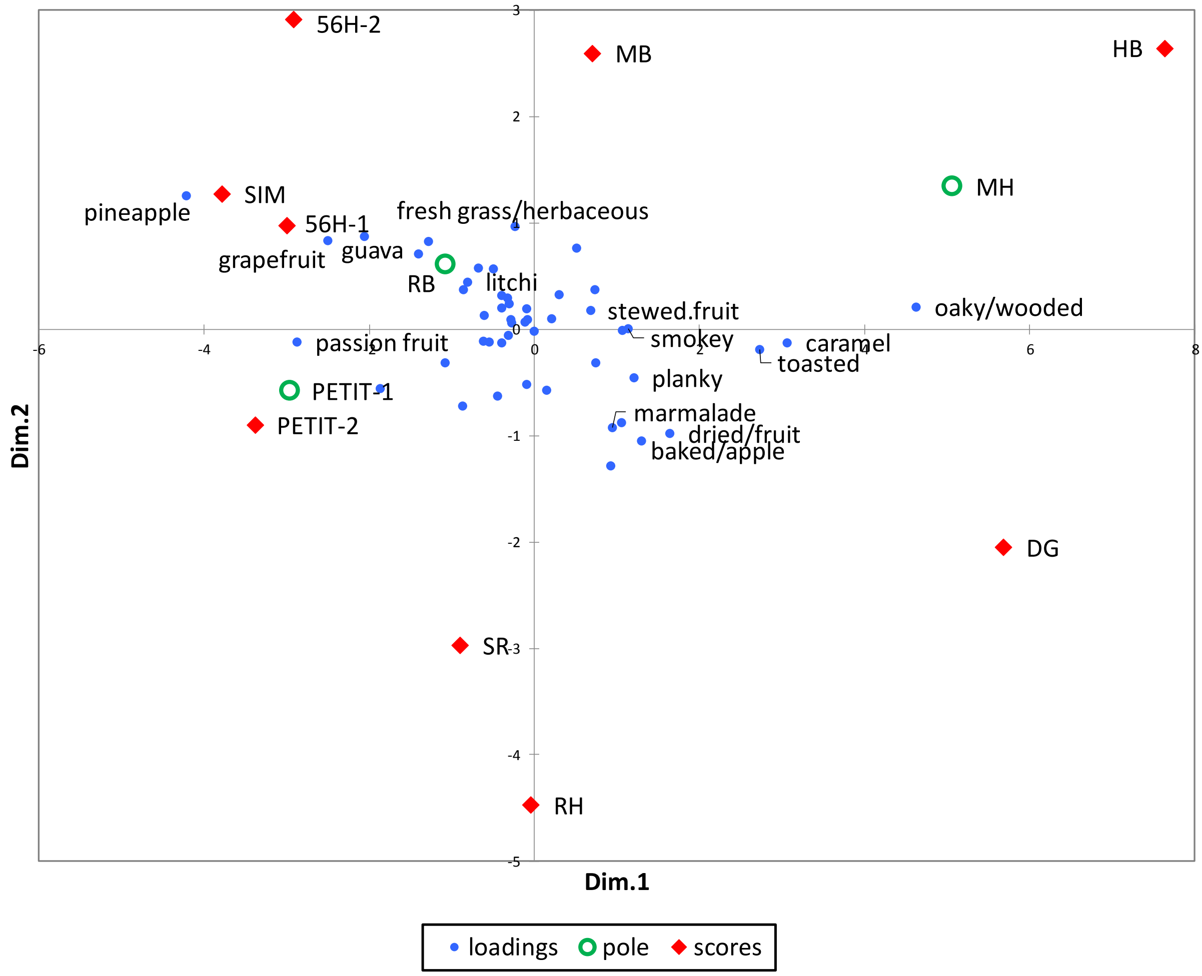

Figure 6.6 MFA biplot of the PPM2 data. Descriptors which are well-represented on the MFA ($cos^2 > 0.6$) are included**.**

The same trends and descriptors were observed in the mixed sets, in PPM3 and PPM4. In all experiments, the blind duplicates showed consistent grouping by the panellists (Appendix D).

Super MFA

Finally, the "Super MFA" (Figure 6.7) shows the results from PPM1 – PPM4 in one single MFA with 48.1% explained variance (Dim 1=41.4%, Dim 2=6.7%). This representation confirmed that over all PPM experiments, there was the same grouping pattern as discussed above. There were two groups separated along Dimension 1, with oaky wines opposing the unoaked wines. A few wines (KZFR, MB, RH, SR) were pulled to the 'oaky/wooded' side of the "unwooded" group, and did have oak contact during vinification. Figure 6.8 shows that wines in the positive direction of Dimension 2 were correlated with more thiol-related or "fresh" descriptors: 'passion fruit', and 'grapefruit', and 'pineapple', while SR and RH in the negative direction were described with more "rich" descriptors: 'floral', honey', and 'peach/apricot'. Considering that neither RH nor SR were included in the first set of wines evaluated in PM and PPM1, the PPM method has allowed us to get a broader picture of the Chenin Blanc wines' sensory space than would have been possible with PM alone.

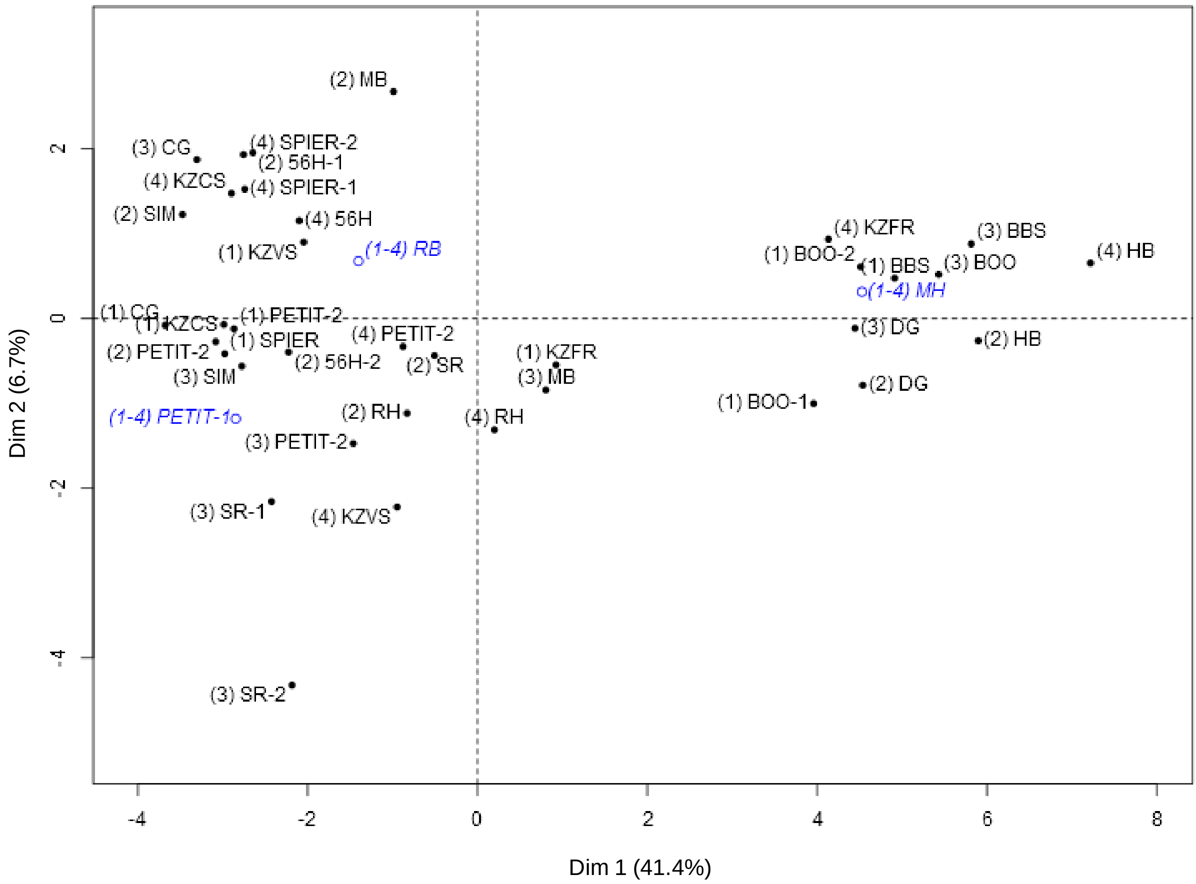

Figure 6.7 "Super MFA" scores plot consisting of the positional data across all PPM experiments from the ten panellists who participated in PPM1-PPM4. The poles are in blue and did not contribute to the construction of this MFA. The PPM experiment number in parentheses, and codes ending in -1 or -2 are blind duplicates.

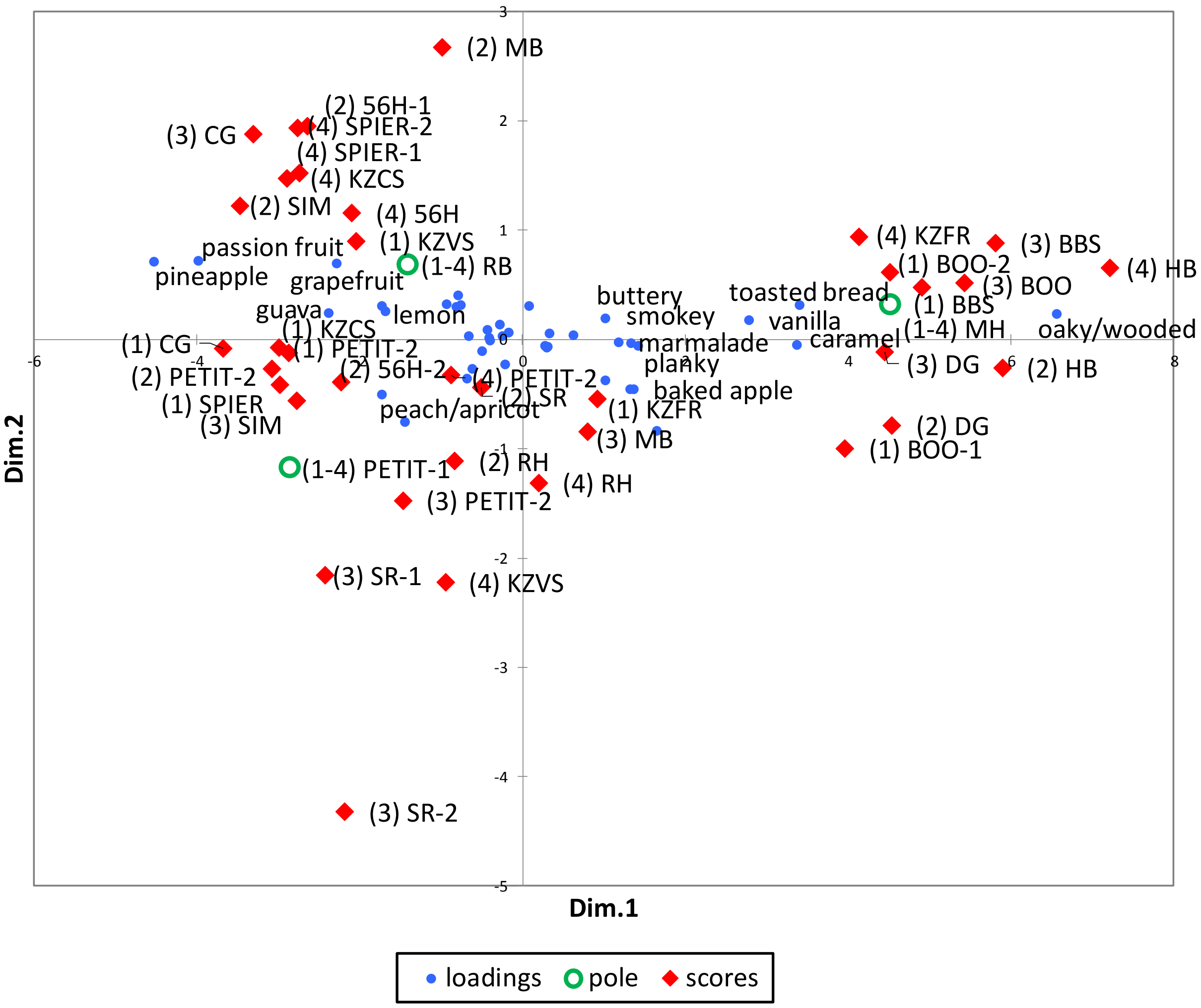

Figure 6.8 "Super MFA" bipot consisting of the data across all PPM experiments from the ten panellists who participated in PPM1-PPM4. Descriptors which are well-represented on the MFA (cos^2 > 0.6) are included**.**

Reviewing whether the same wines evaluated in different PPM experiments group together on the Super MFA (Figure 6.7), the wooded wines show greater consistency between experiments than the partially-wooded or unwooded wines. The lack of agreement along Dimension 2 was not surprising as it only represented 6.7% of the explained variance. While the use of poles should standardize the placement between tasks, it is possible that varying the set of “free-moving” wines could affect placement between different sessions. It is also not ideal that only the data from the 10 judges who participated in *all* PPM experiments could be used to produce this MFA. Groupings between unwooded wines may have been more consistent if more judges’ data was included.

The effect of the choice of poles on PPM MFA configurations has been studied (De Saldamando *et al.*, 2015a), and it was found that as long as the poles represent the sensory space of the product, sample configurations are stable. The poles selected for this set of experiments were the extremes of the sensory space in PM, but the two unwooded poles were more centrally located in PPM. The unwooded poles should have encouraged separation of wines between the sensory space of these two poles. However, no consistent separation of these wines was seen, which suggests that the wines in this portion of the map were very similar to one another. The use of the Super MFA allows for identification of wines that may have been more suitable poles e.g. SPIER for a ‘pineapple’, ‘passion fruit’ pole and SR for a ‘honey’, ‘peach/apricot’ pole.

Chemical analysis

The PCA of all the chemical analyses (esters, volatile alcohols, monoterpenes, thiols and wood-related compounds), explains 55.6% variance in the data in the first two principal components (Figure 6.9). The grouping of the wines by chemical results is similar to the groupings seen in the sensory experiments. The wooded wines are correlated with the positive side of Dimension 1 on the PCA, and oppose the unwooded wines. The wooded wines (BBS, DG, BOO, and HB) are highly correlated with the wood-related compounds such as cis- and trans-whiskey lactones and furfural (Figure 6.9), and were described as 'vanilla', 'nutty', spicy', 'toasted bread', and 'oaky/wooded' (Figure 6.10). The monoterpene β-farnesol, esters ethyl caprate, ethyl caprylate, hexyl acetate and isoamyl acetate, and the thiol 3MHA are correlated with the wines described with more 'fruity' descriptors.

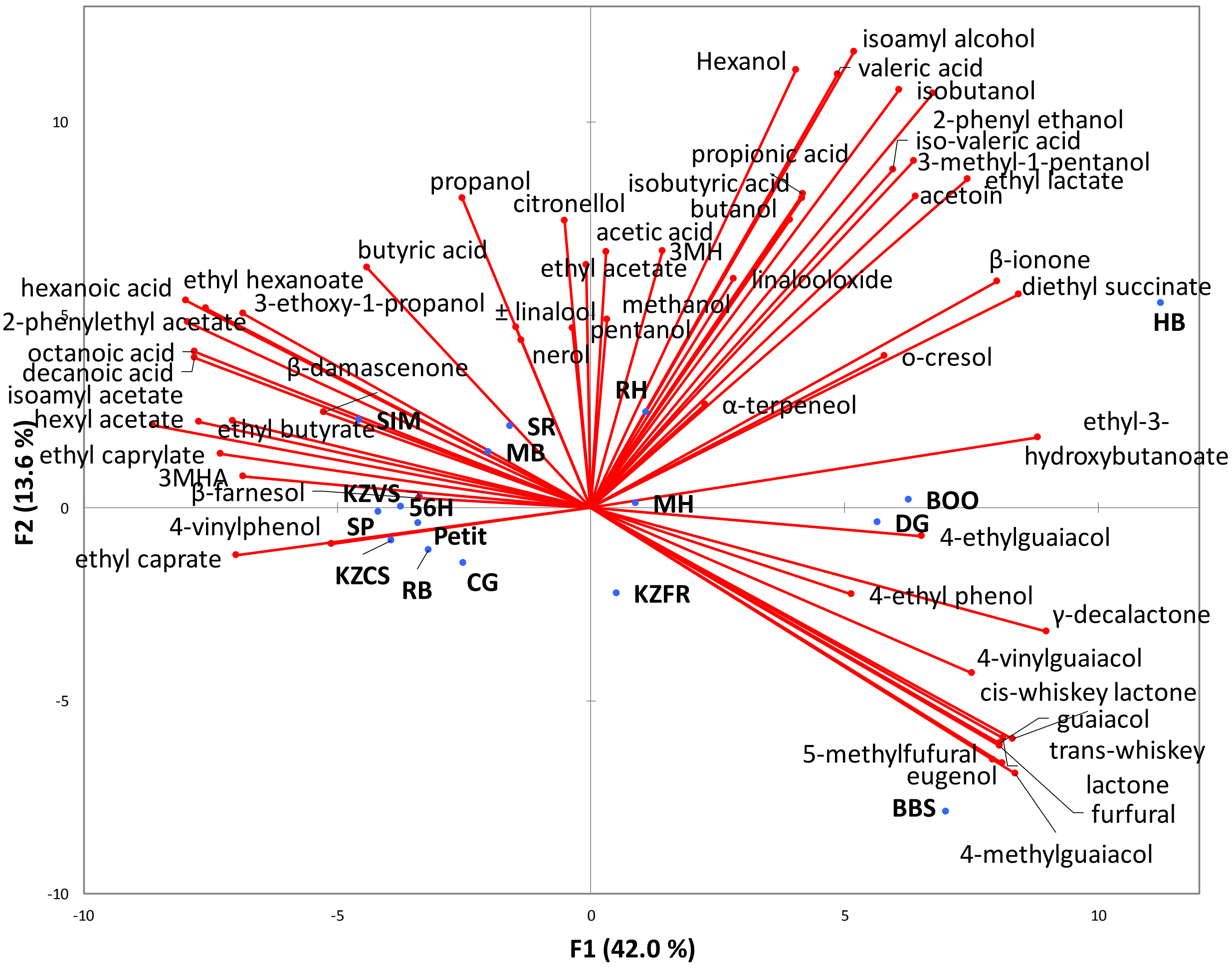

Figure 6.9 PCA biplot of the chemical results including major acids, esters, monoterpenes, and the volatile thiols 3-mercapto-hexan-1-ol (3MH) and 3-mercapto-hexyl acetate (3MHA). The values were standardized by dividing the concentrations by the standard deviation of each compound.

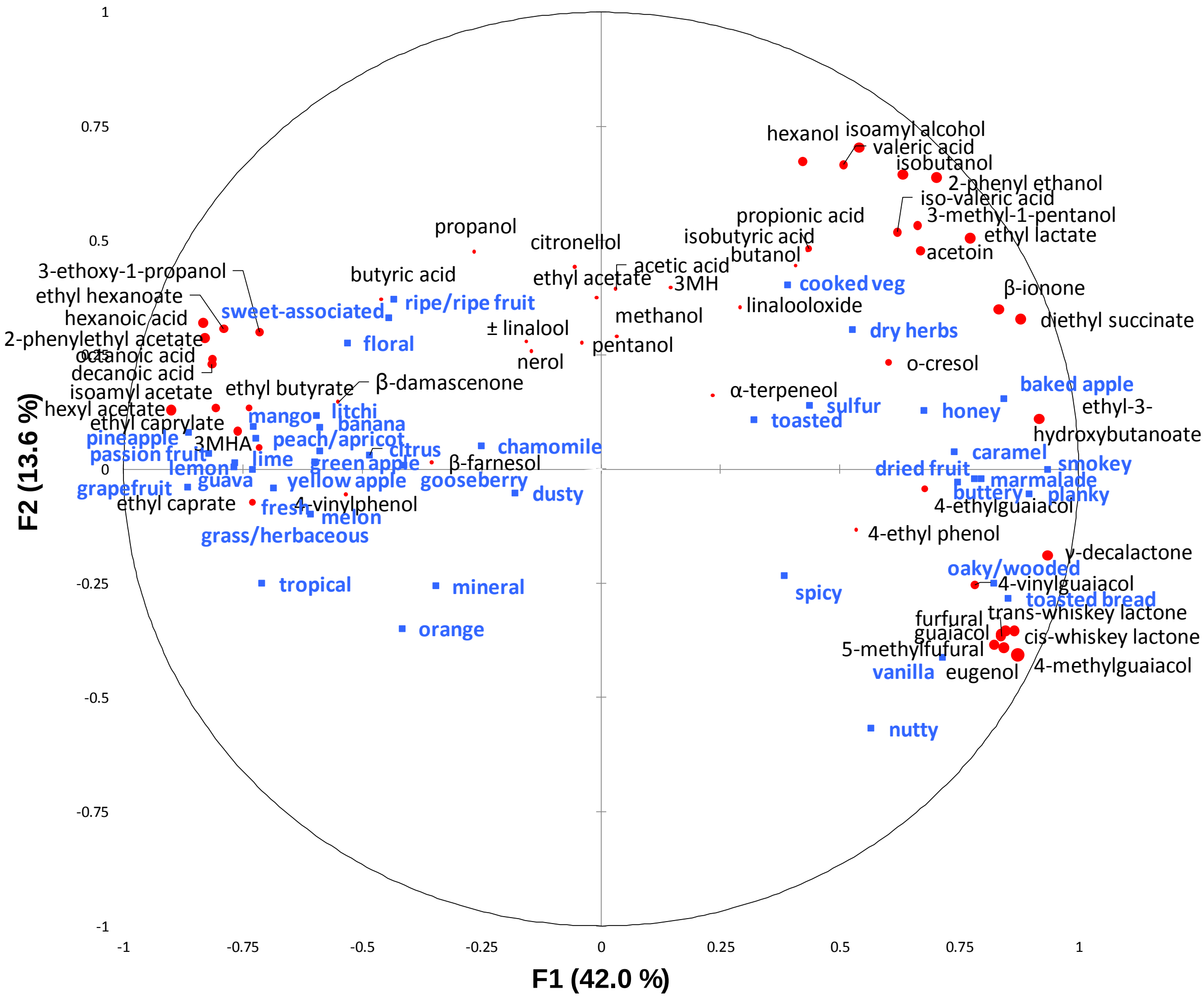

Figure 6.10 Correlations between chemical compounds and frequency of descriptor citations from the Super MFA data

Looking at the thiols in the PCA (Figure 6.9), 3MH is not well-explained in the first two dimensions (cos^2=0.022 in Dim. 1, 0.156 in Dim 2.), but 3MHA is (cos^2=0.512 in Dim, 1). 3MHA is correlated with CG, RB, Petit, SP, 56H, KZVS, KZCS and SIM, which is confirmed by the raw data (Appendix D, Table D.1). The correlations of the chemical data with the Super MFA frequencies show that 3MHA is best correlated with wines described as ‘passion fruit’, ‘guava’, and ‘grapefruit’, which are typical descriptors of thiols (Tominaga *et al.*, 1996, 1998; Dubourdieu *et al.*, 2006; Roland *et al.*, 2011), as well as ‘pineapple’ and ‘lemon’. This suggests that 3MHA was more influential in contributing thiol-related attributes to the wines than 3MH, which may be due to 3MHA’s lower sensorial threshold. The wooded wines form a group which was negatively correlated with thiols (as in Chapter 3), which is not surprising as thiols are relatively unstable compounds, sensitive to oxidation (Herbst-Johnstone *et al.*, 2011; Coetzee & Du Toit, 2012). The exception was HB which was a wooded wine, but had the highest 3MH levels of all the wines analysed (1937 ng/L). HB was perceived as ‘oaky/wooded’, and ‘caramel’ and not as ‘passion fruit’ or ‘grapefruit’ (raw data, not shown). This suggests that wood-derived compounds may suppress thiols, with toasty, woody aromas dominating the more delicate tropical ones.

6.4 Conclusions

In this experiment repetitions, blind duplicates, explained variance, confidence ellipses, and grouping trends were used to establish the reliability of the results. All of these parameters indicated good reliability of the results.

In all PPM experiments, panellists could distinguish wooded from unwooded wines. They were also able to distinguish lightly wooded wines from heavily-wooded and unwooded wines better in PPM than in PM. Among the unwooded wines in all evaluations, there was no clear discrimination. This result does correspond with the recommendations of experimenters who suggest that PM-type analyses are best suited for discriminating based upon the main characteristics of wines, but not well-suited to subtle differences. It is difficult to say whether the poor pairing of unwooded wines was because the differences were too small for this type of analysis, or whether in the context of different samples the perception and placement of a wine will change. It would be interesting to investigate this further by performing PM with only unwooded South African Chenin Blanc wines to see if removing the dominant trait of oak-derived aromas would encourage better discrimination between the other wines.

The descriptors that did drive differences along Dimension 2 within the unwooded wines were ‘pineapple’, ‘passion fruit’, ‘grapefruit’, and ‘fresh green/herbaceous’. All of these descriptors (except for ‘pineapple’) are associated with thiols, and the wines described as ‘passion fruit’ and ‘grapefruit’ were also those with the highest levels of 3MHA. This leads to the hypothesis that thiol levels may be an important character of unwooded Chenin Blanc wines, and an important factor in the differentiation between unwooded wines.

It was reassuring that the use of poles did not artificially force the separation of the wines. In other words, even though three poles were used in the PPM experiments, the MFA configurations did not consist of three separate groups, but rather modelled the same two-group arrangement seen in the PM experiment. Future PPM research could look at applications to other types of wine, and the effect of the position of the pre-located poles on the sheet.

New rapid methods provide significant cost benefits for the wine industry and researchers. For the purposes of method validation only 17 wines were analysed in this set of experiments, but if new samples had been introduced in each experiment (assuming 12 products and 2 blind duplicates), 31 different wines could have been evaluated (10 unique wines in PPM1, and 7 for each subsequent PPM). One issue with data presentation that would have to be addressed is the overcrowding of graphics when different sets are combined.

PPM may not have been successful for differentiating between the unwooded Chenin Blanc wines, but it very consistently separated wooded and unwooded wines with acceptable percentage explained variance and correct groupings of blind duplicates. The overall groupings were also consistent with those found in PM and as such can still be considered a valid method for evaluation of these types of samples. PPM could allow wine researchers to perform sensory evaluation on larger sample sets in a shorter amount of time than is possible with current methods. The success of PPM possibility of analysing large sample sets, even with complex products such as wine, can make this method attractive to researchers.

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Appendix D

Polarized projective mapping (PPM) instructions and additional figures

Appendix D. : Polarized projective mapping (PPM) instructions and additional figures

Polarized projective mapping instructions

You will be evaluating the wines in front of you according to the **similarities** and **dissimilarities** in their **AROMA only**.

You will be asked to mark the positions of the wines **relative to the three poles** on the sheet in front of you so that wines you perceive as **similar** wines are **near (close to each other)**, and wines you perceive as **different** are **distant (far from each other).**

- Familiarize yourself with the three poles. Write the pole number on a post-it. **Smell** the wine and write down **3-5 descriptors per wine** on the post-it. **The poles must not be moved**.
- Then, for the first sample write the 3-digit code of the wine on a post-it. **Smell** the wine and write down **3-5 descriptors per wine** on the post-it.
- Place the wine glass and post-it on the provided paper relative to the poles.
- Write the next wine's code on a new post-it, smell the wine, record your descriptors and place the glass/post-it on the paper according to how similar or dissimilar it is to the other wines.
- You can move the non-pole wines/post-its around as you smell each sample and relate them to one another. You can smell the wines and revise your descriptors as many times as you wish.
- Please use the **whole sheet** to express your opinion in differences among the wines, but stay within the borders of the paper.
- **When you are finished, underneath each glass mark an X on the paper, write down the 3-digit code of the wine, and stick the post-it next to it.**
- Use as much time as you need.
- There are no right or wrong answers. It is important that you use your own criteria to arrange the samples.
- Please do not comment on the wines or discuss them with other panellists.
- Please take a 10-minute break before evaluating the second set of wines.

Have fun, and thank you for your participation!

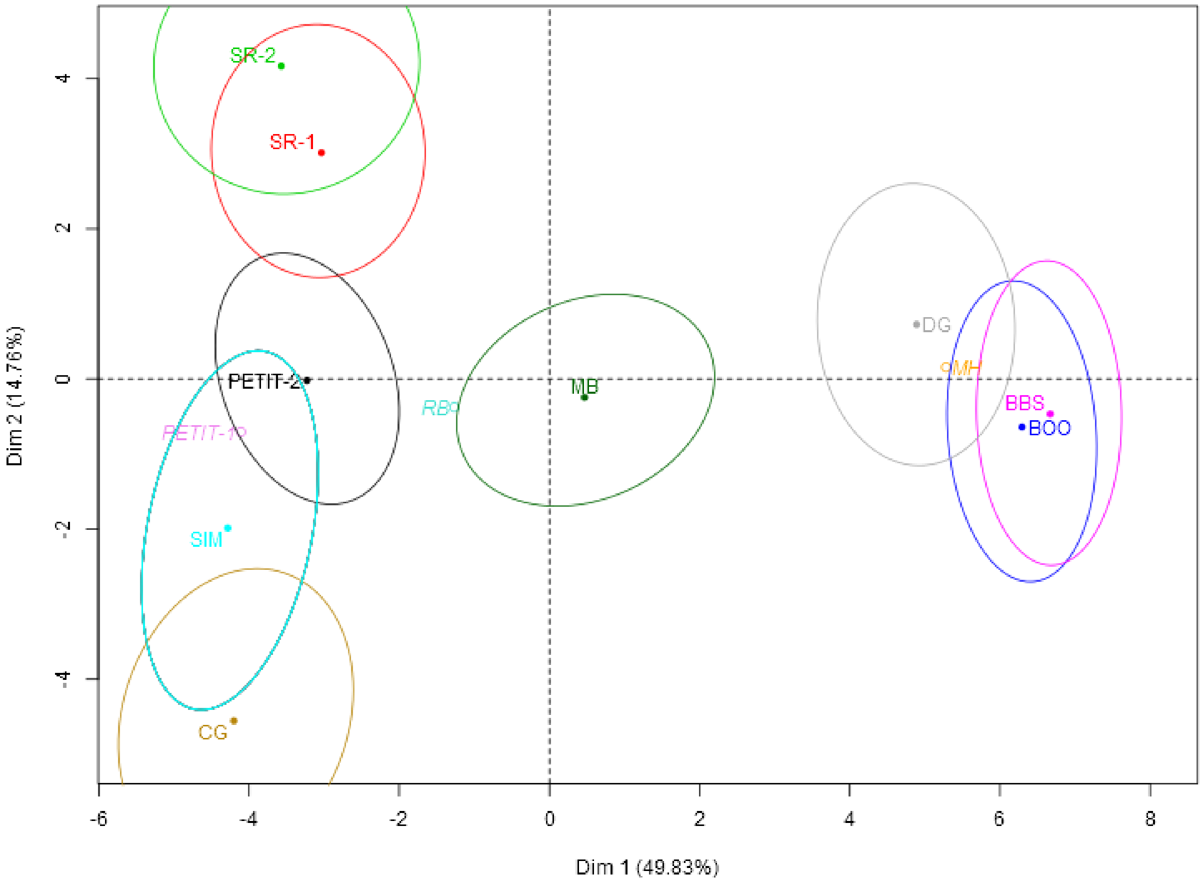

Figure D.1 MFA scores plot of the PPM3 data. Codes ending in -1 or -2 are blind duplicates. The 95% confidence ellipses were created according to Dehlholm *et al.* (2012).

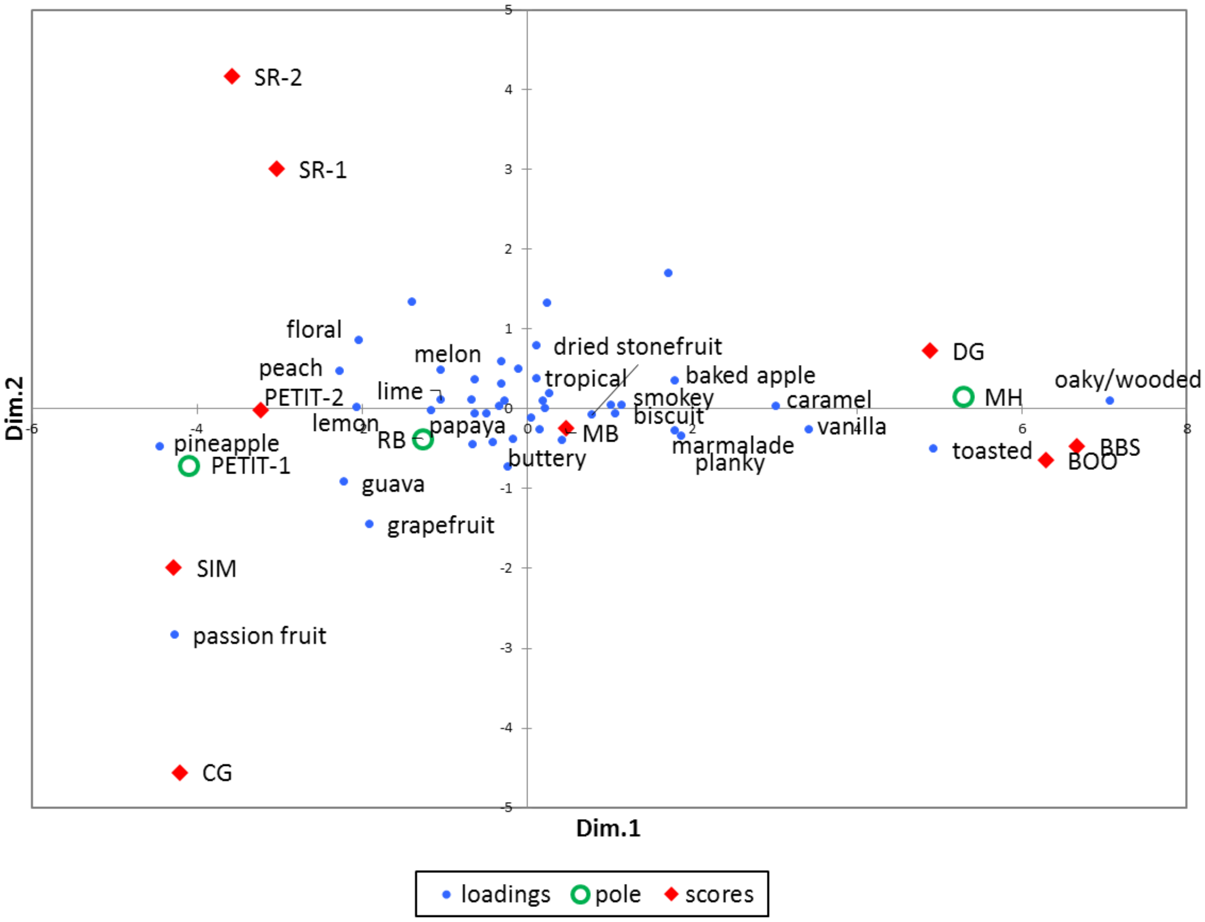

Figure D.2 MFA biplot of the PPM3 data. Descriptors which are well-represented on the MFA (cos2 > 0.6) are included.

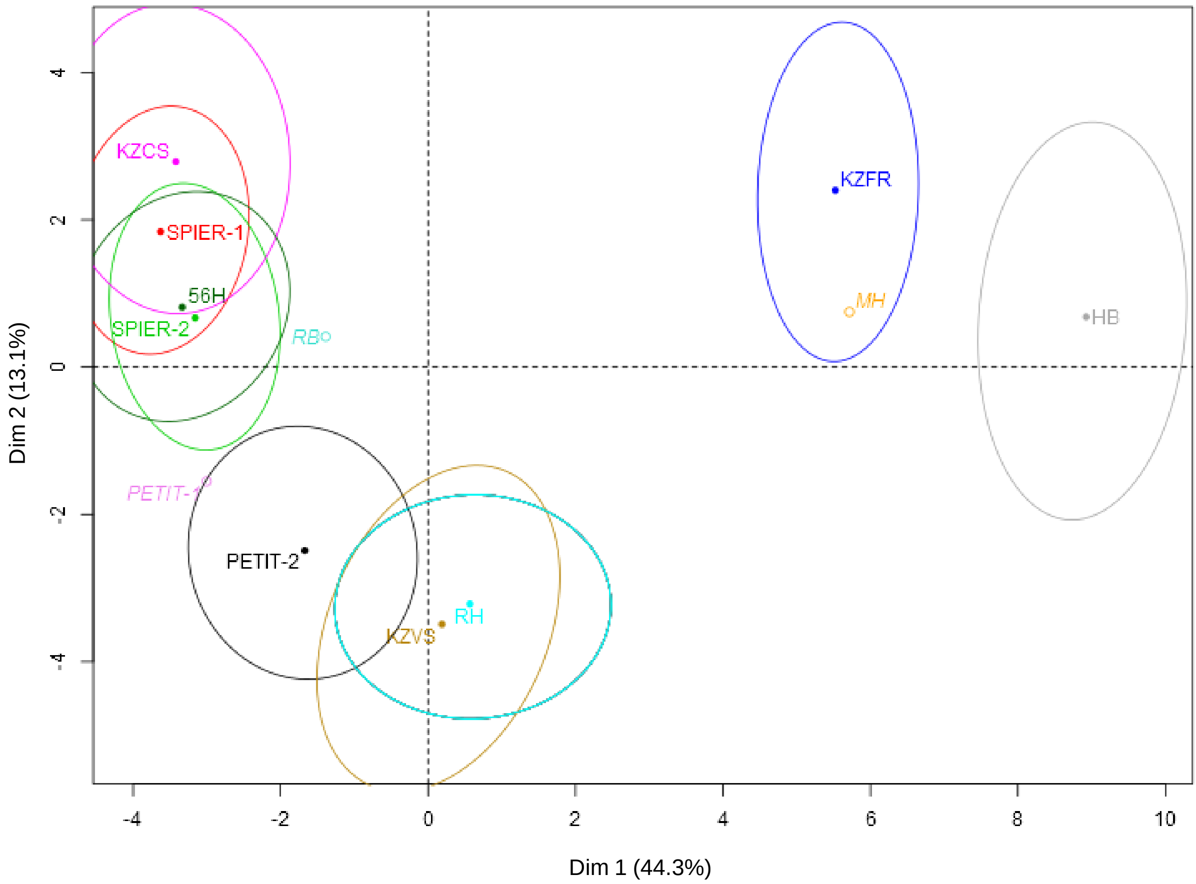

Figure D.3 MFA scores plot of the PPM4 data. Codes ending in -1 or -2 are blind duplicates. The 95% confidence ellipses were created according to Dehlholm et al. (2012).

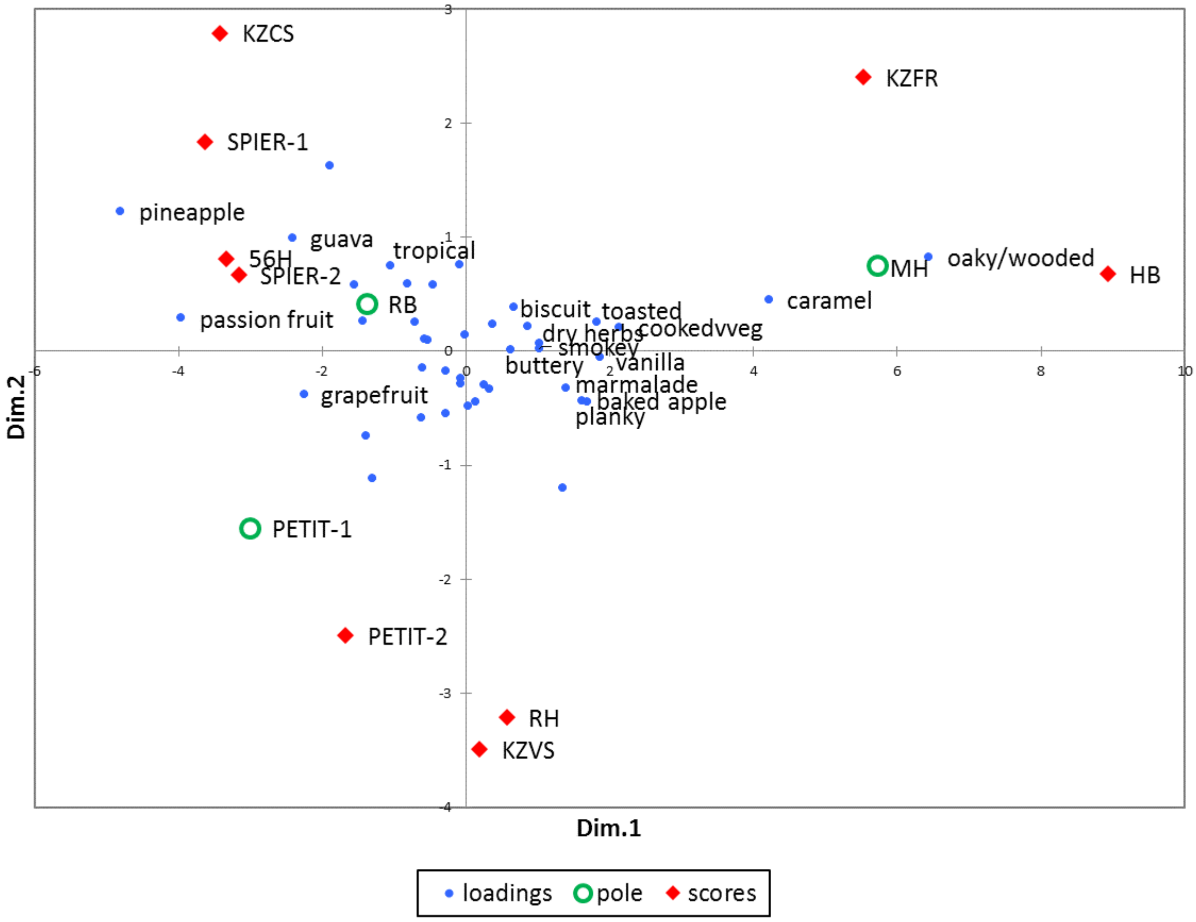

Figure D.4 MFA biplot of the PPM4 data. Descriptors which are well-represented on the MFA (cos2 > 0.6) are included.

Table D.1 3MH and 3MHA levels of the wines evaluated in Chapter 6

Wine	3MHA (ng/L)	3MH (ng/L)
PETIT	27	816
MH	0	739
RB	21	1576
KZFR	15	784
KZCS	33	1024
KZVS	32	757
CG	32	823
SPIER	56	1589
BOO	0	957
BBS	0	827
56H	53	1004
MB	12	856
DG	0	509
HB	0	1937
SIM	72	799
SR	0	1271
RH	0	976

Chapter 7

General discussion and conclusions

Chapter 7 : General discussion and conclusions

7.1 General discussion and conclusions

Volatile thiols have previously been quantified in several cultivars (Chapter 2, Table 2.2), but data on the levels of thiols in commercial Chenin Blanc wines had not been published. The results of the thiol analysis (Chapter 3) showed that both 3-mercaptohexan-1-ol (3MH) and 3-mercaptohexyl acetate (3MHA) were found in South African Chenin Blanc wines at concentrations high enough to impact the aroma of these wines. The average concentrations of 893 ng/L for 3MH and 23 ng/L for 3MHA exceeded their respective odour threshold values and were considered odour active. While in all the wines measured, 3MH was found at levels above its odour threshold, 3MHA was only quantifiable in twenty-four out of sixty-five samples. This finding, in addition to the fact that the maximum odour active value for 3MHA was higher than for 3MH, indicated that 3MHA may be more responsible than 3MH for differences in the thiol-related aromas of these wines.

The combination of chemical data with sensory analysis helped to further understand the sensory impact of volatile compounds on wine aroma. To this end, several sensory experiments were performed using a variety of methodologies. Of the two thiols, 3MHA seemed to have a greater sensorial impact than 3MH, driving the perception of thiols in model wine in Chapter 5, and correlating better with the thiol-related descriptors of commercial wines in Chapter 6. The hypothesis of Du Plessis & Augustyn (1981) that a thiol was responsible for the ‘guava’ aroma characteristic of Chenin Blanc wines was confirmed in Chapters 4, 5, and 6 due to the association of ‘guava’ aroma with 3MH and 3MHA. Thiols in Chenin Blanc matrices were described with various ‘tropical’ and ‘green’ terms previously-established in Sauvignon blanc and model wine with the addition of ‘pineapple’. The ‘pineapple’ attribute was consistently generated for wines spiked with 3MH in Chapter 4 and both thiols in Chapter 5 using different sensory methodologies, different matrices, and different panels.

Knowledge of how thiols interact with other compounds increases the understanding of how they behave as a part of the whole Chenin Blanc matrix. The interaction study in Chapter 4 which included just three of the many volatile compounds present in Chenin Blanc wines showed important interactions within the volatile matrix. Most notably, there was an antagonistic effect between the ‘guava’ character of 3MH and the ‘floral’ attribute of linalool, which was also seen in the commercial wines in the following chapter. Chapter 5 illustrated the importance of both the volatile and non-volatile matrix to sensory perception, as the results in model wine did not correspond with the results in more realistic wine matrices. For researchers, this shows that the choice of the most suitable matrix for sensory studies must be made carefully, considering experimental objectives. Caution must also be taken when extrapolating perception in simple model wine to real wine.

Another potentially important interaction was hypothesized in Chapter 6 due to the chemical and sensorial opposition of ‘oaky’ and ‘tropical’ terms, which suggested a suppression of thiols by wood-related compounds. Considering the prevalence of wood contact in South African Chenin Blanc wines, the effect of wood contact on the chemical concentration and sensory perception of thiols would be an interesting area of further study. In the future, performing interaction studies with methodologies assessing the large-scale interactions of many compounds, such as that used in Ferreira *et al.* (2015) will be of great use to researchers. Rather than dissecting the matrix compound-by-compound, approaches like this can help to understand the contribution of different classes of compounds to the holistic experience of wine aroma. The idea of predicting sensory perception from chemical composition is a distant goal for wine researchers, but one strategy that has been used to more closely link sensory and chemical data is gas chromatography-olfactometry, which could be applied to these wines with interesting results (Campo *et al.*, 2005; Francis & Newton, 2005).

Due to the time and cost required to perform descriptive analysis studies, the innovation and validation of new rapid sensory methodologies provides more accessible options to researchers. In this work, a rapid method with a new modification was used for an interaction study, and another rapid method was performed with wine for the first time. Adding an intensity rating to the ultra flash profiling method allowed for a greater understanding of the drivers between groupings in the MFA, and would be a potentially useful addition to any PM study. As a suggestion for future research, performing a flash profile prior to PM with intensity could provide clearer results by standardizing the list of descriptors rated. Previously, polarized projective mapping (PPM) had only been applied to a simple product set (Ares *et al.*, 2013), but the results of Chapter 6 show that with careful selection of the poles, PPM can be applied to a product as complex as wine. This means that several sample sets can be evaluated using a rapid method, and combined into a single statistical representation. Therefore, this method provides a solution to sample size limitations of current methods and opens up feasible avenues for the characterization of large sets of wines.

There are several other areas of future study that should follow this research. As the ultimate goal of most wine research is the ability to create more enjoyable wines, an important next step in sensory research would be to perform consumer studies to determine what levels of thiols in South African Chenin Blanc are optimal for different segments of consumers. A limitation of the thiol analysis method used is that out of all the different thiol compounds identified in wine, only 3MH and 3MHA were measured. However, other thiols like 4-methyl-4-mercapto-pentan-2-one (4MMP) have been found above odor threshold in several varieties (Chapter 2, Table 2.2) and their presence or absence in Chenin Blanc wines could further the understanding of thiols in wines. Considering the instability of 3MHA, it will also be important to measure thiols in juice and younger wines and replicate studies performed on Sauvignon Blanc on the evolution and stability of thiols through the aging process (Herbst-Johnstone *et al.*, 2011). This, together with greater knowledge of the precursors responsible for these compounds will develop understanding of how thiols form and change over time in Chenin Blanc Wines.

A greater understanding of the chemical drivers of wine aromas can help the industry create wines better tailored to target markets. The knowledge that thiols are present in South African Chenin Blanc wines and contribute to aroma of these wines prompts winemakers to consider how their practices affect thiols, and explore ways they can promote or suppress thiols to change the aromas of their wines. Very little research addressing the effects of winemaking practices on thiols in Chenin Blanc has been performed (Weightman, 2014; Aleixandre-Tudo *et al.*, 2015). Until more studies focus on thiols in Chenin Blanc, research performed on Sauvignon Blanc can help guide the industry. A summary of factors known to affect thiols in Sauvignon Blanc (and potentially Chenin Blanc), such machine harvesting, *Botrytis cinerea* infection, fermentation temperature, SO_2, and nitrogen content can be found in Coetzee & du Toit (2012). Future research tailored to Chenin Blanc regarding the impact of viticultural and oenological practices on thiols will support the industry in crafting their ideal South African Chenin Blanc wines.

7.2 References

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