Linking sensory attributes to selected aroma compounds in South African Cabernet Sauvignon wines

by

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Summary

Cabernet Sauvignon is a widely planted red grape cultivar which produces worldwide, some of the finest and most expensive red wines. The typical aroma of Cabernet Sauvignon wines is often described as either fruity and berry-like or vegetative, herbaceous and green; the latter descriptors are often considered undesirable. High levels of 2-isobutyl-3-methoxypyrazine (ibMP), a powerful grape-derived compound, have been associated with greener notes in Cabernet Sauvignon wines. Over the past two decades, extensive research has been conducted worldwide to identify the active odorants that impact the aromatic profiles of Cabernet Sauvignon wines. These compounds are mostly higher alcohols, esters, C_{13}-norisoprenoids, methoxypyrazines, sulphur compounds and certain terpenes. More recent studies have endeavoured to establish a relationship between the sensory analysis and chemical composition of these wines as it could help to explain the impact of certain odorants on the perception of either fruity or herbaceous notes. Despite the interest shown by the South African wine industry to improve the quality of Cabernet Sauvignon wines, no such study has been conducted in South Africa yet.

The first part of this study gives an overview of the major active aroma compounds which have been identified in Cabernet Sauvignon wines with a particular focus on volatile compounds that could exhibit either fruity berry notes or herbaceous/vegetative notes. Some of the findings of studies conducted in Australia and the United States are also discussed.

The second part of this study investigates the relationship between the volatile composition and sensory properties in 13 mono-varietal Cabernet Sauvignon wines produced in South Africa. The wines were selected to represent a broad range of fruity and herbaceous sensory attributes and were assessed by descriptive analysis. A limited number of volatile compounds (33 in total) that could contribute to either fruity or herbaceous characters, as indicated in the literature, were analysed using either headspace solid phase micro extraction (SPME) and gas chromatography–ion trap mass spectrometer detection (HS-SPME-GC-ion trap-MS analysis) or solid phase extraction (SPE) and gas chromatography coupled with a triple quadrupole detector (SPE-GC-MS/MS analysis). The statistical treatment by multiple factor analysis (MFA) of both compositional data and sensory data showed that certain volatile compounds such as β-damascenone, β-ionone, dimethylsulphide (DMS) and ibMP predicted well some of the aroma attributes used to describe the selected wines.

It was found that the analysis of β-damascenone, β-ionone, 3-mercaptohexyl acetate, dimethyl sulphide and ibMP could be of interest for winemakers wanting to explain certain typical aroma descriptors characterising South African Cabernet Sauvignon wines.

Opsomming

Cabernet Sauvignon is ‘n algemeen aangeplante rooidruif-kultivar wat wêreldwyd sommige van die beste and duurste rooiwyne produseer. Die tipiese aroma van Cabernet Sauvignon-wyne word gereeld as óf vrugtig en bessieagtig óf vegetatief, kruidagtig en groen beskryf; laasgenoemde beskrywende terme word in baie gevalle as ongewens beskou. Hoë vlakke van 2-isobutiel-3-metoksipirasien (ibMP), ‘n kragtige druifafgeleide verbinding, is reeds met die groener note in a Cabernet Sauvignon-wyne geassosieer. Oor die afgelope twee dekades is breedvoerige navorsing wêreldwyd onderneem om die aktiewe geurstowwe wat die aromatiese profiele van Cabernet Sauvignon-wyne beïnvloed, te identifiseer. Hierdie verbindings is meesal hoër alkohole, esters, C_{13}-norisoprenoïede, metoksipirasiene, swaelverbindings en sekere terpene. Meer onlangse studies het gepoog om ‘n verhouding te bepaal tussen die sensoriese analise en chemiese samestelling van hierdie wyne, aangesien dit sou kon bydra tot die verklaring van die impak van sekere geurstowwe op die waarneming van óf vrugtige óf kruidagtige note. Ten spyte van die belangstelling wat deur die Suid-Afrikaanse wynbedryf daarin getoon is om die kwaliteit van Cabernet Sauvignon-wyne te verbeter, is geen sulke studies tot op hede in Suid-Afrika onderneem nie.

Die eerste deel van hierdie studie verskaf ‘n oorsig van die vernaamste aromaverbindings wat reeds in Cabernet Sauvignon-wyne geïdentifiseer is, met ‘n spesifieke fokus op vlugtige verbindings wat óf vrugtige bessienote of kruidagtige/vegetatiewe note kon vertoon. Sommige van die bevindings van studies wat in Australië en die VSA onderneem is, word ook bespreek.

Die tweede deel van hierdie studie ondersoek die verhouding tussen die vlugtige samestelling en sensoriese eienskappe in 13 enkelvariëteit Cabernet Sauvignon-wyne wat in Suid-Afrika geproduseer is. Die wyne is gekies om ‘n breë verskeidenheid van vrugtige en kruidagtige sensoriese eienskappe te verteenwoordig en is deur middel van beskrywende analise geassesseer. ‘n Beperkte aantal vlugtige verbindings (33 in totaal) wat óf tot die vrugtige óf die kruidagtige karakters kon bydrae, soos in die literatuur aangedui, is deur middel van óf lugspasie-analise (*headspace solid phase micro extraction (SPME)*) en gaschromatografie–*ion trap* massaspektrometrie waarneming (*gas chromatogoraphy–ion trap mass spectrometer detection (HS-SPME-GC-ion trap-MS)*) óf soliede fase ekstraksie (*solid phase extraction (SPE)*) en gaschromatografie tesame met ‘n a *triple quadrupole detector* (SPE-GC-MS/MS analise). Die statistiese behandeling deur veelvuldige faktor-analise (*multiple factor analysis (MFA)*) van beide die kompositoriese data en die sensoriese data het getoon dat sekere vlugtige verbindings, soos β-damaskenoon, β-ionoon, dimetielsulfied (DMS) en ibMP, sommige van die aroma-eienskappe wat gebruik word om die geselekteerde wyne te beskryf, goed voorspel het.

Daar is gevind dat die analise van β-damaskenoon, β-ionoon, 3-merkaptoheksiel asetaat, dimetielsulfied en ibMP van belang kan wees vir wynmakers wat sekere van die tipiese aromabeskrywers wil verklaar wat Suid-Afrikaanse Cabernet Sauvignon-wyne karakteriseer.

This thesis is dedicated to Pieter.

Biographical sketch

Emmanuelle Lapalus was born in Chatillon sur Chalaronne France on 1 December 1971. She obtained a 3 year degree in Chemistry at the University of Dijon (France) in 1994 and obtained her HonsBSc-degree in Wine Biotechnology in 2012. She specialized in gas- and liquid chromatography and currently works as a GC/HPLC analyst at VinLAB Pty Ltd.

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Preface

This thesis is presented as a compilation of four chapters.

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Chapter 1

Introduction and project aims

1 Introduction

Vitis vinifera L. cv. Cabernet Sauvignon cultivar is internationally known for the prestigious wines produced from it in the Bordeaux region of France where it originates. Cabernet Sauvignon vines have the ability to grow in a variety of climates and soil types (Carey *et al*., 2008) which explains why Cabernet Sauvignon has become a popular, widespread red grape variety in many other wine producing countries including Australia, the United States of America, South Africa and recently China (Carey *et al*., 2008; Tao & Li, 2009; Robinson *et al*., 2011, Hjelmeland *et al*., 2013). Although Cabernet Sauvignon is a versatile grape cultivar which can thrive in various climatic conditions, it performs at its best in warm regions with well-drained soils. (Roujou de Boubée *et al*., 2000; Oberholster *et al*., 2010). High quality grapes produce tannic wines with an intense dark red colour which often exhibits red berry, black berry and spicy aromas (Oberholster *et al*., 2010). In cooler climates, Cabernet Sauvignon wines tend to develop greener notes described as green pepper, mint and cut grass which are perceived as a lack of ripeness and can be detrimental to their quality (Allen *et al*., 1994; Allen & Lacey, 1998; Roujou de Boubée *et al*., 2000). The overall perceived aroma of wines derives directly or indirectly from the grape composition at the time of harvest (Carey *et al*., 2008; Polášková *et al*., 2008). Thus, a great deal is done in the vineyard so that the grapes reach optimum maturity, translating into the optimal chemical composition (including colour, sugar levels and amino acids) at the time of harvest to produce Cabernet Sauvignon wines that are fruitier and still present an intense darker colour (Oberholster *et al*., 2010).

Worldwide, the sensory evaluations conducted on Cabernet Sauvignon wines have often led to two different sets of descriptors: one characterised by fruity, berry notes and the other by vegetative/herbaceous notes (Heymann & Noble, 1987; Chapman *et al*., 2005; Robinson *et al*.; 2011). Moreover, gas chromatography-olfactometry (GC-O) techniques have helped to identify important impact odorants of Cabernet Sauvignon wines (Lopez *et al*., 1999; Kotseridis & Baumes, 2000; Gùrbùz *et al*., 2006; Falcao *et al*., 2008). The volatile compounds that contribute to the fruity notes are ethyl esters, 2-phenyl ethanol, β-ionone, β-damascenone and 3-mercaptohexan-1-ol (Kotseridis & Baumes, 2000). The vegetative, herbaceous notes described as green pepper, cut grass and mint have been attributed to methoxypyrazines and especially 2-isobutyl-3-methoxypyrazine (ibMP) (Allen *et al*., 1994; Allen & Lacey, 1998; Roujou de Boubée *et al*., 2000), but also to some aldehydes and alcohols C_6 and C_9 derivatives such as hexanol, cis-3-hexenol and nona-2,6-dienal (Kotseridis & Baumes, 2000; Kalua & Boss, 2009; Callejón *et al.,* 2012) and certain monoterpenes such as 1,8-cineole (Capone *et al*., 2011).

Recent studies conducted in Australia and the United States of America have investigated the relationship between sensory attributes and wine composition in Cabernet Sauvignon wines. The authors were particularly interested in the fruity/berry notes and the herbaceous/vegetative notes and how they linked to the chemical composition of these wines (Robinson *et al*.; 2011; Hjelmeland *et al*., 2013; Bindon *et al*., 2014). Despite the fact that Cabernet Sauvignon is one of the most planted red grape variety in South Africa (SAWIS, 2014), grown to produce some of its most expensive and iconic wines, such research has not yet been conducted in South Africa. Little has been published on the perceptual aromatic properties and chemical composition of South African Cabernet Sauvignon wines. A better knowledge thereof could benefit the wine industry and help to produce Cabernet Sauvignon wines with more desirable perceptual properties.

2 Project aims

The aim of this study was mainly to investigate the relationship between the sensory attributes and the volatile composition of a selected number of South African Cabernet Sauvignon wines. The specific aims were as follows:

(i) select single mono varietal Cabernet Sauvignon wines that exhibit a broad range of herbaceous or fruity notes,

(ii) characterise the aroma profiles of the selected wines by descriptive analysis,

(iii) select previously reported aroma-active components, arising mostly from grape composition and yeast metabolism that are responsible for, either the fruity notes or the herbaceous/vegetative notes,

(iv) analyse and quantify the selected aroma compounds in the different wines using gas-chromatography, and

(v) investigate the relationship between sensory attributes and selected chemical compounds.

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Chapter 2

Literature review

Linking volatile composition to sensory attributes in Cabernet Sauvignon wines

1 Introduction

Wine aroma is the result of a complex mixture of chemical compounds derived from grapes, yeast and bacterial metabolism during vinification and, if used, oak wood during barrel ageing (Francis & Newton, 2005). Grape quality at the time of harvest is the foundation for the production of quality wines (Oberholster *et al*., 2010). The grape berry provides most of the substrates needed for the yeast and lactic acid bacteria to function: sugars, fatty acids, nitrogen and sulphur-containing compounds are metabolised into volatile compounds (Callejón *et al*., 2012). Grapes also contain odourless precursors that can be released by the yeast during fermentation. Grape composition depends on the grape variety (Hernandez-Ortez *et al*., 2002) and environmental and viticultural conditions, which include the type of soil, pruning and training systems, density of plantation, etcetera, all of which can have a strong influence on grape composition at véraison and on variations in ripening (Robinson *et al*., 2014).

Worldwide, Cabernet Sauvignon has become very popular and often produces some of the most expensive wines (Tao & Li, 2009; Robinson *et al*.; 2011, Hjelmeland *et al*., 2013). Cabernet Sauvignon is the most widely planted red grape in the United States (Hjelmeland *et al*., 2013), it was ranked the third most planted grape variety in Australia in 2009 (Robinson *et al*., 2011) and accounted for 72% of the total grape-producing areas in China (Tao & Li, 2009). In 2013, 11.7% of the area under vines in South Africa was planted to Cabernet Sauvignon, making it the predominant red cultivar in the country (SAWIS, 2014).

Cabernet Sauvignon wines are often characterised by two antagonistic aromatic profiles: one with fruity, berry-like aromas and the other with vegetative, herbaceous aromas (Heymann & Noble, 1987; Chapman *et al*., 2005; Carey *et al*., 2008; Preston *et al*., 2008; Robinson *et al*., 2011; Bindon *et al*., 2013a). Worldwide, studies have been conducted to establish relationships between the sensory attributes and chemical composition of Cabernet Sauvignon wines (Kotseridis & Baumes, 2000; Ferreira *et al*., 2000; Falcao *et al*., 2008; Escudero *et al*., 2007; Robinson *et al*., 2011; Forde *et al*., 2011).

The active odorants that have been characterised in these studies belong to different chemical groups, consisting mostly of higher alcohols, esters, C_{13}-norisoprenoids, methoxypyrazines, sulphur compounds, aldehydes and terpenes. This literature review will thus focus on the prevalent volatile aroma compounds that have been characterised in Cabernet Sauvignon wines and how they relate to the sensory composition of the wines.

2 Volatile compounds contributing to fruity, berry-like aromas.

Cabernet Sauvignon wines are often described as exhibiting fruity aromas, such as fresh cherry, red or black berry, jam/cooked berry, cooked fruit, dried fruit and raisin (Heymann & Noble, 1987; Chapman *et al*., 2005). The compounds that have been associated, directly or indirectly, with the fruity aromas in Cabernet Sauvignon wines are mostly higher alcohols, esters, C_{13}-norisoprenoids and sulphur compounds.

2.1 Higher alcohols

Higher alcohols are alcohols containing more than two carbon atoms. They are quantitatively one of the most important groups of secondary metabolites formed during alcoholic fermentation. Their concentrations in wine range from less than 100 mg/l to 300 mg/l and above, with white wines generally exhibiting the lowest levels. Levels below 300 mg/l contribute positively to the aromatic profile of the wine, while higher levels impact negatively (Ugliano & Henschke, 2009).

Sugar levels, yeast strain, aeration and fermentation temperature are factors to consider in the production of higher alcohols, but the amino acid composition of the must certainly plays the most important role. Moreover, each grape variety presents a relatively characteristic amino acid profile that will determine the eventual volatile composition of the wine (Hernandez-Ortez *et al*., 2002). Higher alcohols are formed from two intertwined pathways that produce α-keto acids as intermediates for the degradation of amino acids (Ehrlich reaction) or their biosynthesis from glucose (anabolic pathway). The availability or deficiency of amino acids in the must determines which pathway will be used during yeast growth (Lambrechts & Pretorius, 2000; Ugliano & Henschke, 2009). Higher alcohols are constituted of either aliphatic or aromatic alcohols, with propanol, isobutanol, isoamyl alcohol and 2-phenylethanol being the major congeners found in wine.

Two higher alcohols that have been characterised as active odorants in Cabernet Sauvignon wines in a number of studies are 2-phenylethanol (or phenethyl alcohol) and 3-methyl-1-butanol (Lopez *et al*., 1999; Ferreira *et al*., 2000; Kotseridis *et al*., 2000; Falcao *et al*., 2008). 2-phenylethanol has a honey, rose-like aroma and plays an important role in Cabernet Sauvignon wines' aromatic properties when found at above threshold levels (Falcao *et al*., 2008). 2-phenylethanol is produced from phenylalanine by the Ehrlich reaction and its concentration depends on the yeast strain. Higher pH and fermentations at 15 or 25°C rather than 35°C yield higher levels (Rankine & Pocock, 1969). Table 1 lists the major higher alcohols, their sensory thresholds and concentrations that have been detected in Cabernet Sauvignon wines.

Table 1: Main higher alcohol congeners, their sensory thresholds and concentrations found in Cabernet Sauvignon wines.

Higher alcohols	Aroma	Odour threshold (µg/l)	Concentrations in Cabernet Sauvignon wines (µg/l)
1-propanol	Ripe fruit	50 000[1a]	5 824-20 395[4]
isobutanol	Solvent-like	40 000[2a]	31 005-105 212[4]
1-butanol	Powerful, fresh, green grass odour	150 000[1a]	1 556-4 712[4]
Isoamyl alcohol (3-methyl-1-butanol)	Whiskey, malt, marzipan	30 000[2a]	164 391-567 524[4] / 179 000-205 000[5]
2-phenylethanol (Phenethyl alcohol)	Honey, rose	14 000[3b]	30 783-140 086[4] / 42 730-90 160[5]

1 Cullere *et al*., 2004; 2 Guth 1997; 3 Ferreira *et al*., 2000; 4 Tao & Zhang, 2010; 5 Bindon *et al*., 2013b
a In water/ethanol (90+10,w/w); b In model wine

2.2 Esters

Some volatile esters are synthesised in Cabernet Sauvignon grapes throughout the stages of berry development, but their contribution to wine aroma is not significant (Kalua & Boss, 2009). Esters found in Cabernet Sauvignon wines are mostly secondary metabolites of the fermentation. Although volatile esters are present at lower concentrations compared to higher alcohols, they have a greater impact on the wine aroma due to their lower odour thresholds.

Esters are divided into two groups: acetic esters of higher alcohols and ethyl esters of fatty acids. Acetate esters are the result of the reaction of acetyl-CoA with higher alcohols. The higher alcohols are formed from the degradation of amino acids, while hexanol is formed through the lipoxygenase pathway activated at crushing (Joslin & Ough, 1978). Acetate esters have intense fruity aromas: isoamyl acetate has an aroma of banana and hexyl acetate has an aroma reminiscent of apple. Fatty acid ethyl esters are the products of the ethanolysis of the acyl-CoA formed during fatty acid synthesis (ethyl lactate occurs after malolactic fermentation from the formation of lactic acid) or degradation. Both groups of esters contribute to the fruitiness of wine aroma (Ugliano & Henschke, 2009).

Branched ethyl esters, such as ethyl 2-methylbutanoate, ethyl 3-methylbutanoate, ethyl 2-, 3-, 4-methypentanoate, and one cyclic ester, namely ethyl cyclohexanoate, have recently been identified as being important contributors to the sweet-fruity aroma in wine (Escudero *et al*., 2007; Pineau *et al*., 2009). Recent studies show that the fruity aroma in wine arises from a collective contribution, rather than individual contributions by esters. In one study, the berry fruity notes of red wines were related to the addition effect of nine fruity esters (Escudero *et al*., 2007). Pineau *et al*. (2009) reported that blackberry aromas in red wines were associated with higher than average levels of ethyl propanoate, ethyl 2-methylpropanoate and ethyl 2-methylbutanoate while red berry aromas were associated with ethyl butanoate, ethyl hexanoate, ethyl octanoate and ethyl 3-hydroxybutanoate.

Concentrations of esters are dependent on grape variety, grape maturity, yeast strain, temperature of fermentation and the juice amino acid content (Ugliano & Henschke, 2009). Enzymatic hydrolysis of esters occurs during fermentation and chemical hydrolysis occurs during storage and ageing (Lambrechts & Pretorius, 2000). The esters having an important impact on the aromatic properties of Cabernet Sauvignon wines are listed in Table 2.

Table 2: Important esters found in Cabernet Sauvignon wines and their odour thresholds.

Esters	Aroma	Odour threshold (μg/l)	Concentrations reported in Cabernet Sauvignon wines (μg/l)
Ethyl esters of fatty acids			
Ethyl butyrate	Papaya, apple	600[1a]	530–1 941[4]
Ethyl hexanoate	Green apple	440[1a]	373–1 315[4]
Ethyl octanoate	Pear	960[1a]	125–741[4]
Ethyl decanoate	Grape	200[2b]	203-402[1] / 4–109[4]
Ethyl lactate	Raspberry	154 000[3c]	43 284–237 415[4]
Ethyl 2-methylbutanoate	Fruity	5600[1a]	162-426[1] / 9.2-32[3]
Ethyl 3-methylbutanoate	Fruity, anise	3[2b]	20-25[3]/14,4–27,3[5]
Acetate esters			
Isobutyl acetate	Solvent	2100[1a]	70-180[4]
Isoamyl acetate	Banana	1830[1a]	205–2 784[4]
Phenethyl acetate	Roses	250[2b]	83–490[4] / 70–165[5]
Hexyl acetate	Pear	1500[1a]	7–19[4] / 57–77[5]

1 Pineau *et al*., 2009, 2 Ferreira *et al*., 2000, 3 Escudero *et al*., 2007, 4 Tao & Zhang, 2010; 5 Bindon *et al*., 2013b
a In dearomatized red wine b In a synthetic wine; c In wine

2.3 C_{13}-norisoprenoids

C_{13}-norisoprenoids are grape-derived compounds that are formed from the degradation of carotenoids. They are present in the berry as non-volatile, non-odorant glycosidic compounds (Baumes *et al*., 2002). Marais *et al*. (1999) showed that light exposure and leaf removal increase the concentration of C_{13}-norisoprenoids in the grapes. Carotenoids are synthesised between berry set and véraison, then degrade from véraison to maturity, producing C_{13}-glycosylated norisoprenoids. Due to their low odour thresholds, the C_{13}-norisoprenoids are among the most potent aromatic components found in wine and contribute greatly to floral and fruity notes in red and white wines (Schwab *et al*., 2008).

β-damascenone and β-ionone are two major C_{13}-norisoprenoids found in wine. β-damascenone has an apple, rose and honey aroma, while β-ionone has a seaweed, violet and raspberry aroma (Francis & Newton, 2005). Both are stored in the berry as odourless glycosylated precursors and are released under the acidic conditions of the must and through fermentation.

At maturity, C_{13}-norisoprenoids are more abundant in berries exposed to sunshine than in shaded berries (Baumes *et al*., 2002). β-damascenone is the result of the degradation of neoxanthine, and β-ionone is a secondary metabolite of the degradation of β-carotene.

The analysis of β-ionone in red wines from the Bordeaux region showed that it is an important impact odorant. Its levels in the berry tend to decrease during ripening, but the levels found in wine are higher than or near to its odour threshold estimated at 90 ng/l in a model wine (Kotseridis *et al*., 1999b). Reported levels of β-ionone in Cabernet Sauvignon wines vary from 0.08 to 0.37 μg/l (Kotseridis *et al*., 1999b; Falcao *et al*., 2008).

β-damascenone has been identified as being an active odorant in Cabernet Sauvignon wines in many studies (Kotseridis *et al*., 1999c; Lopez *et al*., 1999; Falcao *et al*., 2008; Tao & Li, 2009). Higher levels of β-damascenone impart peach or canned apple notes which positively benefit the aromatic properties of Cabernet Sauvignon wines (Ferreira *et al*., 2000; Falcao *et al*., 2008). According to Pineau *et al*. (2007) β-damascenone mostly acts as an enhancer of red fruit aroma and its odour threshold in red wine probably ranges from 2 to 7 μg/l. Reported levels of β-damascenone in Cabernet Sauvignon wines vary from 1.25 to 17.7 μg/l (Pineau *et al*., 2007; Falcao *et al*., 2008; Bindon *et al*., 2013b).

2.4 Sulphur compounds

Sulphur compounds are present in wine as sulphides, polysulphides, heterocyclic compounds, thioesters and thiols. They have low odour thresholds (from low ppt to low ppb levels) and thus account for some of the most potent odorants in wines (Mestres *et al*., 2000).

Sulphur compounds originate from two main processes that are either enzymatic (degradation of sulphur-containing amino acids, formation of fermentation products and metabolism of sulphur-containing pesticides) or non-enzymatic (photochemical, thermal and other reactions during winemaking and storage) (Mestres *et al*., 2000). Sulphur compounds present different olfactory qualities: some sulphur-containing compounds cause reductive aroma characters ranging from onion to cabbage and burnt rubber, while others, like 4-mercapto-4-methylpentan-2-one, 3-mercaptohexan-1-ol and 3-mercaptohexyl acetate, are impact odorants contributing to the varietal characteristics of certain wines (Mestres *et al*., 2000; Coetzee & Du Toit, 2012).

2.4.1 Reductive sulphur compounds

Reduction in wine is associated with sulphur compounds having aromas reminiscent of rotten egg, cabbage, onion, garlic and burnt rubber. The main volatile sulphur compounds responsible for these off-odours include H_2S, methanethiol, ethanethiol, dimethyl sulphide, and other sulphides and disulphides (Park *et al*., 1994; Mestres *et al*., 2000). H_2S acts as an intermediate product in the sulphate reduction sequence (SRS) pathway, which is activated to feed the metabolic demand for cysteine and methionine, two sulphur-containing amino acids. The yeast cell utilises sulphate and sulphite readily present in must to synthesise sulphur-containing amino acids. When there is a deficiency of nitrogen and precursors of sulphur amino acids (O-acetylhomoserine and O-acetylserine), H_2S is no longer metabolised by the yeast cell and it starts accumulating in the must. During and after fermentation, H_2S reacts with ethanol and methanol to form the corresponding mercaptans: methanethiol and ethanethiol (Swiegers & Pretorius, 2007).

Dimethyl sulphide (DMS) is another low molecular weight sulphur compound linked to reduction in wine. To date, the pathways leading to the production of dimethyl sulphide in wine have not been elucidated fully. It is thought to be formed by the yeast during fermentation from sulphur-containing amino acids such as cysteine, cystine and glutathione. Cysteine supplements in a culture medium subjected to fermentation by *Saccharomyces cerevisiae* led to the production of DMS (De Mora *et al*., 1986). The levels of DMS found in young wines are usually low and below its perception threshold, which is 27 µg/l in red wines (Segurel *et al*., 2004). Levels of DMS increase during ageing and wine storage as a result of the degradation of dimethyl sulphoxide (DMSO) and of S-methyl-L-methionine (Swiegers *et al*., 2005). In storage experiments, bottled wines that had been spiked with DMSO and cysteine presented increased levels of DMS, indicating that DMSO is a potential precursor of DMS during bottle ageing (De

Mora *et al*., 1993). In a survey screening 77 Californian wines, dimethyl sulphide (DMS) was found to be the most widely distributed and most abundant sulphur-containing compound (Park *et al*., 1994). DMS has an aroma reminiscent of asparagus, cooked corn and molasses (Swiegers *et al*., 2005). However, some authors have reported that low levels of DMS exhibit herbaceous, vegetal and quince-like aromas (Mestres *et al*., 2000) and have the ability to enhance the fruity notes of red wines (Segurel *et al*., 2004; Escudero *et al*., 2007).

2.4.2 Varietal thiols

Varietal thiols are a group of sulphur compounds with extremely low odour thresholds, accounting for some of the most powerful aroma notes found in wine. 4-mercapto-4-methyl-pentan-2-one (4MMP), 3-mercaptohexyl acetate (3MHA) and 3-mercaptohexan-1-ol (3MH) have been identified as three major aroma compounds contributing to the varietal aroma of Sauvignon Blanc wines (Darriet *et al*., 1995; Tominaga *et al*., 1998a).

Bouchilloux *et al*. (1998) identified 3MH and 3MHA in the Bordeaux red wine varieties Merlot and Cabernet Sauvignon, and noted that the aromatic complexity of a Cabernet Sauvignon or Merlot wine was significantly decreased by the simple addition of copper sulphate. These compounds exhibit powerful aromas, ranging from black currant bud (4MMP) to grapefruit (3MH) and passion fruit and box tree (3MHA) (Roland *et al*., 2011).

4MMP and 3MH are almost non-existent in grape juice and are released during fermentation from odourless non-volatile precursors synthesised in the grape berry (Dubourdieu *et al*., 2006). 3MHA arises from the acetylation of 3MH during fermentation by the action of the yeast ester, forming alcohol acetyltransferase (Roland *et al*., 2011). Three metabolic pathways leading to the production of 4MMP and 3MH have been identified (Roland *et al*., 2011). Two of these pathways are shared by 4MMP and 3MH and involve cysteinylated and glutathionylated precursors (Tominaga *et al*., 1998a; Peyrot des Gachons *et al*., 2002). The third pathway leading to the formation of 3MH involves trans-2-hexenal as well as trans-2-hexenol, with H_2S as a sulphur donor (Harsch *et al*., 2013). Experimental trials show that a delay in the metabolisation of both trans-2-hexenol and trans-2-hexenal, combined with sufficient levels of H_2S, could significantly increase the production of 3MH. However, these conditions are not easily met in commercial fermentations, as H_2S would have to be produced timeously to react with both C_6 derivatives before they are metabolised in the early stages of fermentation (Harsch *et al*., 2013).

The levels of precursors formed in the berry depend on a number of parameters, such as soil, microclimate, maturity and operations prior to fermentation (Murat *et al*., 2001a), and only a small percentage of the precursors available in the must are released as volatile thiols during fermentation (Murat *et al*., 2001b; Dubourdieu *et al*., 2006). The yeast strains and species play a decisive role in the levels of 4MMP and 3MH released in wine (Murat *et al*., 2001a; Howell *et al*., 2004; Dubourdieu *et al*., 2006), as well as in the conversion of 3MH into 3MHA. The temperature of fermentation also has a strong effect on the production of 4MMP; some strains are able to release up to 100-fold more 4MMP than others when fermenting at 28°C rather than at 18°C (Howell *et al*., 2004). The cysteinylated precursor of 3MH is mainly located in the skins of Merlot and Cabernet Sauvignon grapes (Murat et al., 2001b), and the cysteinylated precursor of 4MMP is present in both the skin and the pulp (Peyrot des Gachons *et al*., 2002). Prolonged juice-skin contact increases the content of the precursor in the must, and this is even more so at higher temperatures (Murat *et al*., 2001b). The thiols are released during fermentation, probably from a *β*-lyase activity of *Saccharomyces cerevisiae* (Peyrot des Gachons *et al*., 2000).

Table 3 lists some sulphur compounds that have an aromatic impact on Cabernet Sauvignon wines, along with their sensory thresholds and concentrations.

Table 3: Sulphur compounds having a positive aromatic impact on Cabernet Sauvignon wines.

Sulphur compounds	Aroma	Odour threshold (µg/l)	Concentrations reported in Cabernet Sauvignon wines
dimethylsulphide	Asparagus, corn molasses herbaceous	10–160[1a] 60[2b]	2–5,3[4] 5–60[5]
4-mercapto-4-methylpentan-2-one	Box tree, guava, black currant	0,003[3c]	–
3-mercaptohexanol	Grapefruit, passion fruit	0,060[3c]	10–5 000[6]
3-mercaptohexyl acetate	Box tree, passion fruit	0,004[3c]	1–200[6]

1 Mestres *et al*., 2000; 2 Swiegers *et al*., 2005; 3 Tominaga *et al*., 1998b; 4 Bindon *et al*., 2013b; 5 Park *et al*., 1994; 6 Bouchilloux *et al*., 1998 (approximate values)
a In wine; b In red wine; c In model wine solution

3 Volatile compounds contributing to herbaceous, vegetative aromas

The vegetative/herbaceous character of Cabernet Sauvignon wines encompasses a number of attributes/descriptors, such as bell pepper, fresh green, fresh, cool, minty and cooked asparagus (Roujou de Boubée *et al*., 2000; Capone *et al*., 2011; Bindon *et al*., 2014). The compounds associated with these descriptors commonly belong to the following chemical groups: methoxypyrazines, C_6 alcohols and aldehyde derivatives and certain monoterpenes. The ambivalent role of DMS, which can contribute to the fruity and vegetative/herbaceous character of red wines, was discussed earlier.

3.1 Methoxypyrazines

Methoxypyrazines are a class of compounds that contribute to the varietal character of Sauvignon Blanc, Semillon, Cabernet Sauvignon and Merlot and impart a herbaceous, vegetal or green aroma (Allen & Lacey, 1998). A comprehensive study of 29 different grape cultivars showed that Cabernet Sauvignon, Merlot, Cabernet franc, Sauvignon blanc and Semillon are the only cultivars presenting significant, measurable levels of 2-isobutyl-3-methoxypyrazine (ibMP) from pre-véraison to harvest. The fact that ibMP only occurs in some cultivars points towards a genetically programmed trait of closely related cultivars (Koch *et al*., 2010). Three principal methoxypyrazines with low odour thresholds contribute the most to wine aroma. These are 2-isobutyl-3-methoxypyrazine (ibMP), 2-isopropyl-3-methoxypyrazine (ipMP) and 2-sec-butyl-3-methoxypyrazine (sbMP), a less important compound (Table 4). While sbMP and ipMP are mostly present in wine at levels nearing their perception thresholds, ibMP is often found at higher, above-thresholds concentrations (Allen *et al*., 1994; Roujou de Boubée *et al*., 2000). ibMP is a potent aroma-active compound: low levels contribute to the aromatic complexity of red wines, but higher levels are perceived as a lack of ripeness and are detrimental to wine quality (Allen & Lacey, 1998; Roujou de Boubée *et al*., 2000). The recognition threshold of ibMP in red wine was established at 15 ng/l (Roujou de Boubée *et al*., 2000).

The levels of ibMP in the berry decrease during fruit maturation (Roujou de Boubée *et al*., 2002; Ryona *et al*., 2008; Scheiner *et al*., 2012). Kotseridis *et al.,* (1999a) observed a 50% decrease in ibMP concentration, with a 15-day delay in harvesting. During the ripening of Cabernet Sauvignon grape bunches, ibMP is found mostly in the stems (53.4%), skin (31%) and seeds (15%), and the levels of ibMP in the skins increases from pre-véraison to harvest, reaching 95.5% of the total ibMP levels, while the levels in the stems and seeds decrease (Roujou de Boubée *et al*., 2002). Some studies have reported concomitant decreases in malic acid and ibMP levels during ripening, suggesting that monitoring the malic acid levels in the berry could be a good indicator of ibMP levels at harvest (Kotseridis *et al*., 1999a; Roujou de Boubée *et al*., 2000). Ryona *et al*. (2008), however, found that malic acid and ibMP levels are not always well correlated.

Marais *et al*. (1999) investigated the effect of average temperature and solar radiation within the canopies of Sauvignon blanc vines in three climatically different South African regions (Stellenbosch, Robertson and Elgin). Some of the vines were manipulated (trained and defruited) in a way that increased shading of the grape clusters, and these were then compared to control vines that were not manipulated. The recording of the temperatures within the canopy and within the clusters, as well as the recording of solar radiation within the canopies, gave an indication of the microclimatic conditions in the vines. In the end, it was found that higher ibMP levels were correlated with cooler seasons and regions.

ibMP levels accumulate in the berry from fruit set to about two to three weeks before véraison, from there on the levels decreased until harvest and it appears that pre-véraison cluster light exposure has a critical impact on ibMP levels at harvest (Roujou de Boubée *et al*., 2002; Ryona *et al*., 2008). Roujou de Boubée *et al*. (2002) reported a significant decrease in ibMP levels (68.4%) at harvest as a result of pre-véraison cluster light exposure. In a recent study, Ryona *et al*. (2008) compared the ibMP levels in shaded and exposed Cabernet Franc vines from three different blocks at ten different time points (from five to 130 days post-bloom). While there seemed to be no significant differences in ibMP levels between the shaded and exposed clusters at harvest, pre-véraison light exposure was shown to effect the accumulation of ibMP in the berries (Ryona *et al*., 2008).

Scheiner *et al*. (2012) reported that vines with less water stress tended to be more vigorous and bear fruit with higher ibMP levels. It appears that soils with a greater water-holding capacity (clay-rich soil) will favour vine growth and yield higher levels of ibMP in the grapes. Cabernet Sauvignon grapes grown on sandy-silt soil were reported to have higher ibMP levels than grapes from gravel soils (Roujou de Boubée *et al*., 2000).

Winemaking practices also affect ibMP levels. ibMP is easily extracted from crushed grape bunches at the beginning of pressing (Roujou de Boubée *et al*., 2002), and prolonged maceration on the skins in the presence of ethanol yields higher levels of ibMP (Kotseridis *et al*., 1999a).

Thermovinification reduces the ibMP levels; however, it is not a selective technique and it also removes desirable aroma compounds from the wine (Roujou de Boubée *et al*., 2002). Settling proved to be efficient for reducing ibMP levels (Roujou de Boubée *et al*., 2002).

Table 4: Aromatic properties and odour thresholds of the main methoxypyrazines that have been detected in Cabernet Sauvignon wines.

Methoxypyrazines	Aroma*	Odour threshold (ng/l)	Concentrations reported in Cabernet Sauvignon wines
ipMP	Green peas	1–2[1a]	2–16[3]
sbMP	Green peas	1–2[1a]	nf
ibMP	Bell pepper	15[2b]	2–24[3]/3,6–56,3[4]

1 Maga & Sizer, 1973; 2 Roujou de Boubée *et al*., 2000; 3 Preston *et al*., 2008; 4 Allen *et al*., 1994
a In water; b In red wine
nf= not found

3.2 C_6 and C_9 derivatives: Green leaf volatiles

Short-chain aldehydes and alcohols such as trans-2-hexenal, cis-3-hexenol, 1-hexanol and nona-2,6-dienal are formed from the dioxygenation of linoleic acid (C18:2) and linolenic acid (C18:3) in the lipoxygenase pathway. The C_6 and C_9 derivatives play an important role in plants, as they are involved in wound healing and pest resistance or have antimicrobial and antifungal activity: The conversion of linolenic acid and linoleic acid to short-chain volatiles is activated by cell membrane disruption caused by crushing. These alcohols and aldehydes are characterised by a fresh green odour and can cause leafy-grassy off-odours in wine (Joslin & Ough, 1978; Schwab *et al*., 2008).

C_6 and C_9 derivatives are produced in the berry and evolve from aldehydes to alcohols in the period from véraison to maturity (Kalua & Boss, 2009). Canuti *et al*. (2009) reported significant concentrations of hexanal, trans-2-nonenal and trans-2-hexenal in grape berries, but only trans-2-hexenal was found in the corresponding wines at levels much lower than its odour threshold. The rapid extraction and degradation or loss of trans-2-hexenal associated with an increase in the levels of 1-hexanol during fermentation, reported by Callejón *et al*. (2012), is in agreement with the rapid reduction of trans-2-hexenal to hexanol during fermentation, as described by Joslin and Ough (1978). The reduction of aldehydes to alcohols during fermentation has a positive impact on wine flavour, as alcohols have higher odour thresholds than their aldehyde counterparts and also have the potential to be converted into esters, which contribute fruity notes. Some of the main C_6 derivatives that have been detected in Cabernet Sauvignon wines are listed in Table 5.

Table 5: Main C_6 derivatives that have been detected in Cabernet Sauvignon wines.

Aldehydes/alcohols C_6 derivatives	Aroma	Odour threshold[a] (µg/l)	Concentrations reported in Cabernet Sauvignon wines
hexanol	Green, cut grass	8 000[1a]	11 382–28 420[2]
Cis-3-hexenol	Powerful, fresh green, grass odour	400[1a]	706–1 522[2] / 6.1-27.3[3]
Trans 2-hexenol	Green, citrusy, orange, pungent odour	400[2b]	151–753[2]

1 Guth 1997; 2 Tao & Zhang, 2010; 3 Bindon *et al*., 2013b
a In water/ethanol (90+10,w/w); b In model wine

3.3 Monoterpenes

Monoterpenes are a grape-derived class of compounds that generally contribute to floral and citrus characters in wines. Terpenes are present in the grape skin, and their levels increase during grape maturation. Red varieties are not characterised by high levels of terpenes (Robinson *et al*., 2014).

The eucalyptus and mint aroma attributes that often characterise Cabernet Sauvignon wines have been positively correlated with 1,8-cineole, otherwise known as eucalyptol, and hydroxyl citronellol (Robinson *et al*., 2011). 1,8-Cineole is described as fresh, cool, medicinal and camphoraceous. The perception and recognition thresholds in a Californian Merlot wine were 1.1 and 3.2 µg/l respectively (Capone *et al*., 2011). 1,8-Cineole is produced in Cabernet Sauvignon grapes during berry development, although levels decrease during ripening and cannot contribute significantly to wine aroma (Kalua & Boss, 2009).

A survey of 190 commercial Australian red and white wines showed that only red varieties exhibited significant levels of 1,8-cineole (Capone *et al*., 2011). The same study reported that an increase in 1,8-cineole occurs during fermentation, but this stops once the skins are removed, indicating that the compound is extracted from grape skins. The proximity of Eucalyptus trees to grapevines can directly influence the concentration of 1,8-cineole in the corresponding wines. It was also shown that 1,8-cineole levels are generally highest in grapevine leaves, followed by the stems and then the grapes (Capone *et al*., 2012).

4 Chemical composition and sensory analysis of Cabernet Sauvignon wines

Several hundred volatile compounds contribute to the overall perceived aroma properties of wine. Gas chromatography techniques still play an important role in the identification and quantification of volatile compounds, but alone do not necessarily provide information on the perceptual properties of the detectable compounds. The introduction of chromatographic analyses coupled with olfactometric detection has enabled researchers to evaluate the odour intensities of the volatile compounds present in wines. In this technique, the sensory properties of the volatile compounds separated by gas chromatography are evaluated by a trained panel (Polášková, *et al*., 2008; Ebeler & Thorngate, 2009).

Typically, impact odorants have high odour intensities and low odour thresholds (low ppb or low ppt levels). Gas chromatography-olfactometry (GC-O), coupled with gas chromatography-mass spectrometry (GC-MS), thus is very useful to identify and quantify active or impact odorants at trace levels (Polášková, *et al*., 2008). A major limitation of GC-O techniques, however, is that they only evaluate the contribution of individual aroma volatiles, not taking into account the additive or suppressive effects that may occur between different compounds. Complex chemical interactions that are not always well understood come into play, expressing suppressing/masking or enhancing/additive effects. Impact odorants at above thresholds concentrations can have suppressive effects, whilst a group of compounds present at below threshold concentrations will have an enhancing effect and contribute to a specific aroma attribute perceived in the wine (Polášková, *et al*., 2008).

Sensory analyses, and particularly descriptive analysis, have been used extensively in combination with chemical analyses to determine the intensities of sensory attributes and how the volatile compounds are perceived in a given set of samples. Statistical modelling procedures, such as principal component analysis (PCA), partial least squares (PLS) and multiple factor analysis (MFA) are applied to sensory and chemical data and provide valuable information on how active odorants are positively or negatively correlated with certain aroma attributes. (Noble & Ebeler, 2002; Francis & Newton, 2005). Omission or addition tests in reconstituted extracts are useful to characterise impact odorants and confirm potential additive or suppressive interactions between compounds (Escudero *et al*., 2007; Francis & Newton, 2005; Plutowska & Wardencki, 2008).

As a red grape cultivar grown to produce some of the most prestigious and expensive wines worldwide, Cabernet Sauvignon has been the subject of extensive research. In the past 20 years, several studies using GC-O techniques combined with GC-MS have been conducted to characterise impact components in Cabernet Sauvignon wines (Lopez *et al*., 1999; Kotseridis & Baumes, 2000; Gürbüz *et al*., 2006; Falcao *et al*., 2008). In these studies, 100+ compounds are detected and identified by matching their linear retention index (LRI) values with their corresponding aroma descriptors. There often are significant differences in the number and type of active components observed in the different studies due to differences in sample preparation

(Gùrbùz *et al.*, 2006; Plutowska & Wardencki, 2008). Nonetheless, a number of volatile compounds have repeatedly been reported as being active odorants in Cabernet Sauvignon wines, as listed in Table 6.

Table 6: Active odorants identified in Cabernet Sauvignon wines.

Active odorants	Odour threshold (µg/l)	Concentrations reported in Cabernet Sauvignon wines
Ethyl hexanoate	440[1a]	1 000-1 400[9]/373-1 315[10]
Ethyl octanoate	960[1a]	125-741[10]
Ethyl butyrate	600[1a]	530-1 941[10]
Isoamyl acetate	1830[1a]	205-2 784[10]
Isoamyl alcohol	30 000[3c]	179 000–205 000[9]/30 783–140 086[10]
β-ionone	0.09[4b]	0.196-0.372[4]
β-damascenone	2-7[5e]	3-21[10]
2-phenylethanol	14000[2b]	30 800–140 100[10]
Dimethylsulphide	10-160[6d]	5–60[11]
3-isobutyl-2-methoxypyrazine	0.015[7e]	0–54[7]
Eucalyptol	1.1[8e]	0,18–28[8]
Hexanol	8 000[3c]	11 382–28 420[10]
Cis-3-hexenol	400[3c]	706–1 522[10]

1 Pineau *et al*., 2009; 2 Ferreira *et al*., 2000; 3 Guth 1997; 4 Kotseridis *et al*., 1999b; 5 Pineau *et al*., 2007; 6 Mestres *et al*., 2000; 7 Roujou de Boubée *et al*., 2000; 8 Capone *et al*., 2011; 9 Bindon *et al*., 2013b; 10 Tao & Zhang, 2010; 11 Park *et al*., 1994
a In dearomatized red wine; b In model wine; c In water/ethanol (90+10,w/w); d In wine; e In red wine

When studies also included a descriptive sensory analysis of the Cabernet Sauvignon wines that had been analysed chemically, two sets of sensory attributes often emerged: either fruity/berry-like or herbaceous/vegetative (Kotseridis *et al*., 2000; Chapman *et al*., 2005; Falcao *et al*., 2008; Preston *et al*., 2008; Robinson *et al*., 2011; Bindon *et al*., 2013a).

Studies have been conducted recently to establish relationships between the sensory attributes and chemical composition of Cabernet Sauvignon wines (Preston *et al*., 2008; Robinson *et al*., 2011; Bindon *et al*., 2013a; Hjelmeland *et al*., 2013; Bindon *et al*., 2014). In a study published in 2008, Preston *et al*. focused on the vegetal aroma characteristics of 16 selected Californian Cabernet Sauvignon wines. A descriptive analysis was conducted and the results were compared to the levels of ipMP and ibMP in the wines. It should be noted that only four of the 16 wines presented levels of ibMP above threshold, and the highest level of ibMP found in those wines was 24 ng/l. The concentrations of the pyrazines alone did not correlate well with any of the sensory attributes, indicating that other volatiles also affected the vegetal character of the wines.

In 2011, Robinson *et al*. studied 30 Cabernet Sauvignon wines (24 from Australian regions, three from Bordeaux and three from the Napa Valley). Three hundred and three volatile compounds were significantly different among the wines, and 232 of these were common to all 30 wines. The statistical analyses of the sensory attribute data with the chemical composition showed a clear distribution of the wines according to fruity and vegetal/herbaceous characteristics. The bell pepper attribute was positively correlated with ibMP and negatively correlated with δ octalactone, γ octalactone, γ decalactone and vitispirane. The red berry and dried fruit aroma attributes were positively correlated with ethyl and acetate esters.

In 2013, Hjelmeland *et al*. studied a total of 24 wines from different vintages and regions in California and Washington State, including 14 monovarietal Cabernet Sauvignon and 10 Bordeaux blends with Cabernet Sauvignon as a main component. The wines were selected based on interest from wine companies and to represent either fruity or vegetal sensory properties. Sixty-one targeted analytes were measured and only 56 were detected. The chemical composition was compared to a descriptive sensory analysis to determine whether chemical analyses could predict sensory profiles. The wines were differentiated in part as a result of varying alcohol levels. Thirty-six of the 56 detected compounds contributed significantly to the prediction of the sensory attributes. These compounds included hexyl acetate, ethyl octanoate, isobutanol, isoamyl alcohol, 2-phenylethanol, β-ionone and linalool. Berry aroma was positively associated with hexyl acetate. Vegetal aroma was negatively associated with ethyl isobutyrate, isobutanol and 2-phenylethanol and positively correlated with ibMP and eucalyptol, although these two compounds did not present a strong, significant correlation with this attribute.

In most of the studies, some attributes were poorly explained by the volatile compounds measured, and some volatile compounds did not correlate well with the attributes. In particular, ibMP often fails to correlate with the vegetative/herbaceous attributes, especially because most wines analysed presented below-threshold concentrations of ibMP (Preston *et al*., 2008; Bindon *et al*., 2013b; Hjelmeland *et al*., 2013). Although 3MH and 3MHA have been described as contributing to the aromatic complexity of Cabernet Sauvignon wines (Bouchilloux *et al*., 1998), none of the studies cited earlier measured these compounds or could report on their levels and their impact on the aromatic profiles of the respective wines studied (Robinson *et al*., 2011; Hjelmeland *et al*., 2013; Bindon *et al*., 2014).

5 Conclusion

Cabernet Sauvignon wines are often differentiated by two antagonistic aromatic profiles: one with fruity, berry-like aromas and the other one with vegetative, herbaceous aromas. The aroma compounds responsible for these two independent profiles are derived directly or indirectly from yeast and bacterial metabolism, and are determined by the grape composition at the time of harvest (Swiegers *et al*., 2005; Carey *et al*., 2008). Advances in analytical techniques have helped identify some of the impact odorants responsible for these typical characters. Thus, the measurement of a selected and limited number of volatile compounds combined with descriptive sensory analysis can help predict the sensory profiles of Cabernet Sauvignon wines (Hjelmeland *et al*., 2013; Bindon *et al*., 2014).

To our knowledge, no such study has been conducted on South African Cabernet Sauvignon wines yet. Bearing in mind that Cabernet Sauvignon is grown to produce some of the most expensive wines, the South African wine industry may gain valuable information from understanding the impact of volatile compounds on the sensory properties of their wines. This information could be used to make decisions at the viticultural and winemaking level to produce Cabernet Sauvignon wines with more desirable sensory attributes (Francis & Newton, 2005; Forde *et al*., 2011).

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Chapter 3

Research results

Linking sensory attributes to selected aroma compounds in South African Cabernet Sauvignon wines

1 Introduction

Over the past two decades, the volatile composition of Cabernet Sauvignon wines has been investigated and certain active odorants contributing to their aromatic properties have been identified. In recent years, researchers have focused particularly on the volatile compounds responsible for fruity and herbaceous/vegetative notes which are often associated with this grape cultivar (Carey *et al*., 2008). The vegetative/herbaceous character of Cabernet Sauvignon wines has been linked to high levels of 2-isobutyl-3-methoxypyrazine (ibMP) (Roujou de Boubée *et al*., 2000) and to a certain extent also to C_6 alcohol derivatives (Bindon *et al*., 2014). 1,8-Cineole (eucalyptol), which is described as fresh, cool and minty, also elicits this type of character, especially in Cabernet Sauvignon wines produced in Australia (Capone *et al*., 2011). The berry fruity notes in red wines have been associated with the presence of esters (Escudero *et al.,* 2007; Pineau *et al*., 2009). It has been reported that higher levels of ethyl propanoate, ethyl 2-methylpropanoate and ethyl 2-methylbutanoate contributed to the aroma of black berries, while the combination of higher levels of ethyl butanoate, ethyl hexanoate, ethyl octanoate and ethyl 3-hydroxybutanoate were associated with the aroma of red berries (Pineau *et al*., 2009).

β-damascenone has been identified as an active odorant eliciting fruity notes in Cabernet Sauvignon wines (Lopez *et al*., 1999; Falcao *et al*., 2008, Tao *et al*., 2009). Its odour threshold depends on the matrix considered and could range from 2 up to 7 µg/l in red wines (Pineau *et al*., 2007). Its impact in red wines could be more indirect than direct, as it has the ability to enhance fruity aromas either by lowering the odour thresholds of esters such as ethyl hexanoate and ethyl cinnamate, or by increasing the odour threshold of ibMP (Pineau *et al*., 2007). Dimethyl sulphide (DMS) is ambivalent in its ability to affect red wines aroma, either directly by imparting a cooked vegetables aroma or indirectly by enhancing certain fruity notes (Escudero *et al*., 2007).

It is sometimes suggested that wine aroma is mostly dependent on a small pool of volatile compounds present at above threshold concentrations (Francis & Newton, 2005; Escudero *et al*., 2007). Compounds that are not odour-active could therefore be disregarded and even eliminated from the data set (Noble & Ebeler). Recent studies have shown, however, that chemicals with weak individual impact could combine with other compounds to have a measurable impact (Bult *et al*., 2001; Miyazawa *et al*., 2008). The contribution of volatile constituents to the overall aromatic properties of wine is major (Polášková *et al*., 2008). The perceived intensities of high-impact odorants can be affected by other high-impact odorants, low-impact odorants and the wine matrix itself (Ryan *et al*., 2008; Ebeler & Thorngate, 2009). The suppressive and enhancing effects of certain high-impact odorants interacting with each other have been characterised in red wine (Escudero *et al*., 2007; Pineau *et al*., 2009). In recent years, the use of statistical techniques, which combine chemical and sensory data, has provided valuable information on how certain active odorants contribute to the typical aroma attributes of Cabernet Sauvignon wines (Preston *et al*., 2008; Robinson *et al*., 2011; Bindon *et al*., 2013; Hjelmeland *et al*., 2013; Bindon *et al*., 2014).

It is widely accepted that unripe grapes produce Cabernet Sauvignon wines of lesser organoleptic quality (Allen *et al.*, 1994; Roujou de Boubée *et al.*, 2000) and, notably in the past five years, the South African wine industry has shown increased interest in the levels of ibMP in Cabernet Sauvignon wines. Many estates have been experimenting in the vineyard (pruning, canopy management) to determine how viticultural interventions affect the ibMP levels in the wines and to improve the quality of grapes at the time of harvest. However, at the same time, little has been published on the volatile composition and the aromatic properties of South African Cabernet Sauvignon wines.

In this study, 13 mono-varietal Cabernet Sauvignon wines produced in South Africa were selected so that a broad range of fruity and herbaceous sensory attributes were represented. A descriptive analysis was performed so that the aroma profiles of the wines could be characterised. Following the outcome of the sensory evaluation, the volatile compounds that seemed most relevant to explain the perceived aromas of the wines were analysed. Finally, the data collected from the descriptive analysis and the chemical analyses was combined to determine possible trends. This study focused on volatile compounds arising mostly from grape composition and yeast metabolism that exhibit either fruity or herbaceous/vegetative notes and are often associated with Cabernet Sauvignon wines (Carey *et al*., 2008); certain major volatile like fatty acids and oak-derived volatiles were deliberately not analysed.

2 Materials and Methods

2.1 Wines

All wines included in the study were mono-varietal Cabernet Sauvignon wines produced in South Africa. At the beginning of 2014, a total of 20 wines were evaluated for their olfactive properties by an expert panel consisting of three academics from the Department of Viticulture and Oenology (Stellenbosch University) so that a broad range of fruity and herbaceous sensory attributes were represented. Initially, 14 wines, coded from A to N, were selected. However, wine L, which presented fresh green notes, was excluded from the study because it also exhibited an unwanted (for this study), noticeable *Brettanomyces* taint. In the end, the study comprised 13 wines: five were commercially available and eight were wines produced during the 2013 harvest, not yet bottled (Table 1).

Table 1: List of the 13 wines, their geographical origin and their vintage.

WINE	VINTAGE	REGION
A	2010	Stellenbosch
B	2013	Stellenbosch
C	2013	Somerset West
D	2013	Franschhoek
E	2013	Stellenbosch
F	2010	Stellenbosch
G	2013	Franschhoek
H	2010	Stellenbosch
I	2008	Somerset West
J	2013	Darling
K	2013	Franschhoek
M	2012	Durbanville
N	2013	Durbanville

2.2 Descriptive analysis

The sensory study was carried out in the month following the selection of the wine samples. Ten judges recruited and remunerated by the Sensory Laboratory of the Department of Viticulture and Oenology at Stellenbosch University performed the descriptive analysis tastings. The panel consisted of eight women (from 25 to 55 years of age) and two men (aged 25). The 13 wines were divided into two flights: the first flight included wines A to G, and the second wines H to N. The wines were presented to the judges in black glasses at a constant volume (30 ml), and were assessed for their olfactive properties only. The sensory assessments took place within 30 minutes from pouring the wines into the glasses, which were closed with a petri dish lid to avoid any loss of aromas. The judges participated in six training sessions of 90 minutes each in a three-week period. During the first three sessions the judges discussed the sensory properties of the wines. Reference standards were prepared to help describe the samples and, in the fourth session, a list of 15 descriptors was generated after consensus was reached (Table 2).

The two last training sessions were dedicated to scaling the intensity of the aromas perceived in the wines. The judges conducted the final evaluation of the wines over two sessions: one session for each flight of wines. The panellists rated the 13 wines using an unstructured line scale, in triplicate. Wines were presented in individual booths, poured into black glasses, and coded in a randomised manner for each repetition, which was different for each judge.

Table 2: List of descriptors and reference standards used during the descriptive analysis

Black berries	Combination of: • Blackberry: solution of five frozen berries ('Hillcrest') + 10 ml distilled water • Blueberry: 2 spoons blueberry sauce ('St Dalfour')
Red berries	Combination of: • Raspberry: fresh fruit of season (two berries) • Redcurrant: solution of five frozen berries ('Hillcrest') + 10 ml distilled water • Strawberry: ½ of a fresh strawberry
Blackcurrant	Solution of five frozen berries ('Hillcrest') + 10 ml distilled water
Prunes	1 dried prune ('Safari') cut into pieces
Violets	Solution of 2 ml of 'Vedrenne' syrup + 4 ml distilled water
Cooked vegetables	10 ml water from a can of 'Koo' canned green beans
Eucalyptus	A few crushed fresh leaves
Mint	2 crushed fresh mint leaves
Bay leaves	1 cut dried bay leaf
Spicy	Combination of black pepper, cinnamon and clove
Fresh green	Half a bottle of fresh grass
Gherkins	Chopped cocktail gherkin ('Goldcrest')
Jalapeno	Chopped green jalapeno chillies ('Mediterranean Delicacies')
Wood	Medium toasted oak wood chips
Planky/dusty	One spoonful of pine sawdust
Hay	Finely cut hay
Sulphur	2% solution of SO_2

2.3 Chemical analyses

All chemical analyses were conducted by VinLAB Pty Ltd, an ISO17025 accredited wine laboratory (Stellenbosch, South Africa).

2.3.1 Conventional oenological parameters

The samples were analysed in duplicate for conventional oenological parameters, including alcohol, pH, titratable acidity (TA), volatile acidity (VA) and residual sugars (RS), using a Foss WineScan FT120 equipped with a 5027 auto sampler (Foss, Hillerød, Denmark).

2.3.2 Volatile compounds

In this study, 33 volatile compounds were selected to be measured in the wine samples. The selection of compounds was based on the outcome of the descriptive analysis and previous studies that reported on impact odorants contributing most to either fruity or herbaceous/vegetative notes in Cabernet Sauvignon wines (Bouchilloux *et al*., 1998; Kotseridis & Baumes, 2000; Falcao *et al*., 2008; Robinson *et al*., 2011). Due to the complexity of the volatile compounds (different chemistry and concentrations) under investigation, it was not possible to use one method to quantify all of them at once; the samples were thus analysed using four different methods requiring two extraction techniques and two instruments. Dimethyl sulphide (DMS), as well as 27 volatile compounds, were analysed by headspace solid phase micro-extraction (SPME) and gas chromatography–ion trap mass spectrometer detection (HS-SPME-GC-Ion Trap-MS analysis). Methoxypyrazines and varietal thiols were quantified using solid phase extraction SPE techniques and gas chromatography, coupled with a triple quadrupole detector (SPE-GC-MS/MS analysis).

2.3.2.1 Reagents, standards and material

All chemical standards were purchased from Sigma-Aldrich (St Louis, MO, USA), except for 4-mercapto-4-methylpentan-2-one (4MMP), 3-mercaptohexyl acetate (3MHA) and 3-mercapto hexan-1-ol (3MH), supplied by Endeavour Speciality Chemicals Limited (Daventry, UK).

ISOLUTE® ENV+ and C18 Solid Phase Extraction (SPE) cartridges were supplied by Biotage (Uppsala, Sweden). Furthermore, 50 ml clear Wheaton™ vials were supplied by Sigma-Aldrich (St Louis, MO, USA). Solid phase micro-extraction (SPME) fibres consisting of a 2 cm divinylbenzene/Carboxen/ polydimethylsiloxane (DVB/CAR/PDMS) 50/30 µm fibre, 23 gauge and an 85 µm Carboxen/polydimethylsiloxane fibre were purchased from Supelco (Bellefonte, PA, USA). Absolute ethanol and hexane were supplied by Riedel de Haen (Sigma-Aldrich, St Louis, MO, USA). Dichloromethane, sodium chloride and sodium sulphate were from Merck (Darmstadt, Germany). Water was purified through an EasyPure water system from Barnstead (Thermo Scientific™, Thermo Fisher Scientific, Waltham, MA, USA).

2.3.2.2 General analysis of wine volatiles

Twenty-seven volatile compounds, namely β-damascenone, β-ionone, rose oxide, eucalyptol, β-citronellol, geraniol, nerol, linalool, eucalyptol, cis-3 hexenol, trans-2 hexenol, hexanol, phenethyl alcohol (or 2-phenylethanol), 1-propanol, 1-butanol, isoamylalcohol, isobutanol, isobutylacetate, isoamylacetate, ethyl butyrate, hexyl acetate, ethyl hexanoate, ethyl lactate, ethyl octanoate, ethyl decanoate, phenethyl acetate and diethylsuccinate were analysed using a method adapted from Hjelmeland *et al*. (2013). The samples were analysed using a 3900 gas chromatograph equipped with a split/splitless 1177 injection port coupled to a Saturn 2100T ion trap-mass spectrometer from Varian (Agilent Technologies, Santa Clara, CA, USA). Sample preparation and sampling are summarised in Table 3. The separation of the volatile compounds was achieved using a 30 m x 0.25 mm x 0.25 µm Zebron WAX plus capillary column from Phenomenex® (Torrance, CA, USA). The carrier gas used was helium at a flow rate of 1.2 ml/min. The injector, MS transfer line and trap temperatures were 230ºC, 245ºC and 170ºC respectively. The oven temperature programme was held for 3 min at 45ºC, then increased at 2.5ºC/min to 80ºC after which it was increased at 4ºC/min to 110ºC and then again at 10ºC/min to 220ºC. The temperature was finally ramped at 25°C/min to 245°C and held for 1.0 min. The detection and quantification were achieved by using full scan mode (mass range: 35 to 250 m/z) and single ion monitoring (SIM) mode. The emission current was 20 µA and the scan time was 0.7 sec/scan. The volatile compounds were identified according to their retention time and their mass spectrum from 10 mg/ml solutions prepared in absolute ethanol. 3-Octanol and undecanol were used as internal standards and were added to the wine samples at a concentration of 250 µg/l. Calibration curves were built by analysing 14% alcoholic solutions containing different concentrations of the volatile compounds and a set concentration of the internal standards (250 µg/l). The range of concentrations was chosen according to the volatile compound considered, as described by Ferreira *et al*. (2000). All wine samples were analysed in duplicate and the means of duplicate analysis were reported.

Table 3: Sample preparation and sampling conditions for the analysis of 27 wine volatiles

<table>
<tr><td>Vial</td><td colspan="2">50 ml clear headspace vial</td></tr>
<tr><td>Sample volume</td><td colspan="2">40 ml of wine</td></tr>
<tr><td>Salt addition</td><td colspan="2">5 g of sodium chloride</td></tr>
<tr><td>SPME fibre</td><td colspan="2">2 cm DVB/CAR/PDMS, 23 gauge</td></tr>
<tr><td rowspan="3">Extraction conditions</td><td rowspan="3">Headspace</td><td>Temperature: 45ºC</td></tr>
<tr><td>Agitation at 400 rpm</td></tr>
<tr><td>Time: 40 min</td></tr>
<tr><td rowspan="2">Desorption conditions</td><td>Injector temperature</td><td>230 ºC</td></tr>
<tr><td>Time</td><td>5 min</td></tr>
</table>

2.3.2.3 Methoxypyrazines analysis

The method used for the analysis of the methoxypyrazines was adapted from various published methods (Allen *et al*., 1994; Roujou de Boubée *et al*., 2000) and validated according to ISO 17025 requirements. 50 ml of wine samples spiked with isopropylethoxypyrazine (ipEP) used as an internal standard were extracted using a C18 solid phase extraction (SPE) cartridge (ISOLUTE). Major interferences were removed by rinsing the cartridges with 5 ml of distilled water, and the analytes were eluted with hexane. The extracts were then concentrated under a gentle flow of nitrogen. The injection of 2 µl of extract was done in splitless mode on a Trace 1300 gas chromatograph coupled to a TSQ8000 mass selective detector (MS/MS) and equipped with an AI 1310 auto sampler (Thermo Scientific, Waltham, MA, USA). The separation of compounds was achieved using a 30 m x 0.25 mm x 0.25 µm Zebron WAX plus capillary column from Phenomenex® (Torrance, CA, USA). The carrier gas used was helium at a flow rate of 1.2 ml/min. The injector and the MS/MS transfer line temperature were 220ºC and 245ºC respectively. The oven temperature programme was held for 1 min at 50ºC, then increased at 5ºC/min to 200ºC, followed by an increase of 25ºC/min to 245ºC. Detection and quantification were achieved using selected reaction monitoring (SRM) mode, details of which are shown in Table 4. A standard calibration curve was created using a 14% alcoholic solution, to which concentrations ranging from 0.005 to 0.12 µg/l of ipMP and ibMP were added, along with a set concentration of the internal standard. The calibration curves showed good linearity in the range of concentrations used, and the correlation factors were above 0,995. The limit of quantification (LOQ) and limit of detection (LOD) were 0.005 and 0.001 µg/l respectively; measurement uncertainty was +/-15% within the calibration range.

Table 4: Detection and quantification parameters used for the methoxypyrazine analysis

Emission current		50 µA	
Electron energy		70 eV	
Scans per peak		20	
Minimum dwell time		0.2 sec	
Compound	Transition	Collision energy	Retention time (min)
ipMP	151.9 to 137 [a]	5	13.70
ibMP	124 to 94,1[a]	10	16.00
ipEP	166.to 151.1 [a]	5	14.44

[a] Quantifier

2.3.2.4 Dimethyl sulphide analysis

Dimethyl sulphide (DMS) was analysed using a method adapted from Mestres et *al.* (1998). The method was validated at VinLAB Pty Ltd according to ISO17025 requirements. Wine samples were extracted by headspace using an 85 µm Carboxen/polydimethylsiloxane SPME fibre (Supleco). The details of sample preparation and sampling conditions are shown in Table 5. The samples were analysed using a 3900 gas chromatograph coupled to a Saturn 2100T Ion Trap-Mass Spectrometer from Varian (Agilent Technologies, Santa Clara, CA, USA). Separation was achieved using a 30 m x 0.25 mm x 1 µm Equity 1 capillary column from Supelco (Bellefonte, PA, USA). The carrier gas used was helium at a flow rate of 1.2 ml/min. The injector, MS transfer line and trap temperatures, were 220ºC, 245ºC and 170ºC respectively. The oven temperature programme was held for 4 min at 40ºC, then increased at 8ºC/min to 130ºC. The temperature was finally ramped at 25°C/min to 270°C and held for 1.0 min. Detection and quantification were achieved by using full-scan mode (mass range: 35 to 150 m/z). The emission current was 20 µA and the scan time was 0.7 sec/scan. Ion mass 62 was selected as quantifier. A standard calibration curve was created using a 14% alcoholic solution, to which concentrations of DMS ranging from 1 to 500 µg/l were added, along with 50 µg/l of ethyl methyl sulphide (internal standard). The calibration curve showed good linearity in the range of concentrations used, and the correlation factor was 0,995. The limit of quantification (LOQ) and limit of detection (LOD) were 2 and 0.6 µg/l respectively, with a measurement uncertainty of +/-20% within the calibration range.

Table 5: Sample preparation and sampling conditions for the analysis of DMS

<table>
<tr><td>Vial</td><td colspan="2">50 ml clear headspace vial</td></tr>
<tr><td>Sample volume</td><td colspan="2">40 ml of wine</td></tr>
<tr><td>Salt addition</td><td colspan="2">5 g of sodium chloride</td></tr>
<tr><td>SPME fibre</td><td colspan="2">85 µm Carboxen/polydimethylsiloxane</td></tr>
<tr><td rowspan="3">Extraction conditions</td><td rowspan="3">Headspace</td><td>Temperature: 45ºC</td></tr>
<tr><td>Agitation at 400 rpm</td></tr>
<tr><td>Time: 30 min</td></tr>
<tr><td rowspan="2">Desorption conditions</td><td>Injector temperature</td><td>220ºC</td></tr>
<tr><td>Time</td><td>5 min</td></tr>
</table>

2.3.2.5 Volatile thiols

The volatile thiols were extracted using the sample preparation method described by Mateo-Vivaracho *et al*. (2009). Briefly, the extraction consists of the selective pre-concentration of the volatile thiols in ENV+ SPE cartridges containing p-hydroxymercurybenzoate. The thiols, which are strongly retained by the organomercury salt, are then eluted with a small volume of dichoromethane containing 1,4-dithioerythritol. The injection of 2 µl of extract was done in splitless mode on a Trace 1300 gas chromatograph, coupled to a TSQ8000 mass selective detector (MS/MS) and equipped with an AI 1310 auto sampler (Thermo Scientific). The separation of compounds was achieved using a 30 m x 0.25 mm x 0.25 um Zebron WAX plus capillary column from Phenomenex® (Torrance, CA, USA). The carrier gas used was helium at a flow rate of 1 ml/min. The injector and the MS/MS transfer line temperature were 220ºC and 245ºC respectively. The oven temperature programme was held for 2 min at 50ºC, then increased to 170ºC at 4ºC/min. The temperature was finally ramped at 25°C/min to 245°C and held for 1 min. Detection and quantification were achieved using SRM mode, details of which are shown in Table 6. A standard calibration curve was created using a de-aromatised red wine to which a mixture of volatile thiols had been added at different concentrations, ranging from 0.05 to 7.5 µg/l.

A set concentration (2.5 µg/l) of 2-mercapto-3-butanol (2MBH) used as an internal standard (IS) and 6-mercaptohexanol (6MH) used as a surrogate, were added to the wine samples prior to extraction. The calibration curves showed good linearity in the range of concentrations used and the correlation factors were at least 0,994 for the three compounds analysed. The limit of quantification (LOQ) and limit of detection (LOD) were 0.05 and 0.015 µg/l respectively with a measurement uncertainty of +/-25% within the calibration range.

Table 6: Detection and quantification parameters used for the analysis of volatile thiols

SRM optimised parameters			
Emission current		50 µA	
Electron energy		70 eV	
Scans per peak		20	
Minimum dwell time		0.2 sec	
Compound	**Transition**	**Collision energy**	**Retention time (min)**
4MMP	132 to 74.8 [a]	10	13.80
2MBH=IS	106 to 62 [a]	5	15.30
3MHA	100.9 to 67.1 [a]	5	23.90
3MH	134 to 82[a]	5	27.19
6MH	100.9 to 67.1[a]	10	30.22

[a] Quantifier

2.4 Statistical analysis of data

2.4.1 Descriptive analysis

A mixed model two-way analysis of variance (ANOVA) testing wines and judges was applied to assess the significance of the attributes, to determine how the intensity scores of all attributes differentiated the wines, and how the judges performed, using both PanelCheck® version 1.2.1 (Nofima, Ås, Norway) and Statistica version 12 (StatSoft Inc., Tulsa, USA). A principal component analysis (PCA) biplot was performed on the means of intensity scores of the attributes from the three repeats to check the relationship between each attribute and all the wines using Statistica.

2.4.2 Linking chemical and descriptive analysis data

A multiple factor analysis (MFA) was performed on the chemical and sensory data to check possible correlations between attributes and volatile compounds using Statistica.

3 Results and discussion

3.1 Descriptive analysis

The Tucker plots (Figure 1) show that there was good consensus amongst eight of the ten judges, who rated the wines similarly for all attributes. As shown in Figure 2, judges 4 and 6 were outliers and were removed from the dataset. The product effect graph (Figure 3) illustrates that there was good consensus between the eight judges for all the attributes (P value of < 0.001). Four attributes, namely hay, prunes, bay leaves and planky/dusty, were almost never observed in the wines (0 observation > 75%) compared to the other attributes (Addendum A). This suggested that these attributes may not have been too relevant to help differentiate the wines. The prunes and bay leaves attributes could be of interest, as they had higher intensity scores in samples A and D and in sample B respectively. Planky/dusty and hay were not deemed relevant for the description of the wines and were thus removed from the dataset.

The least square (LS) means plots illustrate the significant differences across the wine samples for each attribute (Figure 4). At a glance, it can be observed that wine B had the highest intensity scores for the bay leaves attribute; wine G had the highest intensity scores for the violets attribute; wine A had high intensity scores for the black berries, black currant and prunes attributes; wine samples F and J had significantly higher scores for the fresh green/gherkins/jalapeno attributes and wine samples F and I had significantly higher scores for the cooked vegetable (or cooked veg) attribute.

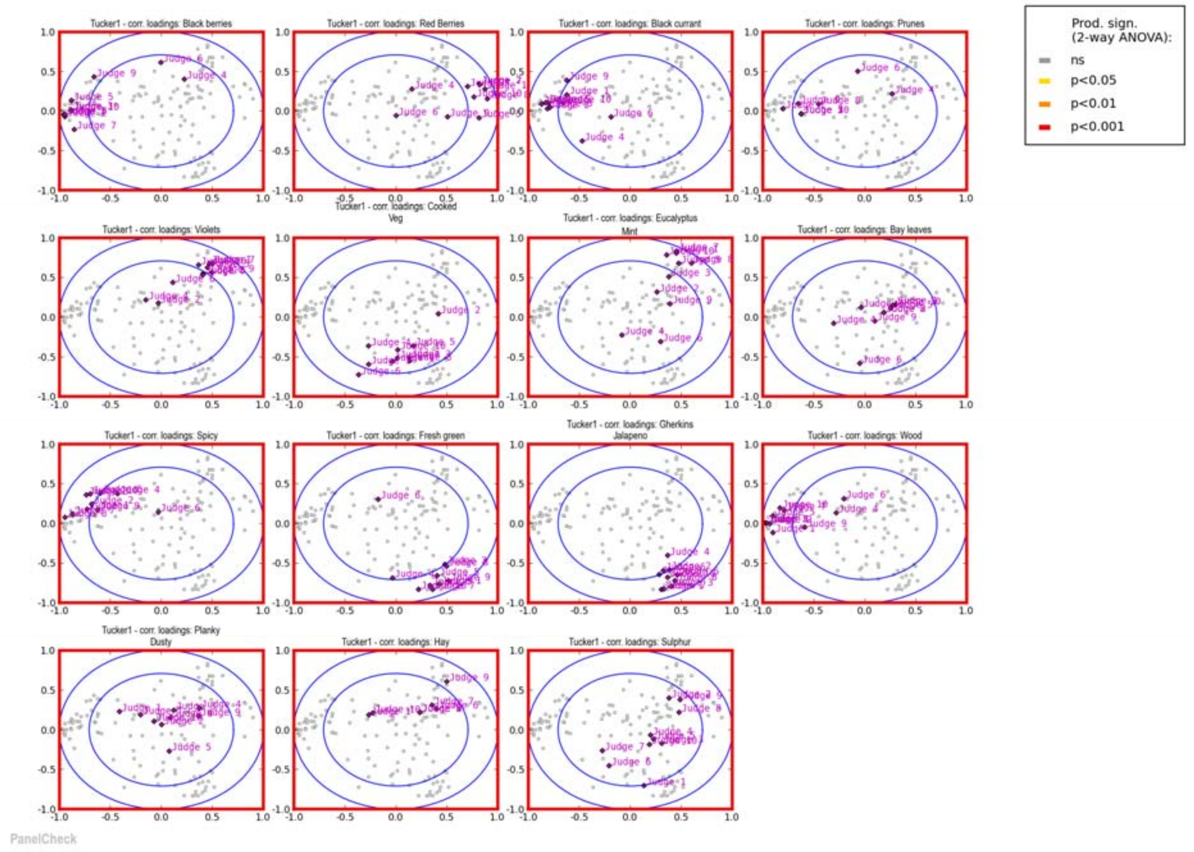

Figure 1: Tucker plots illustrating the judges' performance during the descriptive analysis of the wine samples.

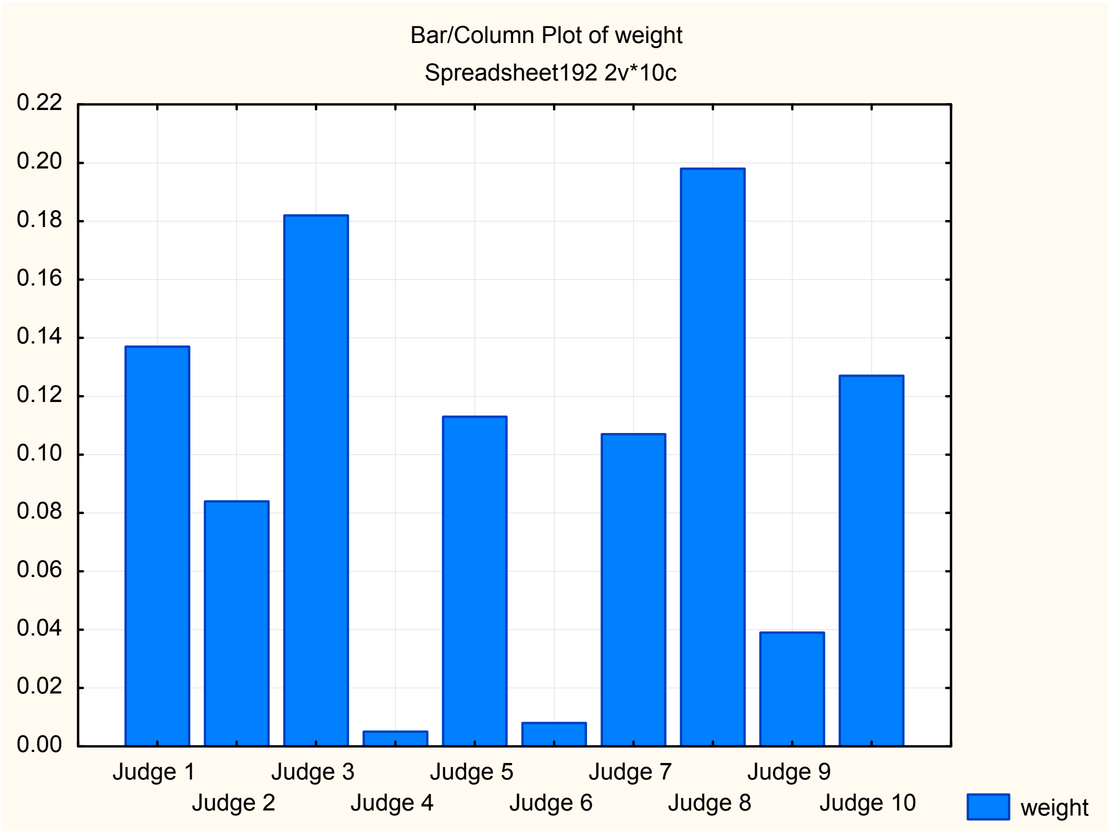

Figure 2: Graph illustrating the performance/weight of each judge during the descriptive analysis.

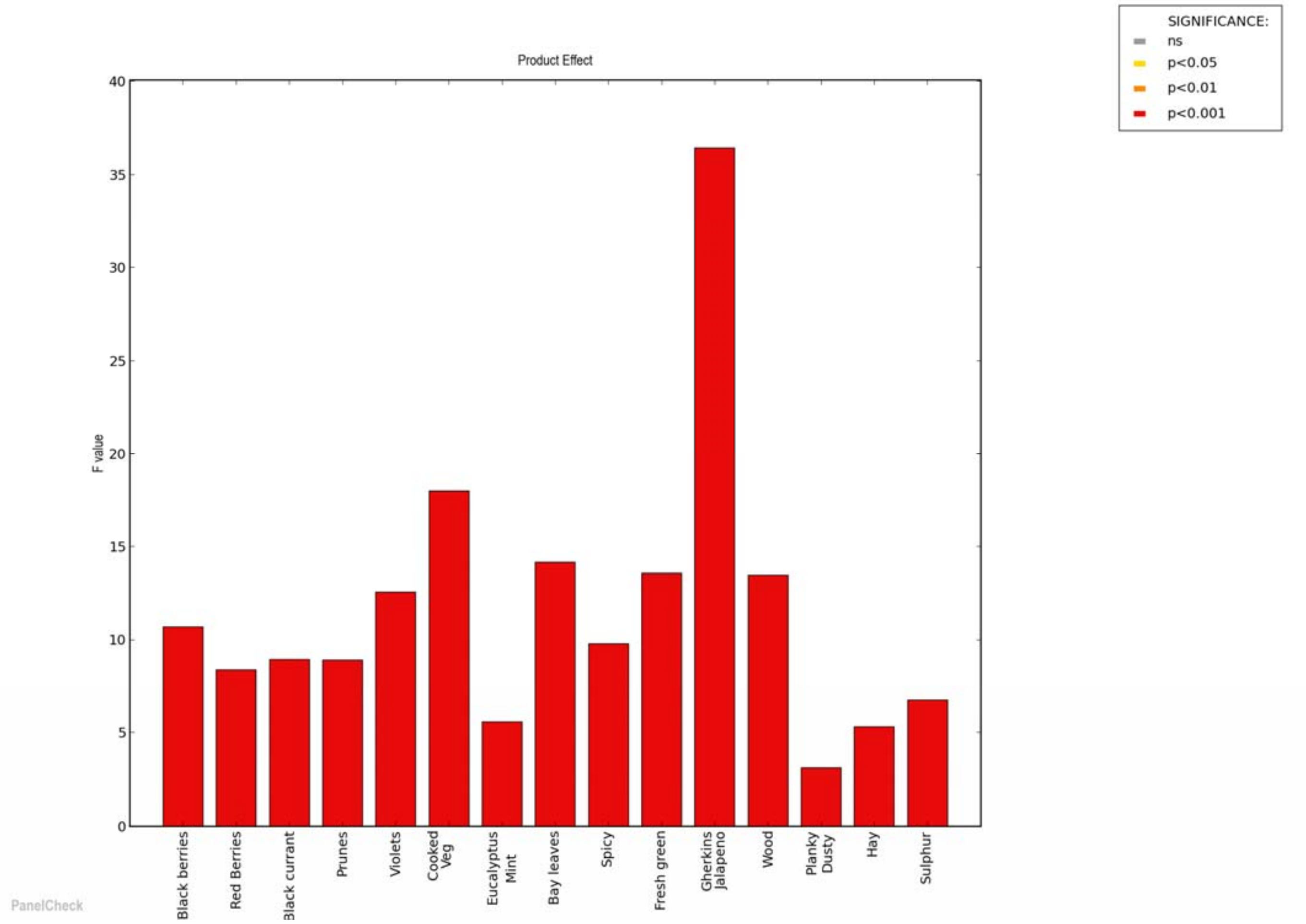

Figure 3: Product effect graph illustrating that judges showed consensus on all attributes.

Figure 4: LS means plots for 13 attributes. Alphabetical letters in the plots denote significant differences ($p<0.05$) between wine samples.

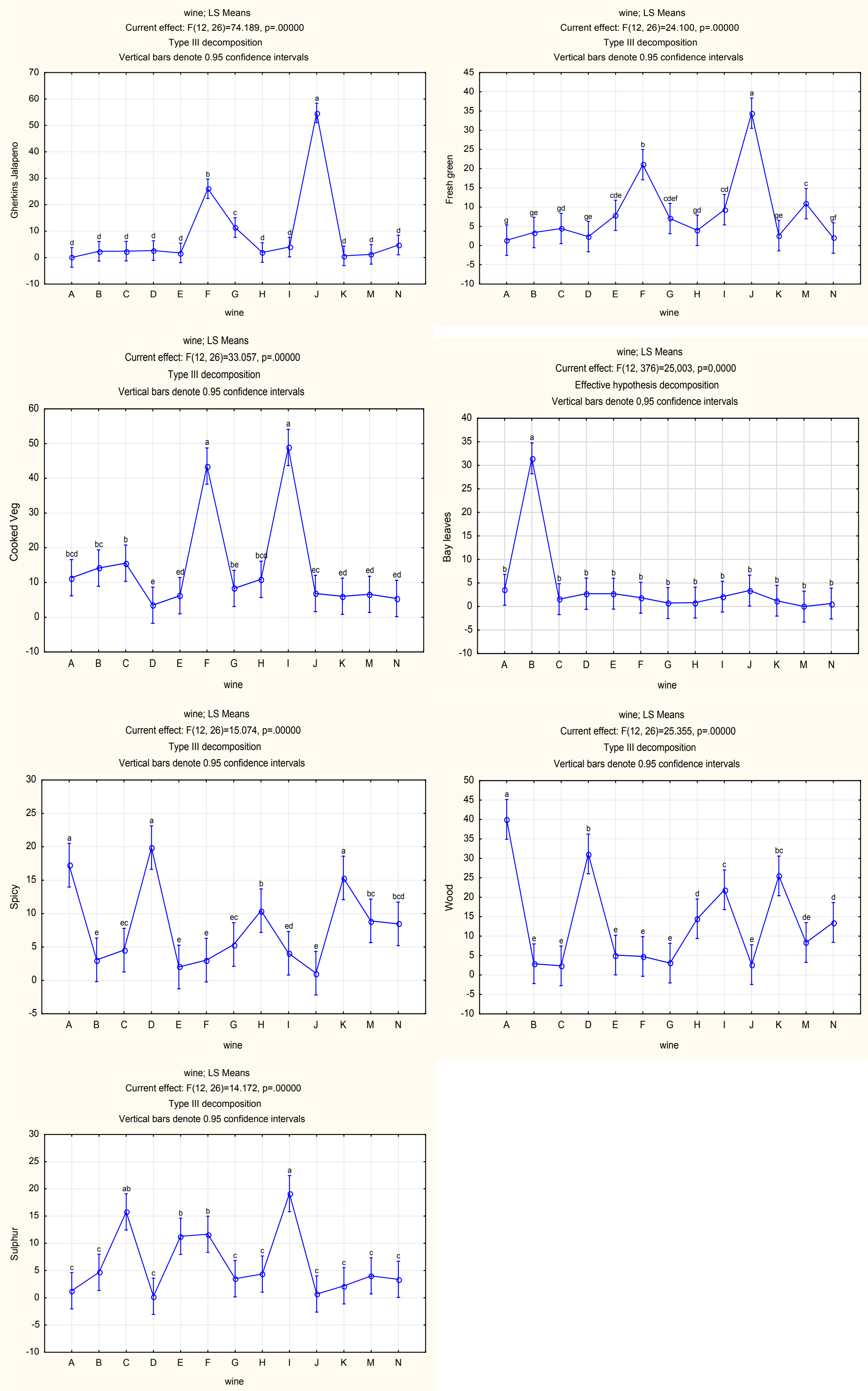

Figure 4 (cont.)

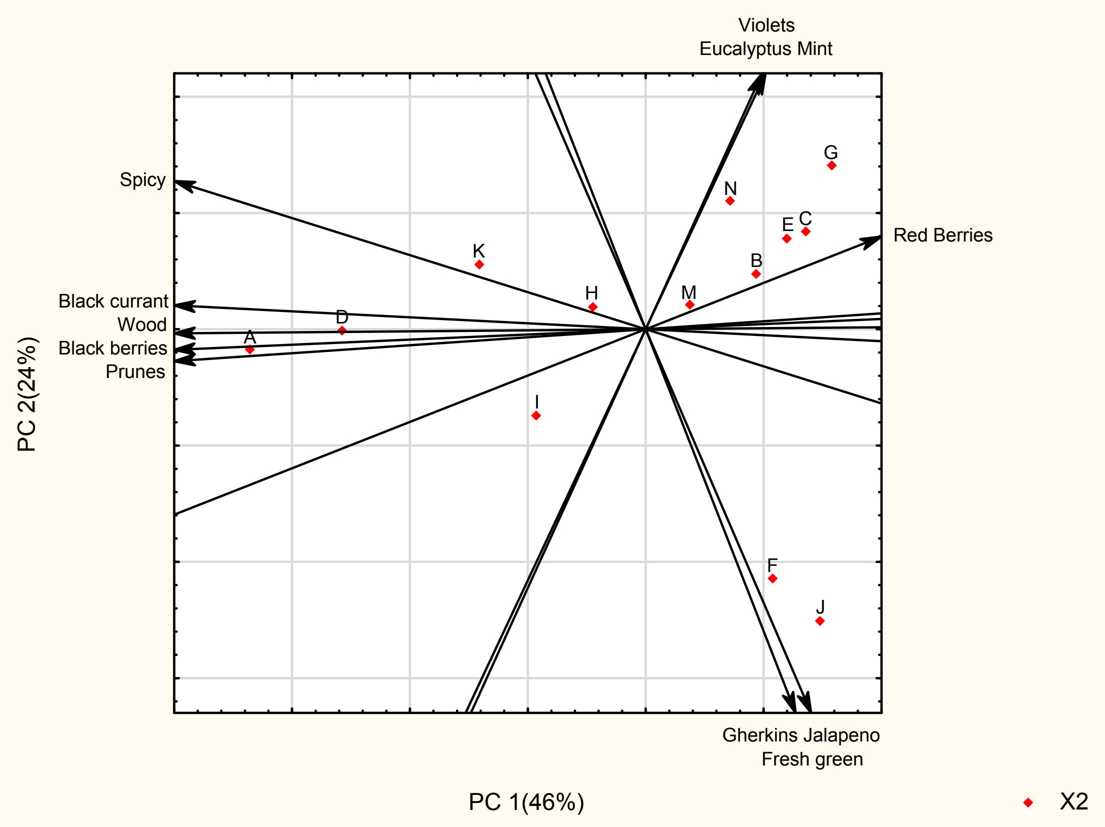

Figure 5: PCA bi-plot representing the relation between attributes(scores) and wine samples (loadings) for principal components 1 and 2.

A principal component analysis (PCA) bi-plot (Figure 5) was generated to illustrate how the wine samples correlated with the descriptors. The first two components explained 70% of the variance, and PC1 (x-axis) alone accounted for 46% of the variance. The black berries, wood, black currant, spicy and red berries attributes contributed to PC1, while eucalyptus/mint, fresh green and gherkins/jalapeno contributed to PC2. Sulphur, bay leaves and cooked veg attributes, which were not well represented in the first two dimensions, do not appear on the bi-plot. These attributes may be represented in a third or fourth dimension. Wines J and F were positively associated with fresh green and gherkins/jalapeno attributes; wines B, C, E and M were positively associated with the red berries attribute and wines H and K were positively associated with the spicy attribute. Finally wines A and D were positively associated with the black berries, wood, black currant and prunes attributes.

3.2 Chemical analysis

3.2.1 Conventional oenological parameters

The alcohol levels measured in the 13 wines ranged from 14.25 to 15.46% v/v. Residual sugars (RS) were all below 4 g/l and ranged from 1.85 to 3.41 g/l (Table 7).

Table 7: Conventional oenological parameters measured in the 13 wine samples

WINE	VINTAGE	Alcohol %v/v	pH	TA (g/l)	VA (g/l)	RS (g/l)
A	2010	14.48	3.40	6.61	0.64	2.83
B	2013	14.47	3.65	5.06	0.59	2.15
C	2013	14.57	3.58	5.65	0.57	2.27
D	2013	14.81	3.78	5.43	0.76	2.80
E	2013	14.72	3.55	5.80	0.63	1.85
F	2010	14.67	3.44	6.19	0.63	3.6
G	2013	15.46	3.91	5.25	0.87	3.18
H	2010	15.11	3.59	5.92	0.58	1.93
I	2008	14.46	3.63	5.48	0.53	3.41
J	2013	15.44	3.90	5.24	0.83	3.14
K	2013	14.62	3.82	5.09	0.66	2.79
M	2012	14.25	3.71	5.90	0.65	2.46
N	2013	14.35	3.79	5.66	0.70	2.38

3.2.2 Volatile compounds

A total of 33 volatile compounds were analysed and 26 were detected (Table 8).

The levels of esters and highers alcohols detected in the 13 wines were on par with the levels found in the literature (Ferreira *et al.,* 2000; Pineau *et al*., 2009; Tao *et al.,* 2009). Esters contribute collectively to the fruity notes of Cabernet Sauvignon wines often described as red or black berries (Escudero *et al*., 2007, Pineau *et al*., 2009). In this study, ethyl lactate was the only ester found at above threshold concentrations; its odour threshold is reported at 154 000 µg/l (Escudero *et al*., 2007). Higher alcohols contribute collectively to the complexity of wine aroma (Ugliano & Henschke, 2009). In this study, 3-methyl-1-butanol (isoamylalcohol) and 2-phenylethanol were found at levels well above their odour thresholds established at 30 000 and 14 000 µg/l respectively (Guth 1997; Ferreira *et al.,* 2000).

Most of the 13 wines presented above threshold concentrations of β-ionone, estimated at 0.09 µg/l, in a model wine (Kotesridis *et al.,* 1999). The levels ranged from 0.04 to 0.25 µg/l.

β-damascenone was found at levels ranging between 1 and 4.3 µg/l, which corresponds to the levels generally reported in red wine (Pineau *et al*., 2007). β-damascenone odour threshold in red wine is estimated between 2 and 7 µg/l (Pineau *et al*., 2007); it is thus difficult to give a definitive interpretation relating to the levels found in this study. However, β-damascenone is a powerful odorant which has been identified as an impact odorant in Cabernet Sauvignon (Kotseridis & Baumes, 2000).

Dimethyl sulphide (DMS) was detected in all 13 wines at levels ranging from 47 to 186 µg/l, the highest levels were found in the commercial wines (2008 and 2010 vintages) which is in agreement with what has been reported in other studies (Swiegers *et al*., 2005). The levels of 3MH and 3MHA, two volatile thiols which contribute significantly to the aromatic complexity of Cabernet Sauvignon wines, were similar to those reported by Bouchilloux *et al.* (1998).

1,8-cineole (or eucalyptol) was detected in three wines at or above its perception threshold in red wine estimated at 1.1 µg/l (Capone *et al.,* 2011); wine B presented a particularly high level of eucalyptol (23.4 µg/l). Other terpenes such as geraniol, β-citronellol, linalool were found at low concentrations as reported by Robinson *et al.* (2014).

The alcohols C_6 derivatives, hexanol and cis-3-hexenol were detected in all 13 wines, at low levels, significantly lower than their odour thresholds estimated at 8000 and 400 µg/l respectively (Guth, 1997). Finally, ibMP levels varied considerably, ranging from 0.006 to 0.045 µg/l. Notably, in this study, six wines (wine samples F, G, I, J, M and N) presented levels at or above the odour threshold of 2-isobutyl-3-methoxypyrazine (ibMP) estimated at 0.015 µg/l in red wine (Roujou de Boubée *et al*., 2000).

Table 8: Volatile composition of 13 South African Cabernet Sauvignon wines and their sensory thresholds (μg/l).

COMPOUNDS	SENSORY THRESHOLD	A	B	C	D	E	F	G
Esters								
Isobutyl acetate	**2100[1a]**	23	63	57	54	34	31	47
Ethyl butyrate	**600[1a]**	202	178	222	182	219	200	138
Isoamyl acetate	**1830[1a]**	274	435	582	483	261	177	417
Ethyl hexanoate	**440[1a]**	236	251	407	280	329	331	202
Ethyl octanoate	**960[1a]**	136	227	272	239	197	234	161
Ethyl decanoate	**200[2b]**	21	38	25	35	33	27	19
Phenethyl acetate	**250[2b]**	33	79	54	78	29	24	5
Ethyl lactate	**154000[3c]**	209159	189537	256040	213634	244547	249424	213960
Diethylsuccinate	**200000[3c]**	49440	32495	31498	26395	30617	39408	22118
Higher alcohols								
1-propanol	**50000[4b]**	60501	16993	26175	26788	27772	75693	32621
1-butanol	**150000[4b]**	2187	1390	1907	1940	2062	2095	1881
isobutanol	**40000[5d]**	48048	78773	80610	66481	51153	33251	65781
Isoamylalcohol	**30000[5d]**	246348	286440	327278	280231	230492	166537	242997
2-phenylethanol*	**14000[2b]**	84000	118000	122000	120000	69000	47000	90000
C6 derivatives								
Hexanol	**8000[5d]**	1787	1217	3016	1910	1174	1643	1858
Cis-3-hexenol	**400[5d]**	29	7	31	39	21	36	34
C_{13} norisoprenoids								
β-Ionone	**0.09[6b]**	0.11	0.08	0.21	0.1	0.15	0.08	0.25
β-Damascenone	**2-7[7e]**	1.8	2.7	3.9	2.7	2.9	1.6	4.3
Sulphur compounds								
DMS	**10-160[8c]**	144	79	47	52	49	186	96
3MH	**0.06[9b]**	1.253	0.554	0.134	0.140	0.730	0.907	1.458
3MHA	**0.004[9b]**	0.158	0.039	0.034	0.046	0.040	0.143	0.060
Terpenes								
Eucalyptol	**1.1[10e]**	1.4	23.4	0.8	0.5	nd	nd	nd
Geraniol	**30[5d]**	4	7	3	3	6	4	3
β-Citronellol	**nf**	2	5	6	9	3	9	7
Linalool	**25.2[2b]**	2	5	7	15	4	3	14
Methoxypyrazines								
ibMP	**0.015[11e]**	0.009	0.006	0.006	0.008	0.008	0.026	0.014

Table 8 (cont.)

COMPOUNDS	SENSORY THRESHOLD	H	I	J	K	M	N
Esters							
Isobutyl acetate	**2100**[1a]	43	51	35	49	48	19
Ethyl butyrate	**600**[1a]	216	270	202	212	264	133
Isoamyl acetate	**1830**[1a]	276	300	499	349	325	297
Ethyl hexanoate	**440**[1a]	302	355	502	298	409	207
Ethyl octanoate	**960**[1a]	235	259	318	314	320	181
Ethyl decanoate	**200**[2b]	21	38	25	35	33	27
Phenethyl acetate	**250**[2b]	49	64	55	109	89	48
Ethyl Lactate	**154000**[3c]	213960	226689	179631	217303	277853	173211
Diethylsuccinate	**200000**[3c]	48261	38734	19044	24530	31325	29890
Higher alcohols							
1-propanol	**50000**[4b]	49957	30434	42529	36204	54279	61689
1-butanol	**150000**[4b]	2675	1875	2324	2263	2425	2002
isobutanol	**40000**[5d]	62346	64452	36116	81980	57683	44990
Isoamylalcohol	**30000**[5d]	290856	282832	236879	362977	320552	271311
2-phenylethanol*	**14000**[2b]	104000	94000	108000	121000	121000	111000
C6 alcohols derivatives							
Hexanol	**8000**[5d]	1523	2368	2482	1433	2446	2556
Cis-3-hexenol	**400**[5d]	31	54	16	26	31	27
β-Ionone	**0.09**[6b]	0.07	0.04	0.19	0.09	0.09	0.17
β-Damascenone	**2-7**[7e]	1.6	1.0	3.9	2.8	1.8	2.7
Sulphur compounds							
DMS	**10-160**[8c]	62	134	91	57	114	80
3MH	**0,06**[9b]	0.558	1.058	1.166	1.226	0.767	1.180
3MHA	**0,004**[9b]	0.022	0.089	0.065	0.144	0.030	0.024
Terpenes							
Eucalyptol	**1.1**[10e]	nd	2.6	0.8	0.4	nd	nd
Geraniol	**30**[5d]	2	2	3	5	4	4
β-Citronellol	**nf**	3	4	7	12	7	7
Linalool	**25.2**[2b]	6	4	8	15	9	10
Methoxypyrazines							
ibMP	**0.015**[11e]	0.012	0.016	0.045	0.006	0.018	0.017

1 Pineau *et al*., 2009; 2 Ferreira *et al*., 2000; 3 Escudero *et al*., 2007; 4 Cullere *et al*., 2004; 5 Guth 1997; 6 Kotseridis *et al*., 1999; 7 Pineau *et al*., 2007; 8 Mestres *et al*., 2000; 9 Tominaga *et al*., 1998; 10 Capone *et al*., 2011; 11 Roujou de Boubée *et al*., 2000
a In dearomatized red wine; b In model wine; c In wine; d In water/ethanol (90+10,w/w); e In red wine
nd= not detected, nf= not found
*2-phenylethanol = Phenethyl alcohol

3.3 Correlation between descriptive analysis and chemical data

3.3.1 Results

Initially eight volatile compounds, namely ibMP, DMS, eucalyptol, cis-3 hexenol, hexanol, β-damascenone, β-ionone and phenethyl alcohol were analysed in an attempt to explain the significant attributes generated during the descriptive analysis. A multiple factor analysis (MFA) combining these eight volatile compounds and 13 attributes was performed and a correlation plot was generated. Compounds and/or attributes that contributed most to the first and the second dimensions were located within the two correlation circles: the inner circle represents a correlation factor (R^2) of 0.7 and the outer circle a correlation factor (R^2) of 1. The first two dimensions accounted for 50.7% of the variance (Figure 6).

Eight attributes, namely the red berries, black berries, prunes, black currant, violets, eucalyptus/mint, wood and spicy attributes contributed to the first dimension while the fresh green, gherkins/jalapeno and bay leaves attributes contributed to the second dimension.

ibMP, β-ionone, β-damascenone, DMS, hexanol and eucalyptol showed some degree of association with at least one particular attribute. The fresh green and gherkins/jalapeno attributes, were strongly associated (R^2>0.7) with ibMP, which was detected at high concentrations in wines F and J. Hexanol was weakly associated with fresh green and gherkins/jalapeno attributes (R^2 <0.7).

The red berries attribute was strongly associated with higher levels of β-ionone and β-damascenone and was negatively correlated with the black berries, prunes, black currant, wood and spicy attributes. The violet attribute showed some degree of association with β-damascenone.

The cooked veg attribute was positively correlated to higher levels of DMS, found in wines F and I. The bay leaves attribute was weakly associated with eucalyptol (R^2<0.7) and the eucalyptus/mint attribute was associated with none of the eight volatile compounds analysed. The black berries, prunes, black currant, wood and spicy attributes, associated with the wine samples A, D, H and K weren't explained by any of the eight volatile compounds.

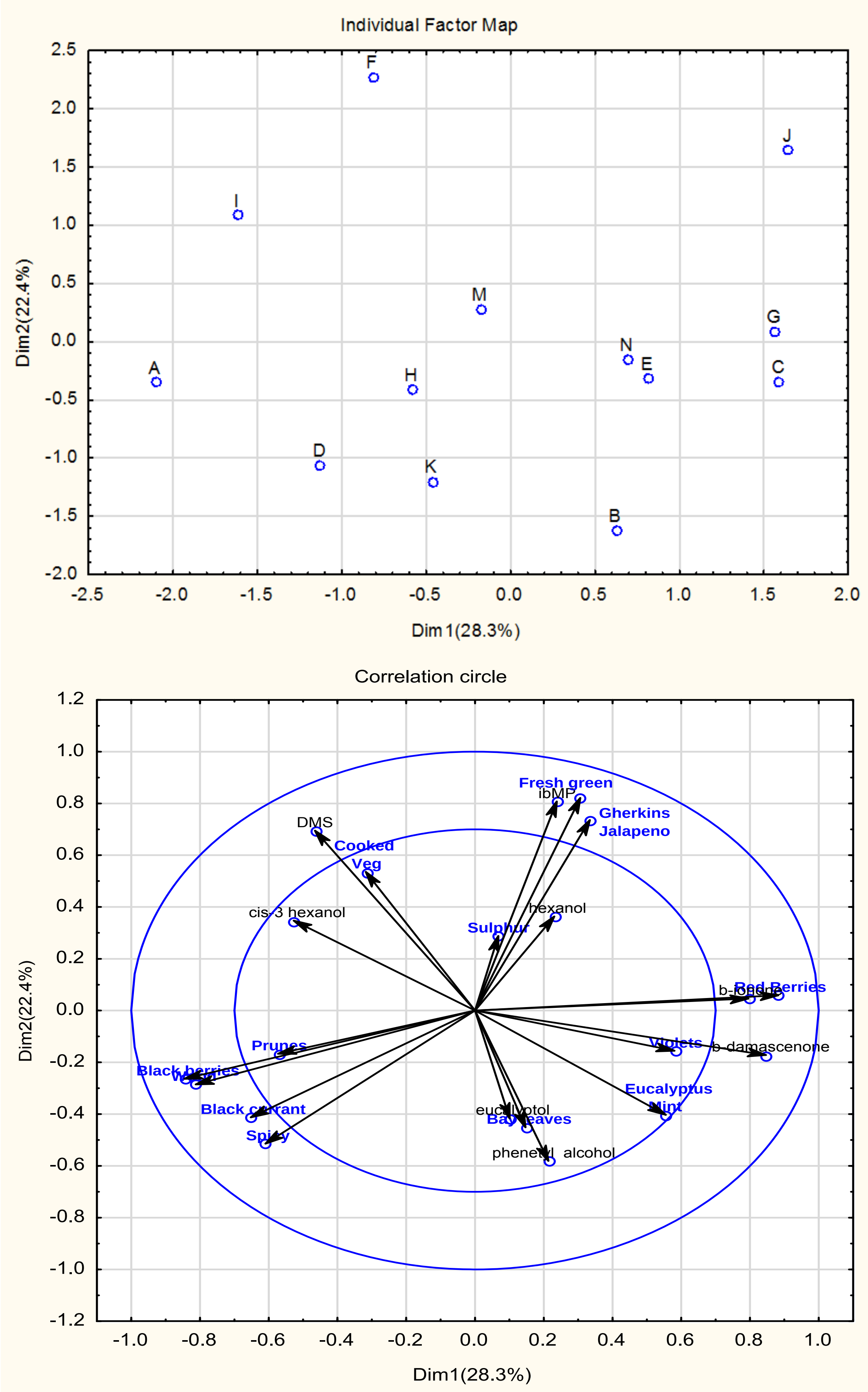

Figure 6: Correlation plots of 13 attributes and 8 volatile compounds of 13 Cabernet Sauvignon wines. (Dim1xDim2)

In an attempt to explain the black berries/prunes/black currant attributes, the concentrations of some esters and volatile thiols were added to the chemical dataset. A new MFA was then performed, combining 18 volatile compounds and 13 attributes and generated a new correlation plot in which the first two dimensions explained only 42.7% of the variance. Little new information was gained in terms of possible relationships between the attributes and the added chemical compounds (Figure 7a). The fresh green and gherkins/jalapeno attributes were positively and strongly associated with ethyl hexanoate. The black berries, prunes and black currant attributes were still not strongly associated with any of the 18 volatile compounds, but diethyl succinate and 3MHA were in close proximity.

To investigate further, correlations in the third dimension were plotted as well (Figure 7b). The third dimension accounted for 14,4% of the variance and brought new insights regarding the relationship between chemical composition and perceptual properties of the wines. Isobutyl acetate, ethyl hexanoate ethyl octanoate, ethyl decanoate, isoamyl acetate and phenethyl alcohol contributed most to the third dimension and were associated with wine samples B, C and M. The fresh green and gherkins/jalapeno attributes were weakly (R^2 <0.7) but positively associated with the red berries attribute. The black berries and black currant attributes were strongly associated with diethylsuccinate. The prunes attribute correlated quite well with DMS and even more so with 3MHA. Finally the violets attribute was positively associated with β-ionone.

Another correlation plot was generated to investigate the relationships between the attributes, the alcohol content and all volatile compounds including the terpenes (β-citronellol, geraniol and linalool) and the higher alcohols (Addendum B). Despite the added chemical data, the first two dimensions explained only 41.9% of the variance. The attributes were poorly explained by the terpenes and the alcohol content. Isobutanol, 3-methyl-1-butanol (or isoamyl alcohol), phenethyl alcohol, phenethyl acetate and isobutyl acetate contributed most to the second dimension together with ibMP and the fresh green and ghekins/jalapeno attributes. The fresh green and ghekins/jalapeno attributes were to a degree, negatively associated with isobutanol, 3-methyl-1-butanol, phenethyl alcohol, phenethyl acetate and isobutyl acetate.

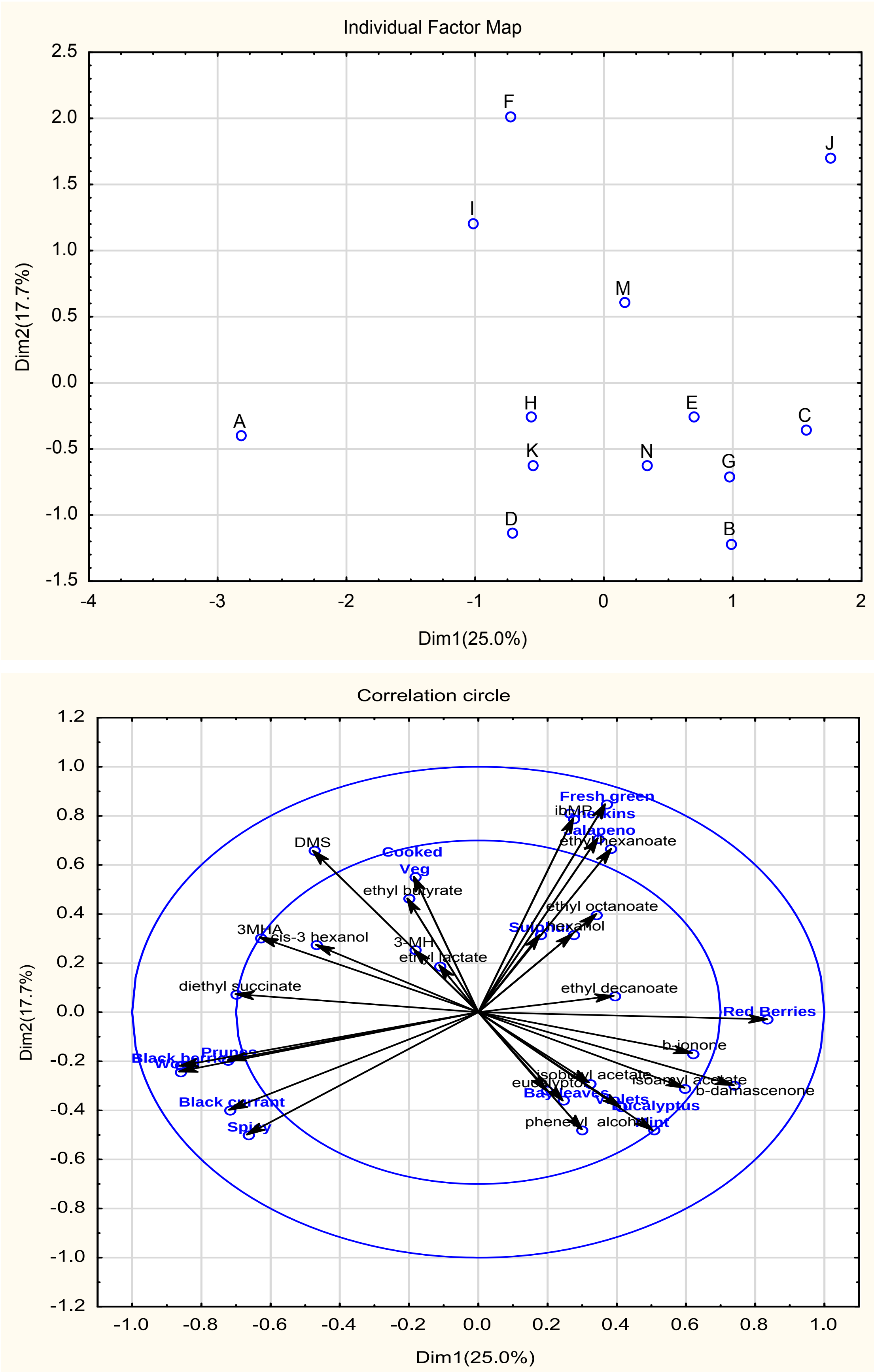

Figure 7a Correlation plot of 13 attributes and 18 volatile compounds of 13 Cabernet Sauvignon wines (Dim1xDim2).

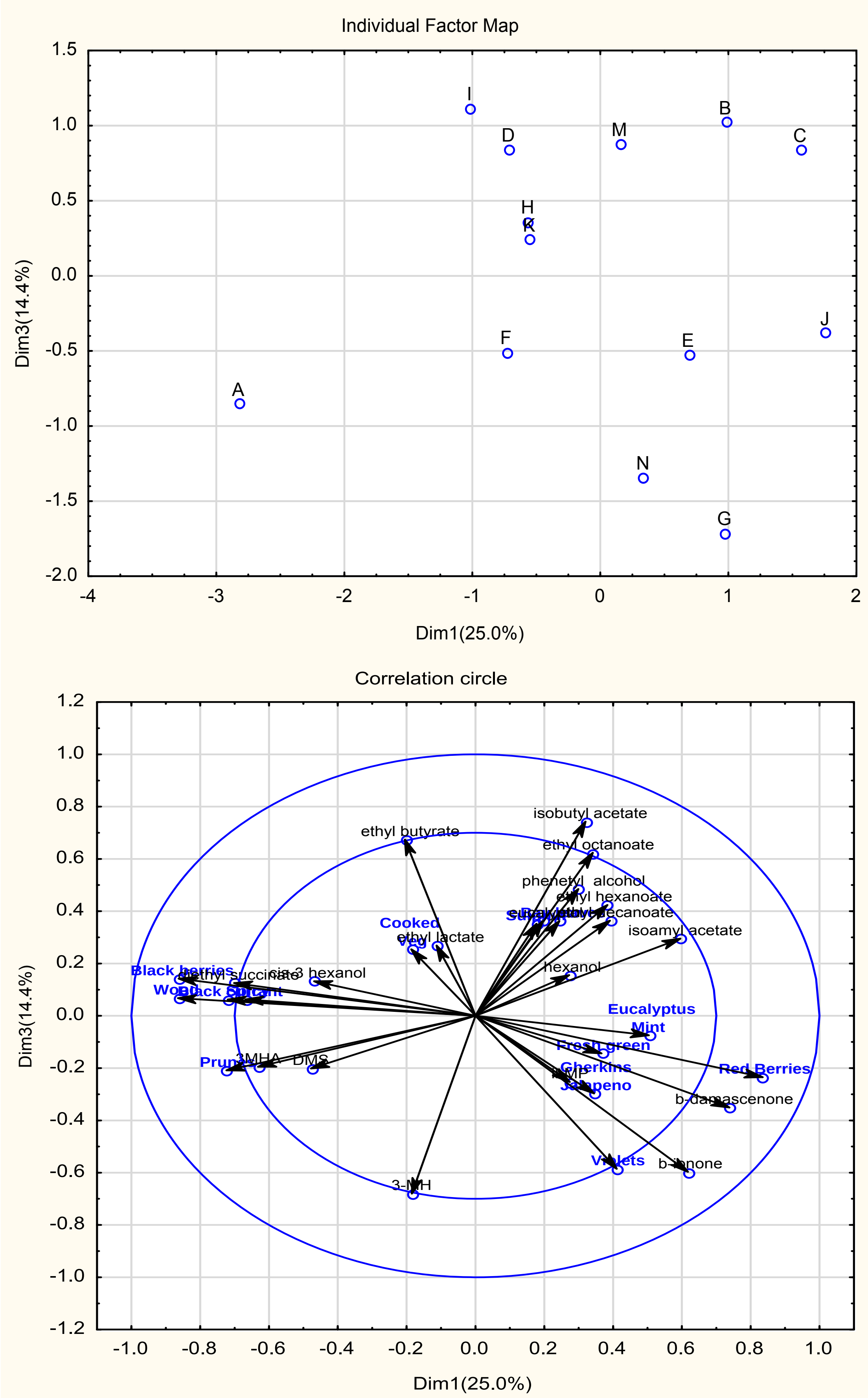

Figure 7b: Correlation plots of 13 attributes and 18 volatile compounds of 13 Cabernet Sauvignon wines (Dim1xDim3).

3.3.2 Discussion

In this study, the fresh green and gherkins/jalapeno attributes were positively correlated with higher concentrations of ibMP. Wines J and F had high, above threshold concentrations of ibMP (45 and 26 ng/l respectively) and had high intensity scores for both the fresh green and gherkins/jalapeno attributes (Figure 4). High levels of ibMP are usually perceived as a lack of ripeness and impart a vegetative/herbaceous character to red wines also described as a bell pepper aroma (Allen & Lacey, 1998; Roujou de Boubée *et al*., 2000). Wine samples G, I, M and N presented relatively high concentrations of ibMP near or above its odour threshold (14, 16, 17 and 18 ng/l respectively); but only wine sample M was described by fresh green as aroma attribute (Figure 4).

ibMP has often been poorly correlated with the vegetative/herbaceous aromas of red wines especially when the reported concentrations are near or below its sensory threshold (Falcao et al., 2008; Preston *et al*., 2008; Bindon *et al*., 2014; Hjelmeland *et a*l., 2013). Hein *et al.* (2009) reported that the bell pepper aroma is less intense in wines spiked with fruity flavorants indicating a masking effect of vegetative aromas by fruity aromas. It has been suggested that β-damascenone could make an indirect contribution to red wine aroma because it lowers the perception thresholds of red fruit aroma compounds such as esters and increases the odour threshold of ibMP (Escudero *et al*., 2007; Pineau *et al*., 2007; Falcao et al., 2008). Aroma models and omission tests performed to help characterise the aroma of Grenache rosé wines showed that the suppression of β-damascenone only affected aroma intensity and the authors concluded that β-damascenone played the role of aroma enhancer although it did not add qualitative properties to the wine as such (Ferreira *et al*., 2002). In the current study the red berries attribute was positively associated with higher levels of β-damascenone and β-ionone and to some extent with ibMP as shown in Figure 3.7b. The correlation of red fruit character with ibMP has also been reported in a recent study (Bindon *et al*., 2014).

In this study, the prunes attribute was positively associated with 3MHA and DMS, two volatile compounds that contribute indirectly to fruity aromas in red wine (Segurel *et al.,* 2004; Escudero *et al*., 2007; Rigou *et al*., 2014). The levels of DMS tend to increase during wine ageing (Swiegers *et al*., 2005), a trend also observed in this study with the highest levels of DMS found in four of the five commercial wines. Two of these wines (wine samples F and I) were described by the cooked veg attribute. However, wine A which presented the second highest concentration of DMS (144 µg/l) had the highest intensity scores for the black berries, prunes and black currant attributes (Figure 4). Other studies have reported the positive contribution of DMS to red wines by enhancing fruity notes (Segurel *et al.,* 2004; Escudero *et al*., 2007; Bindon *et al*., 2014). It was shown that the addition of 100 µg/l of DMS to a mixture of esters and C_{13} norisoprenoids significantly increased the berry fruity notes (Escudero *et al.,* 2007).

Little work has been published on the levels and sensory impact of varietal thiols in Cabernet Sauvignon wines. However, the addition of copper sulphate to Cabernet Sauvignon and Merlot wines proved that both 3MH and 3MHA contribute greatly to the aromatic complexity of these wines (Bouchilloux *et al*., 1998). In this study, the prunes attribute was positively correlated with 3MHA but none of the fruity attributes were associated with 3MH. The fruitiness of rosé wines made from Merlot, Cabernet Sauvignon and Grenache has been significantly associated with 3MH (Murat *et al.,* 2001; Ferreira *et al*., 2002). A recent study reported that 4MMP correlated well with the blackcurrant aroma and that high concentrations of 3MH and 3MHA had an enhancing effect which increased the perception of this aroma in red wine (Rigou *et al*., 2014).

Recent studies reported that the fruity/ berry notes in red wine were related to the addition effect of fruity esters and particularly branched ethyl esters (Escudero *et al*., 2007; Pineau *et al*., 2009). The combination of some esters present at higher than average levels impact on the perception of, either red berries aroma (ethyl butyrate, ethyl hexanoate, ethyl octanoate) or black berries aroma (ethyl propanoate, ethyl 2-methylpropanoate, ethyl 2-methylbutanoate) in red wines (Pineau *et al.,* 2009). In this study most of the esters of interest were measured at concentrations well below their olfactory thresholds determined in de-aromatized red wine by Pineau *et al.* (2009) and were not or only poorly associated with the fruity aromas with the exception of diethylsuccinate which was strongly associated with the black berries and black currant attributes (Figure 7b).

Unlike what other studies have reported, eucalyptol or 1,8-cineole did not correlate well with the eucalyptus/mint attribute (Capone *et al*., 2011; Robinson *et al.,* 2011) but rather with the bay leaves attribute which characterised wine samples B and I (Figure 4). A recent study suggests that 1,4-cineole together with 1,8-cineole could contribute to the hay, bay leaves and black currant aromas reported in Australian wines. In contrast to 1,8-cineole, the occurrence of 1,4-cineole is probably not due to the presence of eucalyptus trees in the vicinity of the vineyard (Antalick *et al*., 2015). Another study reported that eucalyptol and hydroxycitronellol were positively correlated with the eucalyptol and mint aroma attributes (Robinson *et al.* 2011). During the descriptive analysis training sessions in our study, it became clear that the panellists were not accustomed to the eucalyptus/mint aroma. The bay leaves attribute was used instead, but correlated rather weakly with eucalyptol. The eucalyptus/mint attribute was kept to describe the wines, but in the end it could not be associated clearly with any volatile compounds. The relevance of this attribute appeared limited in this study.

Wine aroma is the result of a multitude of physico-chemical interactions involving the volatile compounds and the non-volatile constituents which constitute the wine matrix (Ebeler & Thorngate, 2009). The suppressive or enhancing effects of certain high-impact odorants interacting with each other have a major impact on aroma perception (Ferreira *et al*., 2002; Escudero *et al.,* 2007; Pineau *et al.,* 2009). A number of studies have also reported that high levels of ethanol tend to decrease the intensity of fruitiness in red wines (Escudero *et al*., 2007; King *et al.*, 2013). Indeed, higher alcohol contents decrease the volatility of active odorants in the wine headspace altering significantly aroma perception (Ryan *et al*., 2008; King *et al*., 2013; Bindon *et al*., 2014).

Volatile compounds that have little or no perceptual impact of their own are often disregarded (Noble & Ebeler, 2002). However, some studies suggested that the addition of subthreshold concentrations of volatile compounds could produce a small but measurable increase in the perceived intensity of active odorants (Bult *et al*., 2001; Miyazawa *et al*., 2008). These factors should be kept in mind when chemical and sensory data of wines are combined.

4 Conclusion

This study has investigated the volatile composition and sensory properties of 13 South African Cabernet Sauvignon wines. A descriptive analysis was performed by a trained panel to characterise the aromatic profile of each wine and 33 volatile compounds were analysed.

The statistical treatment of the sensory and chemical data provided valuable insights regarding the relationship existing between the volatile composition and the perceptual properties of the studied wines. The descriptors fresh green and gherkins/jalapeno correlated well with higher levels of ibMP and the red berries attribute correlated well with higher levels of β-damascenone and β-ionone. Finally the black berries and black currant attributes correlated well with diethylsuccinate while 3MHA and DMS were positively associated with the prunes attribute.

A number of attributes were not or poorly explained by the volatile compounds measured; this was the case for the sulphur, spicy, wood, eucalyptus/mint and the bay leaves attributes. Obvious reasons for this would be the limited number of volatile compounds measured and the limited number of wine samples analysed. A larger number of wine samples, and of course the analysis of a larger number of volatile compounds including branched ethyl esters and oak-derived compounds may have helped in giving more insight into the correlations with the attributes by revealing stronger trends.

However, this study has shown that a limited, selected number of volatile compounds could predict the aromatic profiles of Cabernet Sauvignon wines. Winemakers may find some value in the analysis of compounds such as ibMP, DMS, β-ionone, β-damascenone and 3MHA to explain certain descriptors in their wines.

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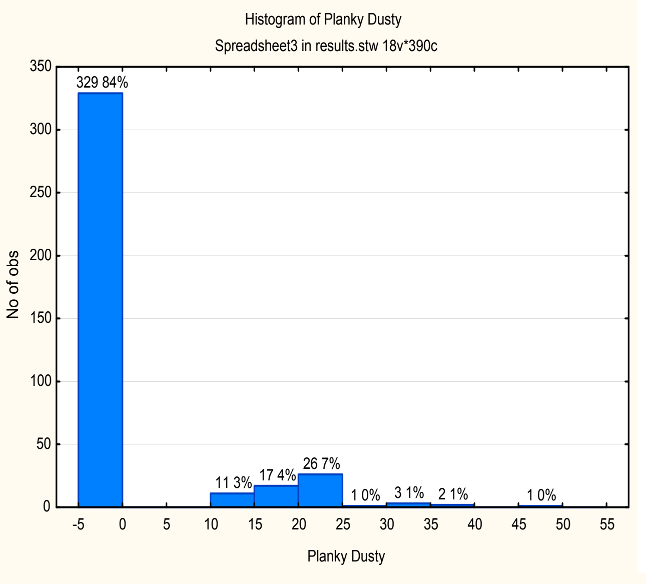
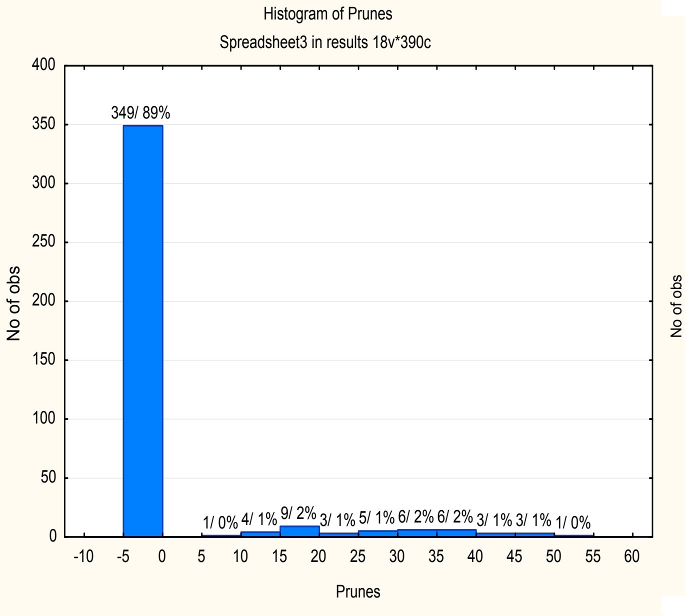
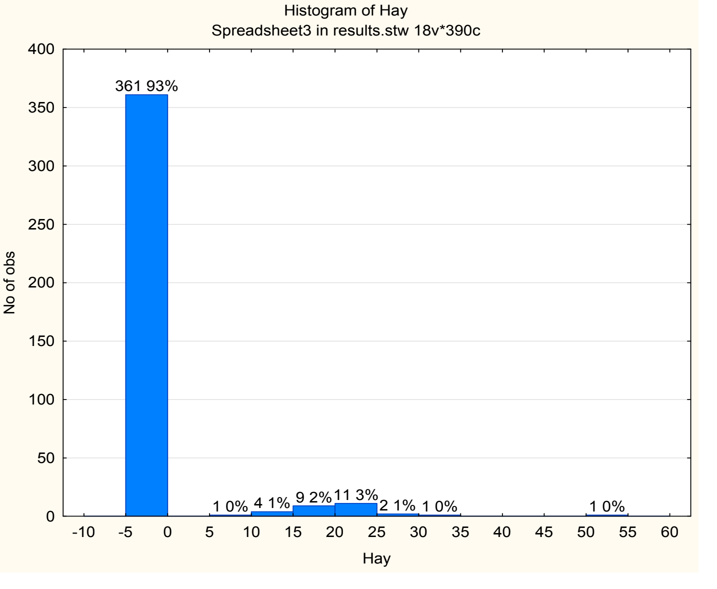
ADDENDUMS

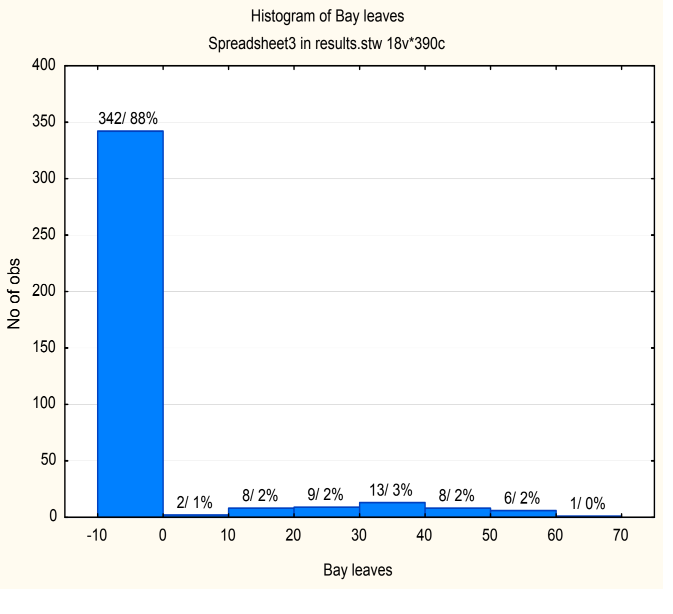
ADDENDUM A

2D Histogramms representing the numer of observations for bay leaves, prunes, hay and planky/dusty attributes.



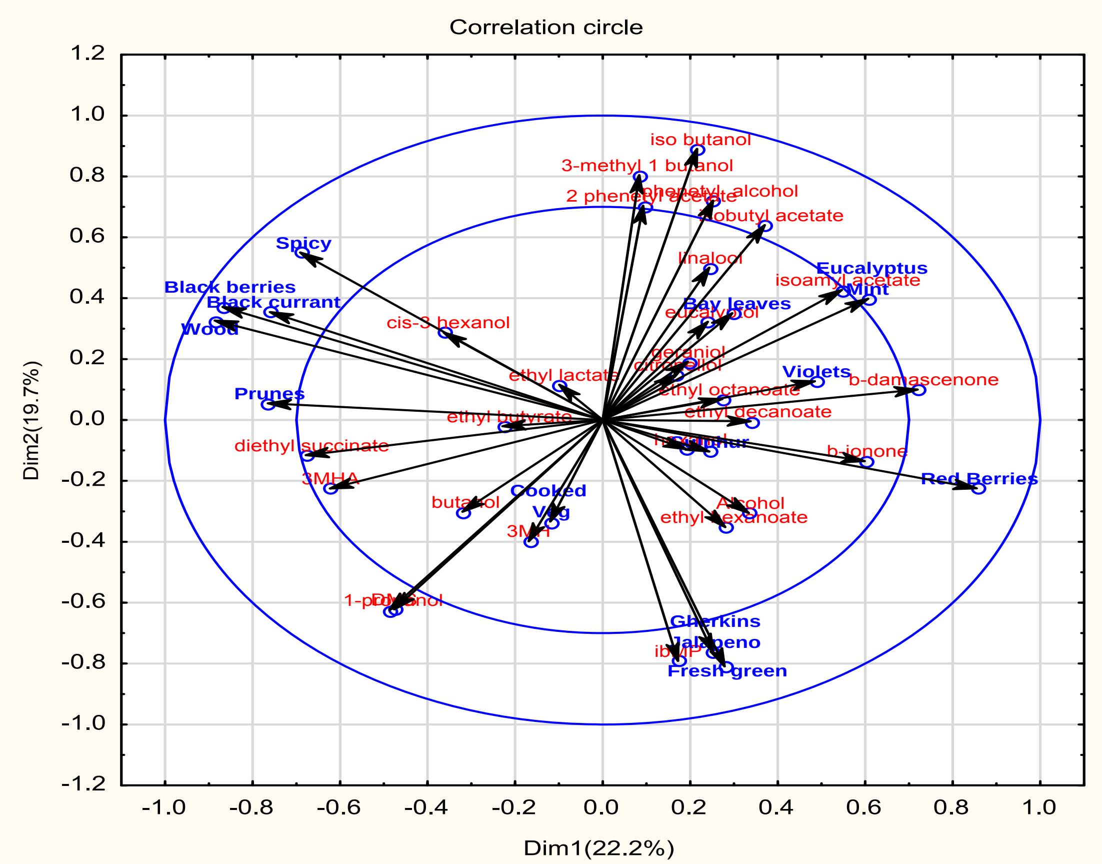


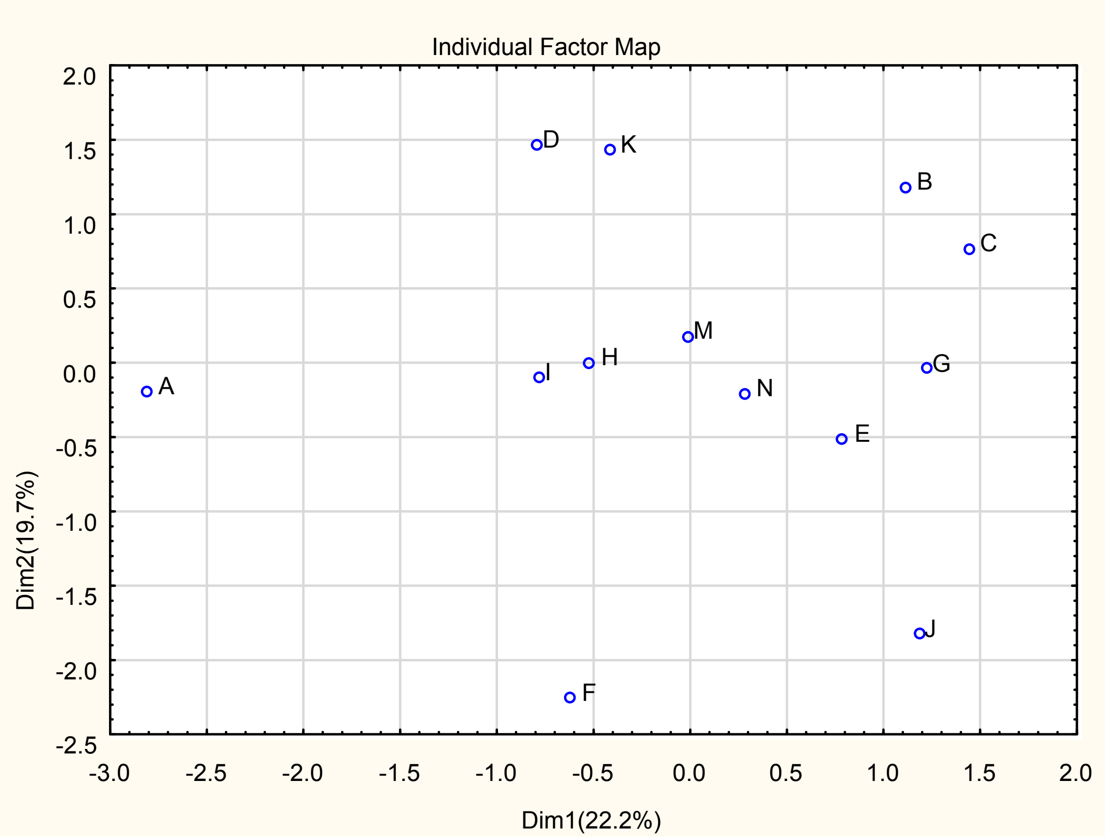




ADDENDUM B

Correlation plot combining 13 attributes, alcohol content and 26 volatile compounds in 13 Cabernet Sauvignon wines





Chapter 4

General discussion and conclusions

1 General discussion and conclusions

Cabernet Sauvignon wines account for some of the finest red wines produced in the world. High quality Cabernet Sauvignon grapes produce tannic wines with an intense dark red colour exhibiting fruity/ berry notes with spicy, roasted and sometimes cedar notes (Bouchilloux *et al.,* 1998; Oberholster *et al*., 2010). However, Cabernet Sauvignon wines can sometimes be characterised by vegetative/herbaceous notes associated with unripe grapes; the produced wines are often considered of a lesser organoleptic quality (Allen *et al*., 1994; Roujou de Boubée *et al*., 2000).

In the past five years, the South African wine industry has shown increased interest in experimenting in the vineyard to improve the quality of Cabernet Sauvignon grapes at the time of harvest. However, little research has been done to characterise the aromatic profiles and measure important volatile odorants in South African Cabernet Sauvignon wines.

The aim of this study was mainly to characterise the sensory attributes and chemical composition of 13 selected South African Cabernet Sauvignon wines.

In Chapter 3 it was found that certain volatile compounds previously linked to the aroma of Cabernet Sauvignon wines (Kotseridis & Baumes, 2000; Falcao *et al*., 2008; Robinson *et al*., 2011) could reliably predict some typical aroma descriptors of Cabernet Sauvignon wines. Notably, higher, above-threshold concentrations of ibMP correlated well with the fresh green and gherkins/jalapeno attributes, higher levels of β-damascenone and β-ionone correlated strongly with the red berries attribute and 3MHA and DMS contributed to the prunes attribute.

From Chapter 3 it also was clear that certain attributes namely eucalyptus/mint, bay leaves, wood, spicy, sulphur, were not explained or only poorly explained, by the compositional data. This study restricted its scope of investigation to the chemical analysis of a limited number of volatile compounds that could best explain fruity berry notes and herbaceous/vegetative notes but the contribution of certain volatile compounds including oak-derived volatile compounds and branched ethyl esters was overlooked.

The aroma of Cabernet Sauvignon wines and red wines in general is extremely complex and far from being understood. A multitude of interactions occur amongst volatile components themselves (suppressive and additive effects) and also between the volatile phase and the wine matrix, which is constituted of polyphenols, tannins, proteins, polysaccarides and lipids (Ebeler & Thorngate, 2009). Several studies have also documented the effect of ethanol on the perception of certain wine aroma and reported that higher levels of ethanol tended to decrease the intensity of fruitiness (Escudero *et al*., 2007) and impacted the overall sensory perception of red wines (Ryan *et al*., 2008; King *et al*., 2013).

A particular volatile compound is likely to have its odour altered due to the physico-chemical interactions taking place in the wine matrix. Thus it is difficult to predict how the different active odorants will interact and will be affected by the wine matrix without modelling procedures (Ryan *et al*., 2008). Omission tests on reconstituted wine models provide much insight into the effect of certain volatile compounds on the perceptual properties of wines (Ryan *et al*., 2008). For instance, using such experiments, Ferreira *et al*. (2002) reported that the elimination of 3MH from a reconstructed mixture of aroma compounds corresponding to Grenache rosé wines led to the suppression of citric and fruity notes and the enhancement of flowery and caramel notes. However, the perceived aromas of reconstructed mixtures often remain different from those of the original wine. These differences may be due to the presence of unidentified, sub-threshold compounds that modulate the perception of impact odorants but cannot be detected by conventional GC-O techniques (Ryan *et al*., 2008).

Moreover, human perception of odours in a complex mixture is limited and is unlikely to discern more than three or four different odours (Murray *et al*., 2001). Sensory perception is not limited to the physicochemical interactions occurring in the wine matrix, but is also dependent on physiological (sensory receptors) and neurological factors that need to be investigated further, possibly by using electroencephalograph (EEG) techniques (Ryan *et al*., 2008).

This study essentially aimed to characterise volatile compounds that could help to explain the fruity versus herbaceous/vegetative notes in a limited number of South African Cabernet Sauvignon wines. It seems possible to do so with a relatively small number of compounds, but a larger set of wines needs to be assessed in the future, as well as the contribution of active odorants such as oak-derived compounds and branched ethyl esters. Techniques such as GC-O could also be used to identify unknown volatile compounds contributing to the aroma of South African Cabernet Sauvignon wines. However, given the results of this study, the analysis of compounds such as β-damascenone, β-ionone, ibMP, DMS and 3MHA seem to hold promise for South African winemakers wanting to explain certain descriptors characterising their Cabernet Sauvignon wines.

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