Volatile metabolic profiling of SA Chenin blanc fresh and fruity and rich and ripe wine styles: Development of analytical methods for flavour compounds (aroma and flavour) and application of chemometrics for resolution of complex analytical measurements

by

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Declaration

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Summary

The aroma and flavour of wine are important aspects that form the basis for consumers' organoleptic experience of wine. Therefore, an understanding of the chemical composition of wine aroma is of major importance, to establish possible links between wine chemistry, sensory attributes and consumer preference for a product. For this purpose analytical chemistry and multivariate techniques are indispensable tools for the metabolic profiling of wine.

Chenin blanc is one of the most important South African export white wine varieties. However, despite its importance, very limited profiling of Chenin blanc aroma compounds has been done and information is restricted to isolated and dated reports on a few chemical compounds only. Therefore, the overall aim of this study was to obtain an in-depth view of the volatile chemical profile of this cultivar.

The first task was to perform targeted volatile metabolic profiling of the three dry and off-dry Chenin blanc styles, fresh and fruity, rich and ripe unwooded and rich and ripe wooded. To this end, a new, simple and robust liquid-liquid extraction technique using dichloromethane was developed and validated for extraction of analytes prior to gas chromatography flame ionization detection (GC-FID) analysis, to quantify 57 analytes in one rapid analytical procedure. This method was applied to profile 48 Chenin blanc wines. Very successful discrimination between the three styles, using the quantified volatile compounds, was obtained with two multivariate methods. These were partial least squares regression-discriminant analysis, (PLS-DA), as well linear-DA, using best subset selection for identifying the most important variables. According to the classification models, a higher content of maturation derived, malolactic fermentation derived and wood derived compounds were predominantly characteristic of the wooded wines. Higher content of some terpenes and ethyl esters were predominantly associated with the rich and ripe unwooded style Chenin blanc wines, while the fresh and fruity style were generally characterized by high levels of acetate esters.

Secondly, untargeted analysis of 21 wines was done with gas chromatography mass spectrometry (GC-MS). Mathematical chromatography, using PARAllel FACtor analysis (PARAFAC and PARAFAC2), was applied to the GC-MS data for resolution of the

complex chromatographic results by multi-way modeling, and to derive unbiased multivariate classification models of the three styles. This approach provided excellent style differentiation, without the arduous task of analysis of numerous standards and setting up of calibration curves, required by the targeted approach described above.

Additionally, the data generated during this study will form part of the current South African wine aroma database, which does not contain any data regarding Chenin blanc at present.

Opsomming

Die aroma en geur van wyn is belangrike aspekte aangesien dit die basis vorm van die wynverbruiker se organoleptiese ervaring van die produk. Derhalwe, is ‘n deeglike kennis van die chemiese samestelling van wynaroma baie belangrik, ten einde die korrelasies tussen wynchemie, sensoriese eienskappe en verbruikersvoorkeure te bepaal. Vir hierdie doel, is die insameling van analitiese chemiese data, tesame met multi-veranderlike tegnieke om relevante inligting uit die data te onttrek, onmisbaar vir die metaboliese profilering van wyn.

Chenin blanc is huidiglik een van Suid-Afrika se belangrikste uitvoer wit wynvariëteite. Ten spyte hiervan, is daar tot hede egter baie min profilering van Chenin blanc se aromakomponente gedoen en die beskikbare inligting is beperk tot geïsoleerde en verouderde navorsingsbevindinge. In die lig van bogenoemde, is die oorkoepelende motivering vir hierdie studie dus om die vlugtige chemiese komponente se profiel in Chenin blanc wyn, in diepte te bepaal.

Die eerste taak was die geteikende bepaling van die metaboliese profiel van drie droë of halfdroë Chenin blanc wynstyle, nl. vars en vrugtig, ryk en ryp ongehout, asook ryk en ryp gehout. Om dit te bereik, is ‘n nuwe eenvoudige en robuuste vloeistof-vloeistof ekstraksieprosedure met dichlorometaan ontwikkel, wat analise met gaschromatografie – vlamionisasie deteksie (GC-FID) voorafgaan, om die konsentrasies van 57 komponente in een vinnige analise te bepaal. Hierdie metode is gebruik om 48 Chenin blanc wyne te profileer. Deur gebruik te maak van multi-veranderlike data analitiese tegnieke, is die gekwantifiseerde vlugtige komponente data gebruik in diskriminant analise. Baie suksesvolle onderskeid tussen die drie style, is verkry deur gebruik te maak van twee multi-veranderlike metodes, naamlik: parsiële kleinste kwadrate regressie, diskriminant analise, asook liniêre diskriminant analise. Vir laasgenoemde analise, is die seleksie van die mees belangrike veranderlikes met beste sub-groep regressie bepaal.

Volgens hierdie klassifikasie modelle is ‘n hoër inhoud van veroudering-, appelmelksuurgisting- en houtverwante aroma komponente baie kenmerkend in die

houtbehandelde wyne. Hoër vlakke van sommige terpene en etiel esters was kenmerkend met betrekking tot ryk en ryp ongehoute Chenin blanc style, terwyl die vars en vrugtige style meer gekenmerk was met hoë vlakke van asetaat esters.

Tweedens is ‘n seleksie van 21 wyne geanaliseer deur gebruik te maak van gaschromatografie – massa spektrometrie (GC-MS) in ‘n ongeteikende metaboliese profileringsbenadering. Wiskundige chromatografiese metodes, spesifiek, parallelle faktor analise (PARAllel FACtor analysis (PARAFAC and PARAFAC2)), was voorts gebruik om komplekse chromatogramme te prosesseer met wiskundige, multi-vlak modellering. Met hierdie nie-selektiewe benadering, is ook suksesvolle klassifikasiemodelle gegenereer vir die diskriminasie tussen die drie verskillende Chenin blanc style. Die voordeel van die ongeteikende GC-MS analise, gekoppel met die data hanterings- en prosesseringsprotokols in hierdie studie gebruik, is dat die arbeidsintensiewe taak om kalibrasiekurwes op te stel vir elke individuele komponent, soos wat vereis word in die geteikende benadering, nie nodig is nie.

Die data wat ingewin is gedurende hierdie studie, sal ook bygevoeg word tot ‘n bestaande Suid-Afrikaanse aroma databasis, wat tans geen data aangaande Chenin blanc wyn bevat nie.

This thesis is dedicated to my whole extended family

Biographical sketch

Nina Lawrence was born in Durban, South Africa, on the 31st of January 1978 and was raised in Denmark, Sweden and South Africa. She matriculated at Västerviks Gymnasium in Sweden in 1997. In 2006 Nina obtained a HonsBSc-degree in Chemistry at Cape Town University. While working for the Institute for Wine Biotechnology at Stellenbosch University, she enrolled for an MSc in Wine Biotechnology in 2010.

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Preface

This thesis is presented as a compilation of 5 chapters. Each chapter is introduced separately and is written according to the style of the journal Analytica Chimica Acta to which Chapter 3 and 4 will be submitted for publication.

Chapter 1	**General Introduction and project aims**
Chapter 2	**Literature review**
Part 1	Wine style research
Part 2	Wine world-wide: Focusing on Chenin blanc
Part 3	Volatile wine compounds
Part 4	Wine analysis
Part 5	Data handling
Chapter 3	**Research results** High throughput quantification of volatile compounds in South African Chenin blanc: Method development, validation and application to wine style characterisation
Chapter 4	**Research results** Untargeted GC-MS analysis of South African Chenin blanc table wines and application of PARAFAC for resolution of complex data
Chapter 5	**General discussion and conclusions**

Contents

Chapter 1

Introduction and project aims

1. GENERAL INTRODUCTION AND PROJECT AIMS

1.1. INTRODUCTION

Wine flavour and aroma are the result of the presence and interactions between a multitude of chemical compounds derived from the grapes, and originating during winemaking and maturation processes as well as during storage (Polaskova *et al.,* 2008; Rapp, 1998). Aroma compounds are considered to be volatile, but are sensed both orthonasally and retronasally (Francis and Newton, 2005), while flavour compounds, that include volatile and non-volatile compounds, are perceived by taste. Continuous research has been undertaken with regards to the chemistry of wine, specifically with respect to aroma compounds, as they contribute significantly to the overall quality and organoleptic experience of a wine (Swiegers *et al.*, 2005; Lambrechts and Pretorius, 2000).

Chenin blanc is a white grape variety that has its origins in the Loire valley of France. Believed to be brought to South Africa by Jan van Riebeeck in the 1650's, it is the most widely planted variety in South Africa (Robinson, 1994; 2004; LaMar, 2002). South Africa is the largest producer of wine under the Chenin blanc label (Floris, 2011). In recent years, South African Chenin blanc has received an increasing amount of media attention (Brower, 2009; Splash, 2009; Eedes, 2011), due to the high quality of the wines being produced, and more interest in the variety spurred on by especially the South African Chenin Blanc Association (CBA). Due to its relatively high acidity, it can be used to produce a wide variety of wine styles, including sparkling, dry and dessert wines; with six unique styles identified by the CBA (Smith, 2004). In terms of dry and off-dry table wines, three styles are recognised by the CBA and used on bottle labels; these being two unwooded styles, fresh and fruity and rich and ripe unwooded, respectively, and one wooded style, referred to as rich and ripe wooded. Winemaking practices vary greatly between different producers, but in general, the fresh and fruity style is made without any malolactic fermentation or maturation, and is intended to be consumed within 18 months after bottling (Loubser, 2008). The rich and ripe styles are made with extended lees contact; sometimes, a limited extent of malolactic fermentation is allowed and the wines are matured with or without wood contact, as the style names specifies.

Chenin blanc style diversity is considered to have caused some confusion for consumers, in the sense that the flavour of the wines is difficult to predict and consumers do not recognise a specific wine on the basis of the current style classification (Brower, 2009; Bester, 2011). Therefore, a more structured and objective approach, using analytical techniques as well as

sensory science and the consumers' point of view, to evaluate these different styles, is eminently required.

The aroma of Chenin blanc has been previously described as reminiscent of guava (Du Plessis and Augustyn 1981), tropical, fruity, honey, floral, bees wax, and earthy mineral flavours (Augustyn and Rapp 1982). Recent sensory evaluation by trained sensory panels elaborated on the sensory lexicon for Chenin blanc, and demonstrated that the sensory attributes vary tremendously between and within the three different styles (Bester, 2011). Indeed, a continuum in perceived attributes and intensities were observed, rather than falling into three distinct categories, as implied by current labeling.

At present, research regarding South African Chenin blanc is part of a large international research project, referred to as the ConsumerCheck project, that combines sensory characterisation (using quantitative descriptive analysis and sorting techniques), consumer research (focusing on preference mapping and drivers of liking), and also chemical characterisation of food and beverage products. In addition, the project has a strong focus on development of advanced data analysis techniques to model the relationships between these three sets of data (Nieuwoudt, 2011).

A review of the literature indicated that chemical information generated over the past four decades with regards to Chenin blanc was very limited in terms of the different classes of chemical compounds analysed and does not cover the current produced styles. No concrete conclusions could thus be formed regarding the distribution of aroma compounds in the cultivar, neither in the specific dry and off-dry styles of interest to current Chenin blanc research. It also became evident that one problem area contributing to this situation was sample preparation prior to gas chromatographic analysis.

The literature review also revealed that robust techniques such as PARAllel FACtor Analysis (PARAFAC), have been developed and applied to specifically chromatographic data during the past decade (Skov and Bro, 2008). These techniques have focussed specifically on handling problems in chromatographic data, such as retention time shifts, baseline drift and co-elution. The application of these techniques to wine chromatographic data is limited and has not yet been applied in South African wine analytical research.

1.2. PROJECT AIMS

Against this background, the specific aims of this study were formulated as follows: (i) to do chemical profiling of aroma compounds in Chenin blanc wines, by focussing on development of a rapid high throughput gas chromatographic flame ionization detection (GC-FID) method for quantification of these compounds; (ii) to develop a protocol for handling the large volume of analytical data created by GC mass spectrometry (GC-MS) analysis, and investigate methods to overcome inherent chromatographic problems such as co-elution of analytes, retention time shifts and baseline drifts, and; (iii) to develop preliminary models, based on targeted as well as untargeted approaches using GC-FID and GC-MS data respectively, to evaluate the current commercial labelling of Chenin blanc dry and off-dry wine styles.

The experimental design to address the aims is summarised in the flow diagram shown in Figure 1.1.

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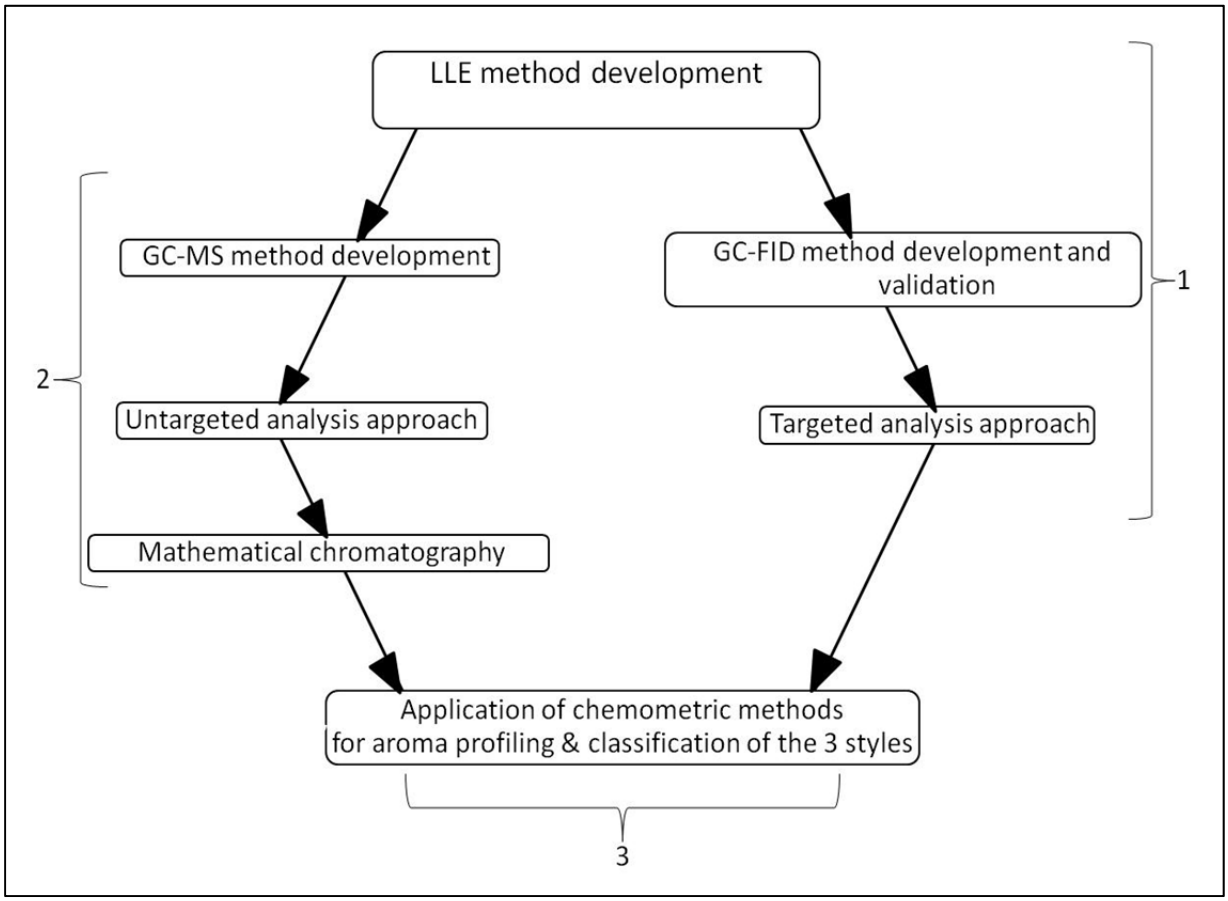

Figure 1.1 A summary of the project experimental design, focusing on the aims and objectives of the project.

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Chapter 2

Literature review

Aroma and flavour of wine with a focus on South African Chenin blanc dry and off-dry styles

2. LITERATURE REVIEW

2.1. INTRODUCTION

Wine is one of the most popular alcoholic beverages consumed around the world (Rodrıguez-Cabo *et al.*, 2011). The sensorial attributes of a wine perceived by smell and taste are important, as together, they form the basis for consumer liking and preference (Noble, 2011; Bruwer *et al.,* 2011). Consumers typically first observe and judge wine by its colour and appearance, followed by sensorial experiences when the wine is consumed. From a chemical perspective, aroma of wine implicates the volatile compounds observed by the sense of smell, while the flavour implicates a combination of volatile and some non-volatile compounds perceived by the sense of taste (Francis and Newton, 2005).

The aroma and flavour of wine are extremely complex as they constitute a synergistic combination of many hundreds of compounds formed and metabolised during various biological processes. These include berry ripening in the vineyard, grape crushing, winemaking and maturation (Bayonove *et al.,* 1998; Perez-Prieto *et al.*, 2003). More than 800 wine volatile compounds have been identified at a wide range of concentrations (from sub-ng L^{-1} to g L^{-1}) (Guth 1997; Rocha *et al.* 2000; Ferreira *et al.* 2000; Ebeler, 2001).

This chapter will review general aspects of wine styles and style selection through winemaking as well as focus on specifically Chenin blanc and the different regions producing this variety world-wide. Additionally this chapter will address the different wine aroma compound classes and their origins, as well as the fundamentally important aspects of wine and data analysis. The subsections include wine style research, Chenin blanc wine styles, volatile wine compounds, wine analysis and lastly data handling.

2.2. PART 1: WINE STYLE RESEARCH

Several factors have a pronounced effect on the style of wine produced from a specific batch of grapes. These include viticultural practices used in the vineyards, as well as the geographic origin, (*terroir*, that implies how and where the grapes are grown), which are of fundamental importance to the final wines chemical make-up. Among these diverse variables, climate, soil, canopy management, trellising, and time of harvesting all affect and contribute to the quality of the grapes. However, as with any product, the result is only as good as the ingredients and the producer; and the transformation of grapes to an exceptional wine is ultimately influenced by the winemaking and maturation processes.

As is the case for all natural products, the inherent flavour and aroma of the variety of grape used is of utmost important to the wine produced. There are many different wine grape varieties contributing to a multitude of different wine styles, with each variety having its own characteristics and flavours. The ripeness of the berries at the time of harvest is another factor influencing the style; the riper and sweeter the grape, the more alcoholic the wine will be. For example, the fresh and fruity style South African Chenin blanc table wines are usually produced using grapes harvested between 21-23 ºBrix; while the rich and ripe styled Chenin blanc wines are produced from grapes harvested between 23-27 ºBrix, which would have an effect on the residual (unfermented) sugars in the final product (O'Kennedy, 2009).

The winemaking process for red wine and white wine differ, as shown in Figure 2.1. For white winemaking (Figure 2.1 (1)) grapes are lightly crushed after harvest, with the solid parts (skins, seeds and stems) separated from the juice, and then pressed before fermentation. For red wine, red grapes are crushed and fermentation occurs with the skins present, which contributes with additional flavour, aroma and colour extraction from the skins.

Primary fermentation, Figure 2.1 (2) is the process whereby yeast converts sugars present in the grape juice or pulp to ethanol and carbon dioxide. The most general description of a wines style refers to its sweetness, which is determined by the residual sugars in the final product, for example sweet Chenin blanc wine styles which contain above 30 g L^{-1} of residual sugars or dry style containing less than 9 g L^{-1}. Fermentation can be arrested with some sugar still remaining in the wine in several ways, for instance, by lowering the temperature significantly (Boulton *et al.,* 1996). Another factor determining the final wine style is the yeast used for fermentation. The most well-known and commercially available yeasts are the related strains and species of *Saccharomyces cerevisiae*. These organisms are widely used for winemaking due to their extremely efficient fermentative catabolism (Swiegers *et al.,* 2005), and a plethora of different yeast strains are available that promote the release of various chemicals which alter and affect the flavours and aromas in the resulting wine. Winemakers producing fresh and fruity Chenin blanc wine styles normally make use of yeasts which promote the release of the mercaptan flavours (passion fruit, grapefruit and guava); while Chenin blanc rich and ripe styles are normally produced with yeasts which are known to promote tropical and floral aromas (O'Kennedy, 2009).

Many other chemical processes occur concurrently during fermentation, with different organic compounds (e.g., fusel alcohols, esters and thiols) previously absent or present in

much lower concentrations in the juice, being formed in the wine (Coetzee and du Toit, 2011).

Other factors (Figure 2.1 (3)) that can influence the style of wine during fermentation are fermentation temperature, juice clarity as well as the type of vessel used (wood or stainless steel). White wine is typically fermented at cooler temperatures (10-18 ºC) than red wine (24-32 ºC); encouraging the formation of esters which are responsible for tropical fruit aromas (Swiegers *et al*., 2005). Chenin blanc fresh and fruity style wines are normally fermented at cooler temperatures (12-13 ºC) than the Chenin blanc rich and ripe unwooded (12-16 ºC) and wooded (16-24 ºC) styles, to retain maximum acetate ester aroma (O'Kennedy, 2009). Fermenting white wines in wooden vessels (typically oak) adds characteristics of paler yellow colours and extra silky textures to the wine. For example, when Chardonnay is fermented in oak barrels, it gets a very distinct aroma profile, reminiscent of coconut, cloves and cinnamon (Guchu *et al.,* 2006; Prida and Chatonnet, 2010). Chenin blanc wines fermented in wood are typically fermented using yeasts used for Chardonnay, which bring out the citrus aromas (O'Kennedy, 2009).

After primary fermentation, malolactic fermentation (MLF) (Figure 2.1 (4)), whereby MLF bacteria convert malic acid to lactic acid, can occur. MLF is generally conducted in the same vessel as alcoholic fermentation, and is undertaken to decrease the acidity, stabilise the wine, soften the taste and improve the mouthfeel. Typically only red wines and selected white varieties, such as Chardonnay and Pinot blanc intended for ageing are subjected to MLF, (Cheynier *et al.,* 2010). White wines such as Riesling, Chenin blanc and Gewürztraminer, as well as other styles noted for their aromatic characteristics, do not usually undergo MLF. This is due to malolactic bacteria interacting with the secondary yeast metabolites, as well as some of the non-volatile grape derived flavour precursors, which would mask the varietal character op the wine (Bartowsky *et al*., 2004). MLF can take place either spontaneous or deliberately initiated, by making use of commercially available starter bacteria cultures (Arnink and Henick-Kling, 2005). Surprisingly, it has been reported that MLF can also give rise to oaky aromas in wines that were not fermented or aged in oak barrels (Arnink and Henick-Kling, 2005).

The final aroma profile of the product is further influenced by any post fermentation treatments (Figure 2.1 (5)), such as filtration, maturation and bottle ageing. Filtration, whereby suspended particles are removed from the wine, can be done to clarify and stabilise the wine before bottling (Ouch, 1992). However, not all wines are filtered as it can affect the

aroma, mouthfeel and body of the wine. In fact, current winemaking trends are such that limited clarification or filtration of wine is undertaken (Arriagada-Carrazana *et al.,* 2005).

All wines may benefit from ageing to a certain extent. Ageing (Figure 2.1 (5)) can occur in oak barrels, stainless steel tanks or bottles, depending on the desired style. The duration of maturation depends on the style of wine that is being produced, but most wines are matured for six to eighteen months (Boulton *et al.,* 1996).

Some wine styles are meant to be drunk within a few months of their bottling, and are not suitable for long ageing. These youthful wines are characterisitc of their fruity fermentation aromas derived from acetate esters (Ouch, 1992). After a couple of years, these styles lose their appeal due to aroma and flavour loss, which occurs due to metabolism and chemical reactions of the fermentation derived compounds during ageing (Boulton *et al.,* 1996). For example Zinfandel, a variety which is grown in cooler regions as well as in California, has a strong raspberry aroma that disappears after a few years. This style is aged for only a short time and bottled at the latest a year after being made (Ouch, 1992). As mentioned by Boulton *et al.,* (1996) and more recently reviewed by Penacho *et al.,* (2012), styles such as Chardonnay and Champagne are aged *sur lie*s (on the lees, i.e., the dead yeast cells), which leads to distinctive yeasty aromas. Extended lees contact (6-8 months) is also very common for barrel fermented Chenin blanc wines, which increases mouthfeel and complexity of the product, as well as enhancing ageing potential (O'Kennedy, 2009). Furthermore, ageing Chenin blanc on lees also enhances nuances of bread, citrus and butter aroma and gives a more full body to the wine (Marais, 2005).

During maturation in wood, subtle aromas of vanilla, toasty, caramel, sweet and smoke are imparted from the wood to the wine. Controlled oxidation also takes place during barrel ageing, which decreases astringency and adds to the stabilisation of the wine. Some winemakers make use of oak staves, briquettes or chips added to wine, instead of (or in conjunction with) using oak barrels (Campbell *et al.,* 2006). The advantages of using oak adjuncts instead of barrels are their availability in various toast levels and prices compared to oak barrels. They can also provide faster maturation speeds. However, disadvantages of using adjuncts include their lack of consistency and quality, as lower grade timber and oak cut-offs are often used. They are perceived by some winemakers and critics to impart harsh flavours and aromas to the final profile of the wine (Campbell *et al.,* 2006).

Ageing of wine in oak significantly changes the character of a wine, due to new and additional mellowing and complexing flavours and aromas being formed (Boulton *et al.,*

1996; du Toit *et al.,* 2011). However, wine spoilage can take place during barrel maturation due to the presence of yeasts and other organisms in the pores of the wood, as well as the presence of oxygen. Therefore, the risk of spoilage increases as the barrel ageing period is extended (Suarez *et al.*, 2007).

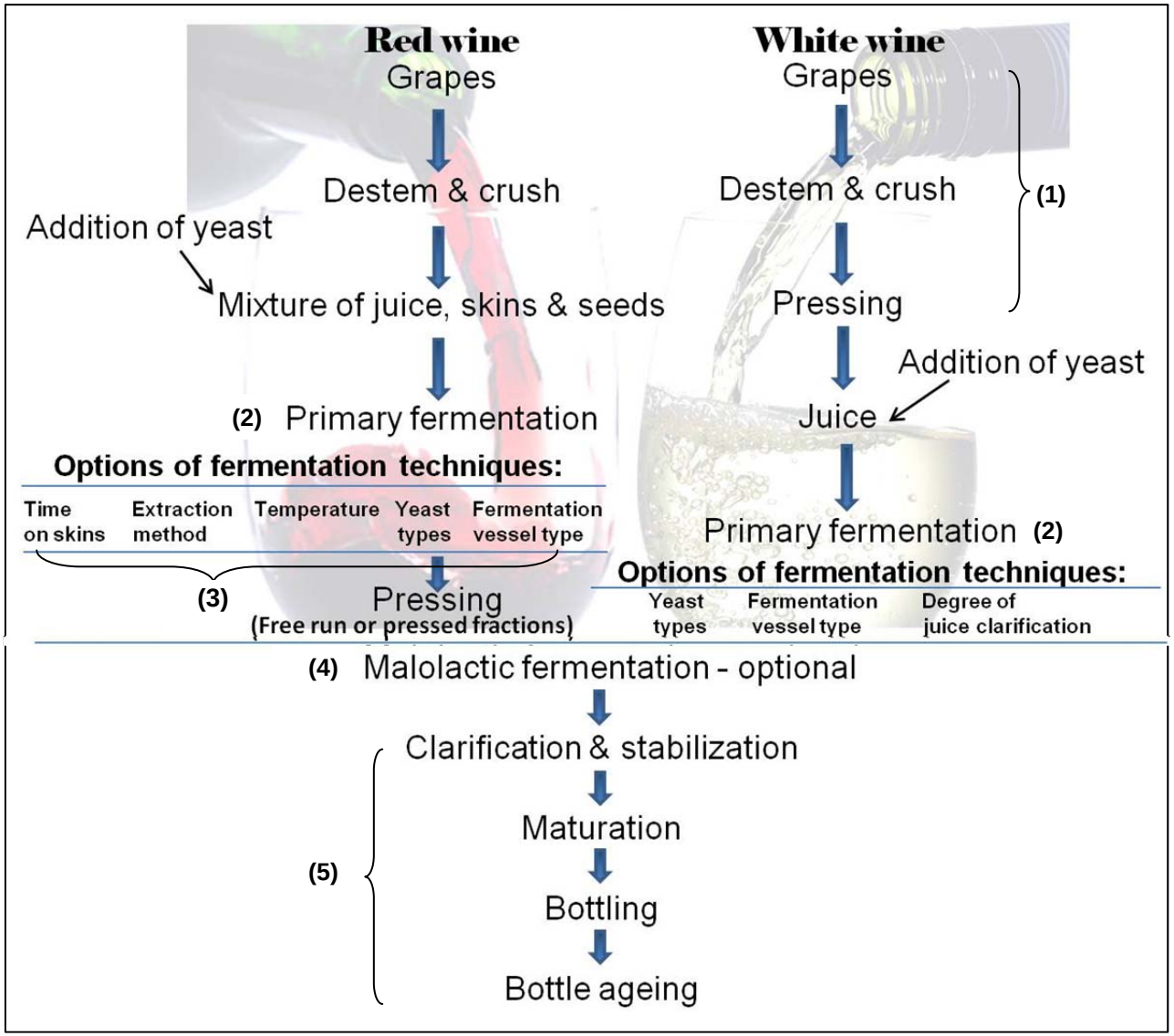

Figure 2.1 Graphical illustration of the winemaking process of red wine and white wine (adapted from Pretorius, 2000).

The unique flavour and aroma profile of a particular wine is affected by many hundreds of compounds, including fatty acids, esters, alcohols, thiols and monoterpenes, and in recent years increasing research has been devoted to the analysis of chemical profiles of wine (Weldegergis and Crouch, 2008; Louw *et al.,* 2010; Vilanova *et al.,* 2010; Weldegergis *et al.,* 2011; Coetzee and du Toit, 2011).

Similarly, sensorial research is of critical importance for understanding how chemical constituents affect the sensorial characteristics of the wine as well as the consumer perception thereof (Chollet *et al.,* 2011). Sensorially speaking, all the components act in synergy to yield the overall sensory experience. Sensorial, chemical and consumer analysis is done to aid in product development, quality control and marketing, and much emphasis is generally being placed on these techniques to analyse wine, in terms of linking consumer preferences with product development (Bester, 2011; Lombardo, 2011; Chollet *et al.,* 2011).

As the wine industry is extremely competitive, with an abundance of different wine styles available to consumers, the sensory characteristics of a wine are critical in determining repeat purchases. There are increasing demands set by the consumers on wines especially with regards to quality and price. The most important part of sensory profiling is to enable description and to quantify sensory attribute differences between a range of products, similarities within a group of products, as well as preference drivers for specific attributes and products (Bester, 2011).

There are two different research methodologies used to investigate products in terms of their sensory properties: classification tests and descriptive profiling tests (Næs *et al.,* 2010). Quantitative descriptive analysis (QDA) makes relative judgements between products (Mirarefi *et al*., 2004; Chollet *et al*., 2011), and in the free sorting method (Parr *et al.,* 2007), products are sorted into categories based on similarities. Both these methods can be used to describe and discriminate between products (Chollet *et al.,* 2011). QDA results in highly reliable and precise qualitative and quantitative data, whereas sorting tasks lead to qualitative data (Næs *et al.,* 2010). These results are of crucial importance to the wine industry to identify target markets (Bester, 2011).

Wine quality is usually defined by chemical analysis since sensory characteristics of a wine are more difficult to analyse, as it relies on human perception. The use of analytical techniques, such as gas chromatography (GC) coupled to various detection methods, for example olfactometry (GC-O) and mass spectrometric (GC-MS), has strongly contributed to the identification of odour active compounds in wine, and aided in characterising the aroma

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profiles of many wine styles. The use of advanced statistical multivariate techniques in combination with analytical data has been used increasingly over the past decade (Paul, 2009; Weldegergis *et al*., 2011). Chemometric techniques are indispensible for interpreting data and extracting relevant information from multivariate data, including sensorial, chemical and consumer analysis data (Malherbe, 2011).

2.3. PART 2: WINE WORLD-WIDE : FOCUSING ON CHENIN BLANC

2.3.1. The global wine situation

The grapevine is one of the oldest cultivated plants, and in fact, earliest viticultural practices predate written history (Jones, 2004). Archaeological findings in ancient Persia, indicate that cultivation of vines occurred as early as 4000-6000 B.C. Cultivation of *Vitis vinifera* started in ancient Persia but soon spread to Assyria, Babylon and the shores of the Black Sea (Robinson, 1994; Jones, 2004).

When discussing wine origins, it is important to distinguish New World wine regions from Old World wine regions. New World wines are those produced in regions outside the traditional European regions (the Old World), following European exploration and/or colonization in the 15th century. The New World regions are in particular South Africa, New Zealand, Australia, the United States, Argentina and Chile. Old World wines are traditionally more *terroir* (which refers to natural factors, including soil, rock, altitude, orientation towards the sun as well as weather patterns) driven, whereas New World wines are typically fruitier and generally more varietal driven (Gorman, 2011). However, in modern times the divide has become less obvious, with Old World producers adopting many of the technological advances developed in the New World, while the New World are gaining new interest in *terroir* (Gorman, 2011).

At the end of 2010, the two countries producing the majority of the almost 25 billion liters of wine produced worldwide, were Italy and France, producing 17.9 % and 17.1 %, respectively as illustrated in Figure 2.2 (Floris, 2011). South Africa produced 3.7 % of the total volume, placing the country as the 7th greatest wine producing country, together with Chile (Floris, 2011).

Shown in Figure 2.3, the leading red and white wine varieties produced globally in 2010 were Cabernet Sauvignon and Chardonnay (Floris, 2011). The production of wine styles links up with current consumer trends and preferences. The current trend has not changed considerably over the past few years. In 2007 Tinney reported that Chardonnay and Cabernet Sauvignon were the two top selling wine styles of the so called “big three”, which

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incorporated Cabernet Sauvignon, Chardonnay and Merlot. This name was due to the fact that together the three styles commanded 40 % of total wine sales (Tinney, 2007). Although the top four wine styles change places intermittently, Chardonnay and Cabernet Sauvignon have dominated the global wine market for many years (Tinney, 2007; Floris, 2011).

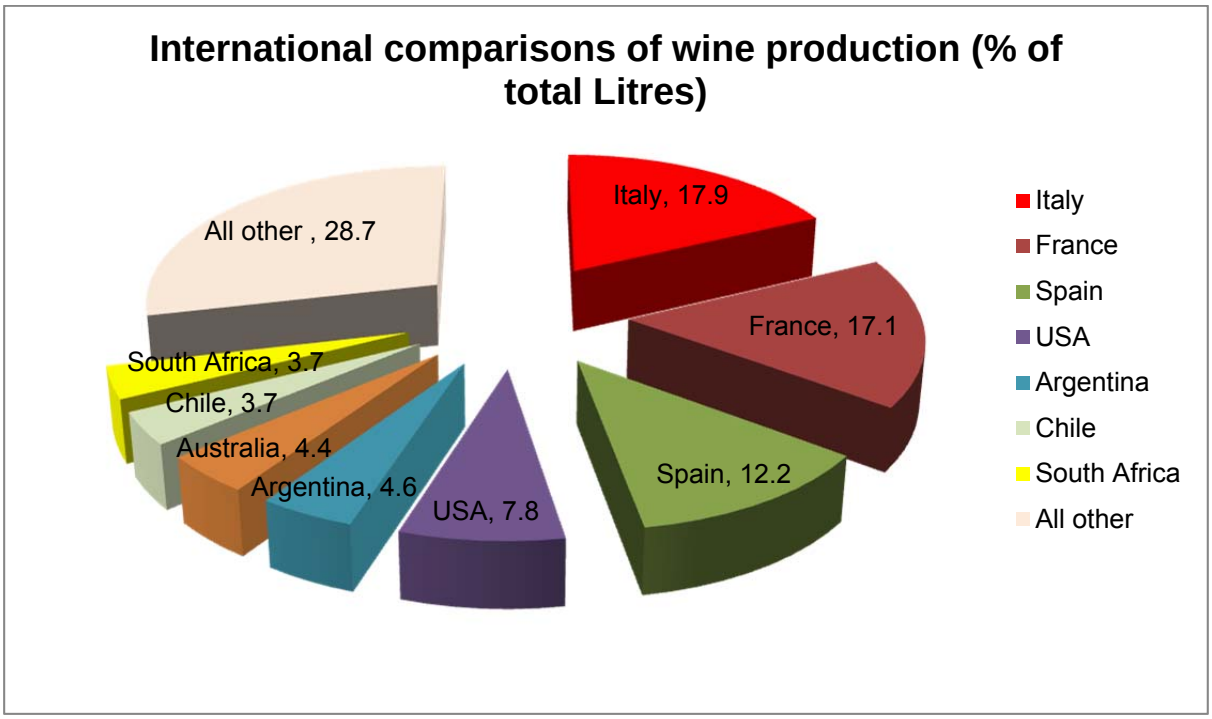

Figure 2.2 A comparison of the global wine production, illustrating that South Africa produces 3.7 %, of the total volume produced; with Italy and France being the major wine producers, supplying 17.9 % and 17.1 % respectively (adapted from Floris, 2011).

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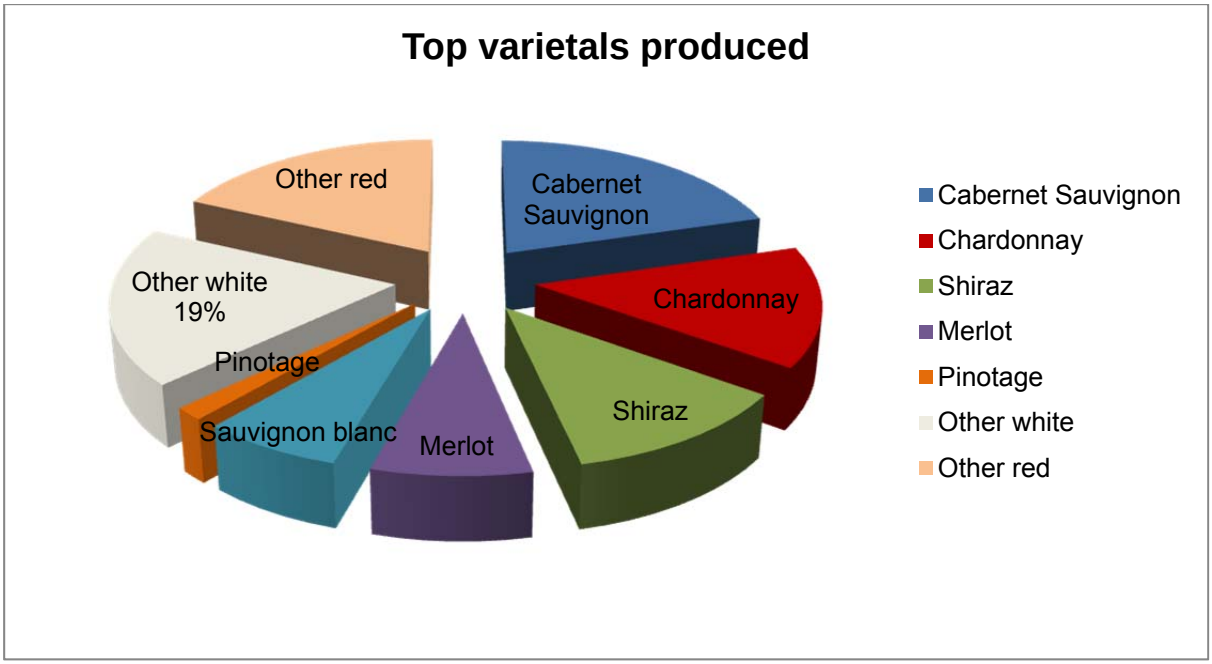

Figure 2.3 Leading wine varieties produced globally (adapted from Floris, 2011).

From the above statistics however, it is not obvious that of the approximately 370 million liters of wine produced in South Africa in 2010, almost 22 % was Chenin blanc. Furthermore, this variety was the most exported wine in 2010, amassing 12 % of the total wine exported from South Africa. This was double the volume of South Africa's second highest exported variety, Chardonnay (Floris, 2011). Despite this, Chenin blanc still is not as well known as other varieties, such as Sauvignon blanc and Chardonnay.

2.3.2. Chenin blanc – The current situation

Chenin blanc is a resilient variety of grape, that can be grown in many different *terroirs* (Marais, 2003). For this reason, it is extremely multi-faceted and can be used to produce a wide array of wine styles, thereby providing a degree of flexibility to wine producers (Ponte and Ewert, 2009).

The aroma of Chenin blanc has been described using the following descriptions: guava, tropical, fruity, honey and floral with bees wax, earthy and mineral flavours (Augustyn and Rapp 1982; du Plessis and Augustyn 1981; Giorgione, 2011). The aroma and flavour of Chenin blanc can vary tremendously between the different styles as well as within the styles, from fresh, fruity, tropical and floral flavours to rich and ripe fruit flavours (Smith, 2004). Chenin blanc wine produced from grapes grown in cool climates with long growing seasons offer the best acid and pH levels, which modulate the sweetness and promote good ageing (Robinson, 2004). No matter what style however, a high quality Chenin blanc will have the

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sensory characteristics of honey, floral as well as zesty acidity. Under the correct conditions, botrytis cinerea can also add complexity with aromas of stone fruit and extra dimensions to the palate, as well as intensity (LaMar, 2002; Thibon *et al.,* 2010).

The international Rendez-vous du Chenin, held under the auspices of the Union des Oenologues de France, in the Loire Valley, is the major Chenin blanc awards ceremony that has been put in place to aid in promoting the image of Chenin blanc world-wide as well as to try to distinguish between the array of expressions of the different styles (Robinson, 2004; Chainier, 2004). Although the aim is not to be prescriptive about style, the wines are judged in four different categories (Table 2.1), with the intention of identifying the most outstanding wines per category that exhibit prominent Chenin blanc characteristics (Robinson 2004; Chainier, 2004).

South African Chenin blanc have been winning many awards in the past few years. In 2003, at the Rendez-vous du Chenin ceremony, 12 of 49 award winning wines exhibiting the best characteristics were South African. In the dry Chenin blanc category, South African wines were awarded more medals than any other country (Budd, 2003). At the 2004 Chenin blanc awards, 210 wines from eight different countries including France, South Africa, New Zealand, Australia, USA, Thailand, India and Mexico were tasted by a panel of 53 international judges (Chainier, 2004). Of the competing wines, 51 wines from four countries were identified as exhibiting great expression of Chenin blanc. Awards were given to 33 wines produced in the Loire valley, 16 South African Chenin blanc wines, one Australian, and one American Chenin blanc (Robinson, 2004). This indicates that South African Chenin blanc, especially the dry styles, have the potential to compete against many white wines on the global wine market (Asimov, 2007).

2.3.3. Chenin blanc world wide

2.3.3.1. France – the Loire Valley

The native region for Chenin blanc is the Loire Valley, where the grape is often erroneously referred to as *Pinaeu de la Loire* (Kerridge and Antcliff, 1999; LaMar, 2002). First mentioned in 845 AD, in the abbey records of Glanfueil, which was a French Benedictine Monastery in Anjou, a former province in Western France (Oz Clarke Encyclopedia of Grapes, 2001), Chenin blanc probably originated as a mutant of Chenin Noir in Anjou (Talbot, 2003).

The Loire Valley region (Figure 2.4) includes several areas along the Loire River from the Muscadet region on the Atlantic coast to Sancerre and Pouilly-Fume in the northern part of central France. However, with approximately 9000 ha of Chenin blanc plantations, there is

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less of the varietal planted in all of France than in some of the New World Wine producing areas (Robinson, 2004; LaMar, 2002).

For many centuries a variety of stylistic different Chenin blanc wines have been produced in the Loire Valley (Table 2.1), with the style for that particular year being decided by the weather patterns. Sparkling wines are typically produced, by méthod champenoise (Stevenson, 2003), when the weather is cooler, while the sweeter wines (Moelluex) are produced during warmer years (Wilson, 1998). Loire wines tend to exhibit a characteristic fruitiness with fresh, crisp flavours (Fallis, 2006). The wines are noted for exhibiting incredible complexity of aroma and high acidity, which promotes good ageing potential, if properly cellared and periodically reworked (Halliday and Johnson, 1992).

Dry and off-dry Chenin blanc wine from the Loire valley region can have aromas of greengage, chalky and apple that with ageing develop into honey, acacia and quince aromas (Robinson, 2004). In some parts of the Loire Valley the plant can be affected by noble rot, and produce luscious high quality sweet wine, with notes of peaches and honey that develop into barley sugar, marzipan and quince as they age (Kerrige and Gackle, 2004).

Loire winemakers loyal to the Old World wine production would therefore not allow any style of Chenin blanc to undergo malolactic activity or age the wines in oak (Lawrason, 2009). Therefore the Chenin blanc styles from France and any New World Chenin blanc producing country would tend to have extremely differing aroma profiles.

Table 2.1 Loire Valley Chenin blanc styles (Friedrich, 1996).

Style type	**Residual sugar levels (g L^{-1})**
Sec	< 4 (can be up to 9)
Demi-sec	4-12
Moelluex	12-45
Doux/liquoreux	> 45

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Figure 2.4 The Loire Valley wine region of France (Thompson, 2006).

2.3.3.2. Australia

Chenin blanc is grown throughout Australia's wine regions (see Figure 2.5) however, in recent years there has been a general decline in area under Chenin blanc vine in Australia (Hughes, 2006). In contrast, production in the Western Australian (WA) Margaret River area more than doubled between 2001 and 2006, with the region accounting for 67 % of Australia's Chenin blanc production (Hughes, 2006). In 2003 there were between 500-748 ha of Chenin blanc planted in Australia (Robinson, 2004; Kerrige and Gackle, 2004).

The soil types across Australia differ greatly, as does the climate; although throughout most of Australia there is little rainfall, which necessitates the need for irrigation. The interior regions of the Riverlands are extremely hot, while the coastal regions are cooler. The Swan district of Western Australia (WA) is one of the hottest winegrowing regions in the world, whereas the Margaret River region in the South Western area offers much more temperate climates due to the influence of the Antarctic currents (Bonnardot, 2005; Johnson and Robinson, 2001). Interestingly, the west coastal area of Witchcliffe (part of the Margaret River region), shares similar weather patterns of daily cloud cover, and shares the same maritime influences as Elgin, a wine growing area in the Western Cape of South Africa. They also have similar latitudes (33 to 35 º South) (Bonnadot, 2005; Johnson and Robinson, 2001).

The most popular variety of Chenin blanc is found in the WA Swan District and Margaret River area, where winemakers produce high quality styles, and the lightly oaked wines can

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gain complexity during up to 10 years of ageing (Halliday and Johnson, 1992). Western Australian Chenin blanc styles have been described as having pronounced fruit salad notes (Robinson, 2004). New world styles are often made to be consumed young and exhibit rich tropical and fruity, guava and pineapple aromas, as they are usually fermented at slightly cooler temperatures than Old world Chenin blanc styles (Croll, 2011).

Figure 2.5 Australian wine regions (Hughes, 2006).

2.3.3.3. America

The Californian wine producing region is famous for wines produced in the Napa and Santa Rosa regions, amongst others (Figure 2.6). With 8000 ha of Chenin blanc planted in the state of California, it is home to more plantations than the Loire Valley (Robinson, 1994; 2004). Chenin blanc is the third most widely planted white grape variety in California (LaMar, 2002), with the best styles being from the Clarksburg and Monterey Counties (Ramey, 2011). The primary type of Chenin blanc produced in California is sweet wine of lower quality that is often blended with other grapes such as Colombar (Robinson *et al,* 1994).

In the 1970's Californian Chenin blanc was the most popular wine in America (Ramey, 2001). Californian styles have won various awards at wine competitions, including the 2010

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American Wine Society Commercial Wine Competition, where the 2008 Vineland Estate Chenin blanc won the bronze medal (Koch, 2011).

In the British Colombia region of Canada, some intriguing Chenin blanc wines have been produced, including icewine (Kelm, 2011), although the main focus is on Shiraz and Gewurztraminer (Kelm, 2011).

In South America (specifically Brazil, Chile, Mexico, and Argentina) where the variety is known as *Pinot Blanco,* over 10,000 acres of Chenin blanc is planted (LaMar, 2002) Argentina alone houses approximately 3600 ha of this total (LaMar, 2002; Robinson, 2004).

Figure 2.6 A map of the Californian wine region of the USA (Ramey, 2001).

2.3.3.4. New Zealand

Mainly grown in the regions of Gisborne and Hawkes Bay (Figure 2.7), New Zealand is home to 200-500 ha of Chenin blanc vines (Robinson, 2004; Robinson *et al.*, 1994; Campbell, 2010), which in fact represents no more than 1 % of the total plantations (Giorgione, 2011).

The soil in the Hawkes Bay region of New Zealand is of such a high quality that winemakers often mention it on the label. The climate in that area too is significantly warmer than the surrounding wine producing areas of New Zealand, due to the area being a former riverbed with stony soils which capture the heat, tempering the cool sea breeze (Johnson and Robinson, 2001).

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New Zealand Chenin blanc wines are typically full bodied wines with a fresh pineapple aroma and high acidity. Although mainly used as a blending variety (Campbell, 2010) there are some award-winning New Zealand wines that exhibit well balanced, complex aromas of lime, honey and lively mineral notes (Giogione, 2011).

Figure 2.7 Map of the wine regions of New Zealand (Campbell, 2010).

2.3.3.5. South Africa

South Africa is one of the oldest New World winemaking countries, as the first wines were produced in the Cape over 350 years ago. In the late 20th and early 21st centuries, nearly a third of all vines grown in the South African wine regions (Figure 2.8) were Chenin blanc, or *Steen* as the variety is also known (LaMar, 2002). In 1999, Chenin blanc represented 27 % of South African vine plantations (van der Merwe, 2007). Although it is still the most widely planted variety in South Africa, Chenin blanc represented only 18.3 % of all plantations in 2010 (Floris, 2011). As Chenin blanc is adaptable to all climactic regions in South Africa, it has been referred to as the "work-horse" of the industry.

It is an extremely versatile grape variety, capable of being used in the production of high quality wines of many different styles, including noble late harvest, sparkling wines, dry white wines, sherries and brandies (Marais, 2003; Smith, 2004). In South Africa the diversity of Chenin blanc is such that the South African Chenin Blanc Association (CBA) has recognised 6 distinctive South African styles, shown in Table 2.2 (Smith, 2004).

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Table 2.2 Chenin Blanc styles according to the Chennin Blanc Association (Smith, 2004).

Style name	**Residual sugar levels (g L^{-1})**
Fresh and fruity	< 9
Rich and ripe – unwooded	< 9
Rich and ripe - wooded	< 9
Rich and ripe – slightly sweet	9 – 30
Sweet	> 30
Sparkling	Tank fermented or Cap Classique

The winegrowing region of South Africa is situated on the southern hemisphere and experiences a Mediterranean style climate with annual weather conditions ideal for grape growing. There are many different soil types, valleys and mountainous regions, creating a multitude of different micro-climates, and providing a rich opportunity of different *terroirs* for vineyards (Toerien, 2000). Due to the fact that the sensory profile of Chenin blanc grapes is neutral, there is great scope for manipulation of the aroma and flavour profile of the final wine during the winemaking and maturation processes. Fermentation esters play an enormous role in the aroma profile of Chenin blanc styles. A wide variety of yeast strains can be used by the winemakers to obtain desired flavours. Fresh and fruity style style Chenin blanc are mainly fermented using yeasts that promote the mercaptan flavours (passion fruit, guava and grape fruit) as well as fruity esters (O'Kennedy, 2009).

According to Lawrason (2009), in keeping with Old World winemaking traditions, Loire traditionalists would not age a delicate Chenin blanc in wood; however, some South African Cape styles, especially from low yielding vines found in warmer areas where alcohol levels are higher, benefit greatly from barrel ageing (Lawrason, 2009). In the case of the wooded Chenin styles, 30-70 % MLF is allowed, as it can give complexity to the wine. Letting a Chenin blanc undergo full malolactic fermentation, on the other hand, is prevented as producers believe that the delicate flavours of Chenin blanc would be masked (O'Kennedy, 2009). MLF of the unwooded styles is avoided, as the varietal aromas would be overpowered by malolactic characteristics.

The rich and ripe wooded styles have ageing potential of between two and ten years, and some of the South African Chenin blanc wines have been found to develop a Loire Valley type character after four years of ageing. These types of wines should in fact be released only after two years of ageing, however, due to high market demands, they are often bought and consumed before developing to their full potential (O'Kennedy, 2009).

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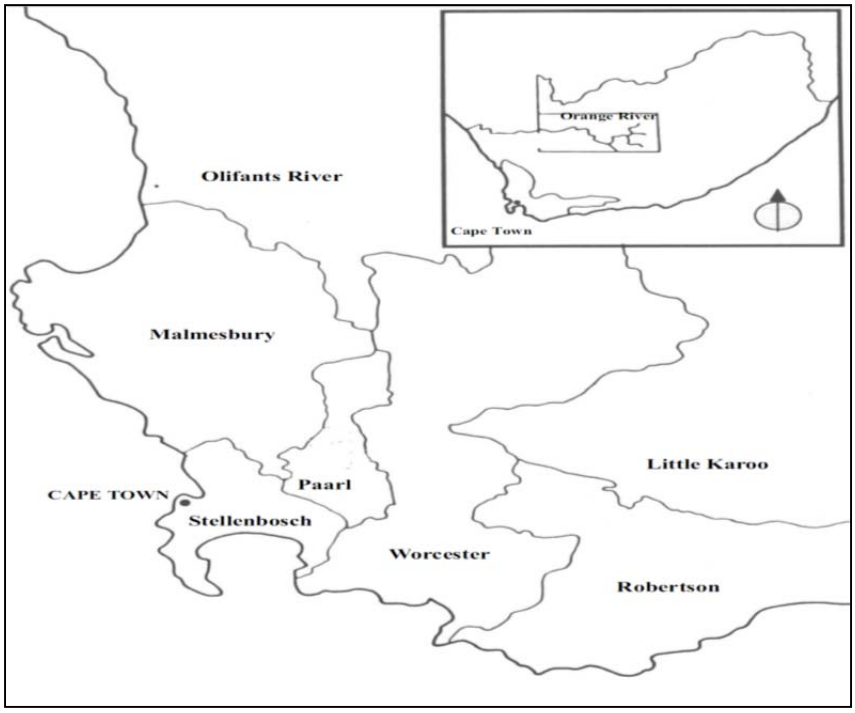

Figure 2.8 Wine regions of South Africa (Floris, 2011).

This diversity of styles and flavour characteristics in South African Chenin blanc alone has the wine consumer extremely confused, and further efforts are needed to educate the consumer to the variety of styles on the market (Gray, 2007; Brower, 2009; Bester, 2011). Goode, a wine journalist since 2001, recently asked the question “What does Chenin [blanc] taste like?” after which he stated “I don’t know what typical Chenin [blanc] is” (Goode, 2011). Gray (2007), a wine expert and wine blogger explained that it would be easier for wine consumers to purchase a bottle of South African Chenin blanc, if they knew what to expect.

The surge of interest in South African Chenin blanc (Lorch, 2011; Goode, 2011; Bester, 2011) is clearly visible from the significant increase in Chenin blanc exports over the past decade, Figure 2.9 (Floris, 2011). As illustrated in Figure 2.9, exports of Chenin blanc increased dramatically from 2003 to 2006, showing a sudden slump in export during the years 2006-2007 (Floris, 2011). This slump in exports could perhaps be explained in terms of wine production, as planting of new vines had decreased steadily from 2003 until early 2008 (Figure 2.10), while uprooting of vines had not been reduced (Floris, 2003-2011). In fact, in 2002 Botha warned that insufficient new plantings from 1997 to 2001 would result in a backlog of established and producing Chenin blanc vines within the next few years (Botha, 2002). Added to the general decrease in production, wine consumption within South Africa saw a great increase during that same period, Figure 2.11 (Floris, 2011).

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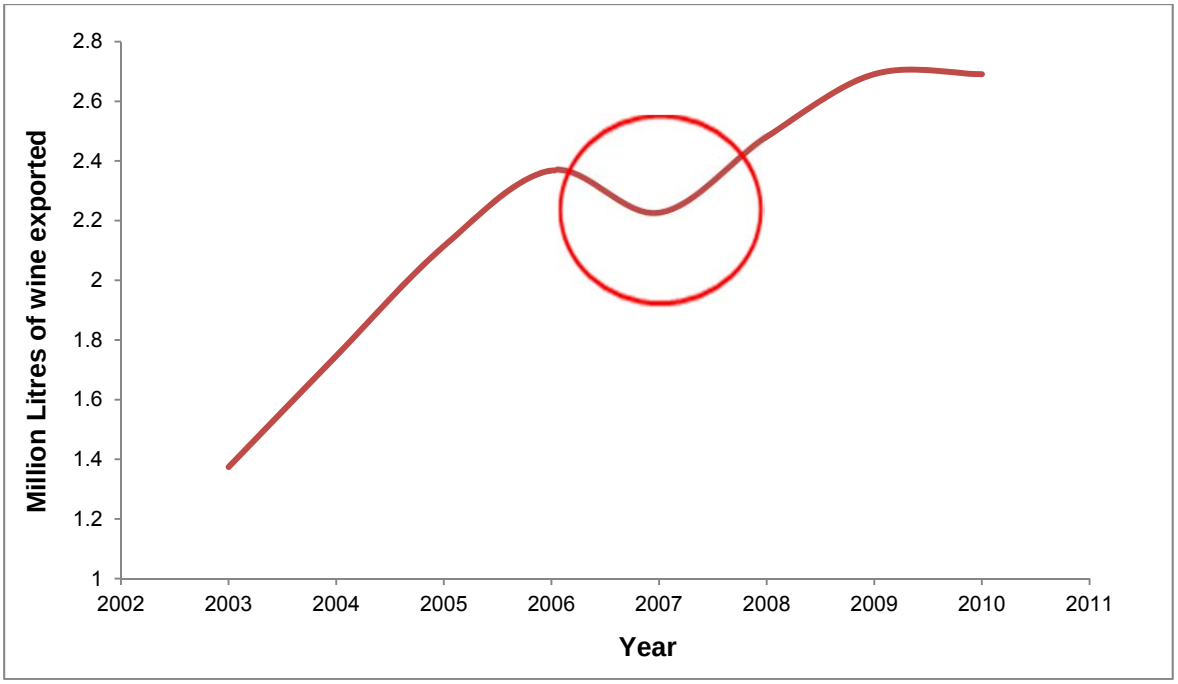

Figure 2.9 Chenin blanc exports 2003-2010 showed dramatic increase from 2003-2006 with a sudden slump between 2006-2007 (adapted from Floris, 2011).

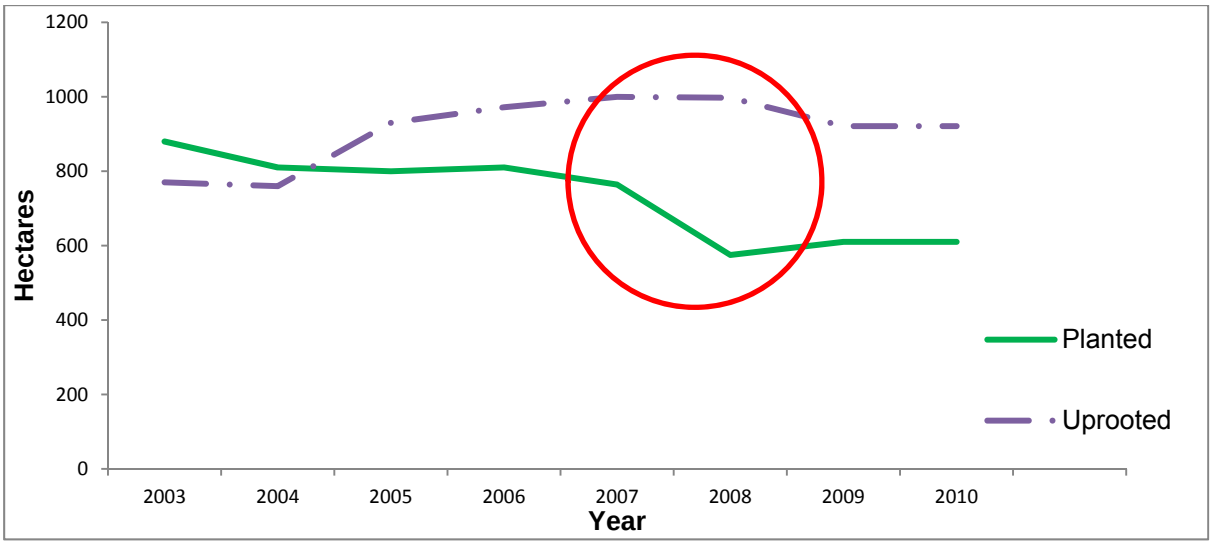

Figure 2.10 Total hectares of Chenin blanc grape vineyards planted and uprooted in South African vineyeards between 2002-2010. The red circle illustrates that between 2005 and early 2008 uprooting of vines remained constant, while planting of new vines had steadily decreased from 2003 to middle of 2008 (adapted from Floris, 2003-2011).

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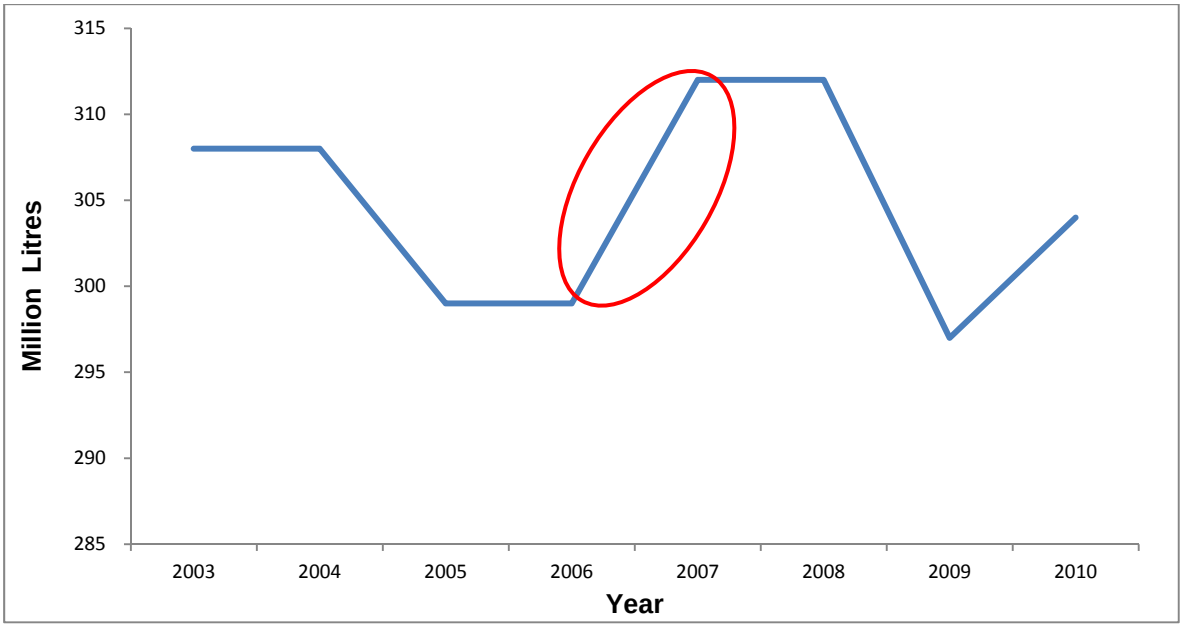

Figure 2.11 Total wine consumption in South Africa, from 2003 to 2010 showed a dramatic increase in 2006/7 (red circle) which remained constant during 2008/9 (adapted from Floris, 2011).

Despite this obvious interest in South African Chenin blanc, limited research has been undertaken on the chemical characterisation of this variety and its many styles, in comparison to other white varietals such as Chardonnay and Sauvignon blanc.

Early studies involving South African Chenin blanc included pattern recognition and factorial analysis studies by van Rooyen *et al*., (1982), in which they studied the chemical profiles of 128 South African Chenin blanc wines which had undergone 5 different fermentation treatments. It was determined that the fermentation time of Chenin blanc could be shortened considerably by increasing grape solid levels and/or temperature, as well as using sub-optimal nitrogen levels. However in doing so, the resulting wine had elevated levels of fusel alcohols and low levels of esters.

The guava aroma is generally regarded by many authors as a very desirable aroma, and sensory panels usually rate wines which exhibit it higher than those which do not (van Rooyen *et al.* 1982). On the contrary, according to South African winemaker Kershaw (2011), South African Chenin blanc producers endeavour to rather produce Chenin blanc styles with aromas of richer fruit, as the guava-like aroma is not necessarily indicative of high quality Chenin blanc. In a study performed by van Rooyen *et al.,* (1982) it was found that certain ratios of volatile compounds were involved specifically in the guava aroma that is linked to South African Chenin blanc and Colombar wines. These compound ratios were deemed to be that of ethyl butyrate to ethyl decanoate as well as ethyl butyrate to ethyl

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octanoate. In contrast, the guava-like aroma associated with Chenin blanc has also been attributed to the thiol 4-methyl-4-mercapto-pentan-2-one (4MMP) by du Plessis and Augustyn (1981). Furthermore, they observed that with ageing the intensity of the guava flavour of South greatly reduced.

O'Kennedy explained that both the rich and ripe unwooded and the fresh and fruity styles are produced using ascorbic acid and in most cases sulphur dioxide and carbon dioxide. The use of ascorbic acid can enhance the conversion of mercaptans from their precursors, which tend to give guava and passion fruit aroma to wine. However, ascorbic acid use in winemaking can lead to sulphur-like off flavors and oxidation, and therefore has become quite controversial (O'Kennedy, 2009). Nonetheless, despite the obvious interest in these characteristics of Chenin blanc, added to the technological developments of analytical instrumentation and techniques, no recent work has been published on the levels of thiols in South African Chenin blanc wines.

In 1982 Augustyn and Rapp reported that South African Chenin blanc contained no measurable levels of monoterpenes (Augustyn and Rapp, 1982). However, no more recent studies regarding the content of monoterpenes or norisoprenoids in South African Chenin blanc wines have been carried out. With the significant technical advancements over the past three decades, there are now analytical instruments with much higher sensitivity as well as better extraction and sample preparation techniques, which should be used to further explore varietal terpene and norisoprenoid levels in Chenin blanc.

In a recent study, linked to this current work which incorporated classifying the three styles of dry South African Chenin blanc by sensory descriptors and consumer preferences, Bester (2011) found that it was not possible for consumers or wine experts to clearly distinguish between the two non wooded styles (i.e., fresh and fruity and rich and ripe unwooded). As illustrated in Figure 2.12, the PCA scatter plot of 11 dry South African Chenin blanc wines according to the significant sensory descriptors, illustrated that the unwooded styles were highly associated with fresh fruit flavours, light body and tropical aroma, whereas the wooded style was strongly associated with high intensities of wood aroma and flavour, spicy aroma as well as the descriptors mature and full bodied.

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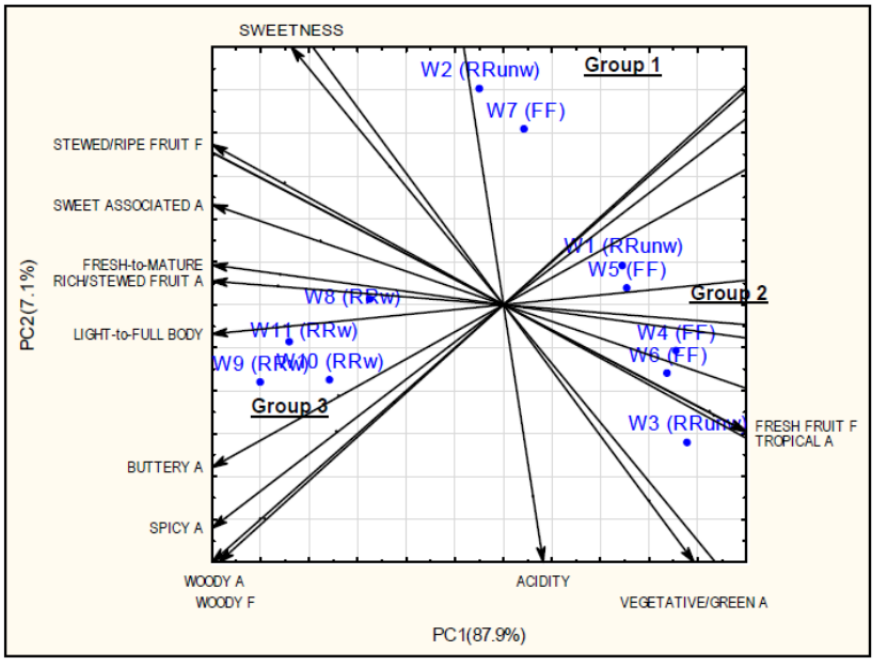

Figure 2.12 PCA scatter plot of 11 Chenin blanc wines according to their significant descriptors. Where RRunw are rich and ripe unwooded, FF are fresh and fruity and RRw are rich and ripe wooded wines (Bester, 2011).

This work revealed that according to the sensory analysis, the dry South African Chenin blanc wines should be divided into only two categories, namely wooded and unwooded styles, with the rest of the dry Chenin blanc spectrum forming a continuum between the two extreme styles. During the consumer preference analysis, it was found that consumers from generation Y (millennial generation, born in the late 1980's and forward) that preferred red wine, also preferred the wooded styles, while the consumers that typically preferred white wine favoured the fresh and fruity styles. It was concluded that marketing strategies should be in place to target the correct style of Chenin blanc towards the correct type of consumer (Bester, 2011).

2.4. PART 3: VOLATILE WINE COMPOUNDS

When studying wine aroma a distinction is generally made between (i) grape derived compounds, (ii) pre-fermentative compounds (which are released or developed during crushing, pressing and maceration), (iii) fermentation derived and, (iv) post-fermentative compounds. In this review the volatile compounds have been grouped according to the various winemaking stages, from which they generally are believed to originate. It is important to note, however, that there are some unavoidable overlaps. For example, some compounds such as terpene-related compounds are normally considered to be indicative of the varietal character however, although some have been shown also to be formed during

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barrel ageing. Similarly, esters are mainly formed during fermentation. However, some esters are also formed during MLF and some from esterification reactions during ageing. Furthermore, they are already present in the grape itself, albeit at significantly different concentrations.

2.4.1. Grape derived compounds

According to Schreier (1979) and a recent review by Moreno-Arribas and Polo, (2009), metabolites of grape derived compounds provide the basis for the specific varietal character of a wine. This large group of constituents includes volatile sulphur containing compounds such as thiols, terpenes and related compounds, volatile phenols and methoxypyrazines. Although many of these compounds occur in most grape varieties, they only exhibit distinctive varietal aromas when they are present above their odour activity thresholds (Moreno-Arribas and Polo, 2009). According to Kinzer and Schreier (1980) and as recently discussed by Vilanova *et al.,* (2008), these constituents are not altered during the fermentation process and instead pass unchanged into the wine, although they can become modified over time depending on the pH of the wine and the storage temperature.

Grape derived compounds occur in either free or glycosidically bound form, and in aromatic grape varieties the bound fraction of aromatic compounds are usually more abundant (Rocha *et al.,* 2000). Glycosidically bound volatile compounds, including terpenes, lactones and norisoprenoids have been identified as aroma precursors responsible for some varietal attributes in many grape varieties. They are released into the wine through the action of endogenous or exogenous glycosidase enzymes during fermentation (Ugliano and Moio, 2008), adding to the flavour profile and enhancing the fruity, floral aroma.

2.4.1.1. Volatile sulphur compounds

Volatile sulphur compounds (thiols) have not been reported to have been isolated from grape must (Howell *et al.,* 2003), yet they have been identified in the grapes of many wine varieties including Sauvignon blanc, Riesling, Colombar and Merlot (Tominaga *et al.,* 2000; Howell *et al.,* 2005). During fermentation, the yeast mediates the cleavage of non-volatile, odorless cysteine-conjugated precursors in the must to release volatile thiols (Tominaga *et al.,* 1995; Howell *et al.,* 2005). These precursors do not increase or decrease during ripening (Peyrot des Gachons *et al*., 2000), however, using different yeasts during fermentation results in different amounts of volatile thiols produced (Murat *et al.,* 2001; Howell *et al.,* 2004).

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Examples of these compounds are 4-methyl-4-mercaptopentan-2-one (4MMP), 3-mercaptohexan-1-ol (3MH) and 3-mercaptohexyl acetate (3MHA) which are potent aroma active compounds that at low concentrations exhibit positive aromas of boxtree, tropical fruit, passion fruit and grape fruit (Goniak and Noble, 1987; Tominaga *et al.,* 1996; Tominaga *et al.,* 1998). It has been observed that these thiols contribute to the typical varietal aromas of Sauvignon blanc (Darriet *et al.,* 1995). In 1981 du Plessis and Augustyn suggested, based on an olfactory analogy that the guava-like aroma associated with Chenin blanc was due to the volatile thiol 4-methyl-4-mercaptopentan-2-one (4MMP) released during fermentation (du Plessis and Augustyn, 1981). Ribereau-Gayon *et al.,* (2000) speculated whether or not the guava aroma in Chenin blanc wine might also be due to 3-mercaptohexyl acetate (3MHA) which exhibits similar aroma characteristics. However, no recent work with regards to the volatile thiols present in particular Chenin blanc has been undertaken, despite significant advances in analytical chemistry.

2.4.1.2. Terpenes

Terpenes, norisoprenoids and their derivatives are also associated with varietal character of wine (Komes *et al.,* 2007). They are known for their fruity, citrus, floral and perfume-like aromas, which are generally associated with the aromatic attributes of geraniol, linalool, nerol and α-terpeneol (Marais, 1983; Gamero *et al.,* 2011). Several authors have proven that terpenes are glycosidically bound to sugars located in the skin of grapes, and play a significant role in the varietal flavour of wine (Gamero *et al.,* 2011). The terpene concentration of grapes increases with ripening but as they reach the overripe stage, the terpene concentration starts to decrease (Marais, 1983). According to Dharmadhikari (1999), during wine ageing, the glycosidic bonds between the sugars and terpenes are often broken, adding to the flavour profile enhancing the fruity, floral aroma of the wine.

At present approximately 50 terpene compounds are known (Mateo and Jimenez, 2000), of which linalool, nerol, citronellol, geraniol and α-terpenol have been found to be play a significant role in for example muskat varieties (Shimizu *et al.,* 1981). In 1976 Schreier *et al.* identified that the norisoprenoid β-ionone imparted violet, woody and raspberry character especially to white wine (Winterhalter *et al.,* 2002). Cabrita *et al*. (2006) observed that β-damascenone which lends flowery, tropical fruit and apple aromas to wine and β-ionone are the two aromatic compounds contributing most significantly to wine made from neutral grape varieties. Linalool, which lends wine a floral aroma, has been observed in significant concentrations in Riesling wine (Dharmadhikari, 1999). In 1982, Augustyn and Rapp were not able to detect these compounds, based on gas chromatographic mass spectrometric (GC-MS) analysis. However, despite significant advances within analytical instrumentation

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over the past three decades, no further work has been undertaken regarding the content of terpenes and related compounds in Chenin blanc wine styles, and their possible contributions to the varietal aroma of these styles.

2.4.1.3. Methoxypyrazines

Methoxypyrazines (MPs) are nitrogen containing secondary plant metabolites, which at low concentrations are known to contribute to the herbaceous or green and vegetative perception of wine (Allen *et al.,* 1991; Koch *et al.,* 2010). On the other hand, at high concentrations they play a detrimental role towards wine quality. Although 2-methoxy-3-isobutylpyazine (IBMP) is present in low (0.5-50 ng L^{-1}) amounts in grapes, it is considered the main contributor to green, grassy and bell pepper aromas in Sauvignon blanc, due to its low odour detection threshold value of 2 ng L^{-1} (Marais, 1994; Koch *et al.,* 2010). 2-methoxy-3-isopropylpyrazine (IPMP) and 2-methoxy-3-sec-butylpyrazine (SBMP) have been identified at lower concentration in Sauvignon blanc grapes as well, exhibiting aroma characteristics of earthy and asparagus (Koch *et al.,* 2010).

Based on mass spectrometric analyses, levels of between 0.3-1.1 ng L^{-1} of IBMP were observed in four South African Chenin blanc wines (Alberts *et al.,* 2009). However, the contribution of IBMP to the aroma of Chenin blanc wines is inconclusive due to the limited number of samples. Koch *et al.,* (2010) found indications, based on the presence of the IBMP quantitative ion (*m/z* = 124) through mass spectrometric analyses, that IBMP may be present in Chenin blanc grapes. Nevertheless, confirmation, by means of the qualification ion for IBMP (*m/z* = 94) was not possible. More research is required regarding this group of compounds and their possible contribution to the aroma and flavour of the different South African Chenin blanc styles.

2.4.2. Fermentation derived compounds

An extremely important part of wine aroma arises during alcoholic and malolactic fermentation, when yeast and bacteria play a vital role in the release and metabolism of a large number of volatile aroma compounds in the wine (Robinson, 1994; Boulton *et al.,* 1996; Rapp 1995). The compounds arising from primary fermentation are considered to be the major group of volatiles present in wine. As they are present above their odour detection thresholds, they contribute significantly to the sensory character of the wine. The most abundant yeast fermentation derived volatiles are primarily ethanol (8-16 % (v/v)), ethyl acetate (22.6-63.5 mg L^{-1}, dependent on style), other esters and fusel alcohols (Cabrita *et al.,* 2006; Moreno-Arribas and Polo, 2009). Of these compounds, fatty acid ethyl esters (e.g., ethyl hexanoate and octanoate) and fusel alcohols (e.g., isoamyl alcohol and 2-

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phenolethanol) are of great importance to the aroma of specifically white wine (Mateo *et al.,* 2001), as they contribute to the perceived fruity aroma of the wine.

2.4.2.1. Volatile fatty acids

The volatile acidity of wine usually refers to a group of volatile, short carbon-chained organic acids. The total fatty acid concentration of wine normally spans the concentration range of between 500-1000 mg L^{-1}, of which approximately 90 % is acetic acid (Perestrelo *et al.*, 2006). Due to their relatively low perception thresholds (Table 2.3), they are of great importance to the aroma and flavour of wine (Francis and Newton, 2005; Perestrelo *et al.*, 2006). They are believed to add complexity to the wine bouquet at lower concentrations; however, at higher concentrations they can have a negative impact, as they impart unpleasant fat-like, rancid, cheese and pungent odours (Francis and Newton, 2005).

Selli *et al.,* (2008) analysed dry white Narince wines from Turkey, known for their neutral aroma profile, with low varietal character. They observed that, although hexanoic, octanoic and decanoic acids were the most abundant acids their contribution to the aroma of wine was of little importance, as they were below their respective odour detection threshold values (Table 2.3.) Three decades ago, Marais and Pool found significant positive correlations between the concentrations of hexanoic, octanoic and decanoic acids in Chenin blanc and its characteristic aroma of the wine (Marais and Pool, 1980). However, no recent work has been carried out on the contribution of volatile fatty acids to the aroma and flavour of South African Chenin blanc.

Table 2.3 Odour threshold (OTH) values, concentrations and odour quality of some prominent volatile fatty acids in wine (Francis and Newton, 2005; Clarke *et al.,* 2004).

Fatty Acid	Odour quality	Concentration in white wine (mg L^{-1})	OTH (mg L^{-1})
Acetic Acid	sour, pungent, vinegar	30-489[1]	200
Decanoic Acid	rancid, fat	0.5-5[2]	1
Hexanoic Acid	sweat	1-73[1]	0.4
Octanoic Acid	sweat, cheese	2-717[1]	0.5

[1]Francis and Newton, 2005; [2]Clarke *et al.,* 2004

2.4.2.2. Esters

Esters, which are mainly produced during yeast fermentation, are of sensorial importance as they represent the primary contribution to the fresh, fruity and tropical characteristics of young wines (Swiegers *et al.*, 2005; Ebeler, 2001). They are formed when an alcohol and an acid react and a water molecule is eliminated (Etievant, 1991). Esters present in wine can be classified into two groups: those formed enzymatically whereby the synthesis is catalysed by

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lipases, esterases as well as alcohol acetyltransferases (Sumby *et al.,* 2010); as well as those formed by an acid catalysed esterification reaction during wine ageing. The main source of esters production is, however, through enzymatic synthesis. As there are a large number of different alcohols and acids available in wine to react to form esters, a wide variety of different combinations are possible (Sumby *et al.,* 2010).

Given in Table 2.4 are some of most prominent esters present in wine. These are the ethyl esters of fatty acids and acetate esters of higher alcohols, which are particularly pronounced, adding fruity aromas in young white wines (Cabrita *et al.,* 2006). Ebeler (2001) considered acetate esters to be the main, if not exclusive esters responsible for the fruity aroma of wine, and Marais and Pool (1980) observed that the pleasant fresh (acidic and crisp) and fruity characters of South African Chenin blanc was derived in large part from higher alcohol acetate esters, such as isoamyl acetate and 2-phenylethyl acetate.

Table 2.4 Esters odour threshold (OTH) values, reported concentrations in white wine, odour and odour quality (Francis and Newton, 2005).

Ester	Odour quality	Concentrations reported in white wine (µg L^{-1})	OTH (µg L^{-1})
Ethyl Hexanoate	Fruit, apple peel	280-1022	4-14
Ethyl Octanoate	Fruit, fat	270-820	2-5
Ethyl Butyrate	Apple	184-700	20
Isoamyl Acetate	Banana, pear	163-4740	30
2-Phenylethyl Acetate	Honey, rose, tobacco	89-475	250

The formation of esters is not cultivar specific, but rather, as noted initially by Bertrand (1968) and reviewed by Gil *et al.,* (2006) influenced by various fermentation factors including fermentation temperature, yeast strain, must clarification and oxygen levels. Low fermentation temperatures (approximately 10 ºC) favour the formation of fruity esters (e.g., isoamyl acetate and hexyl acetate), while higher temperatures (15-24 ºC) promote the formation of higher molecular weight esters (e.g., ethyl octanoate and phenylethyl acetate).

Gil *et al.,* (2006) observed that the concentration levels of higher alcohol acetate esters in white, red and rosé wines from Spain, were significantly different, and could therefore be used to discriminate between the wine styles. On the other hand, Louw *et al.,* (2009) observed that, with the exception of hexyl acetate, no significant differences were found in the concentration levels of esters between South African Chardonnay and Sauvignon blanc wines. Nevertheless, the different winemaking practices used to produce the three South African Chenin blanc styles (as mentioned in section 2.1), including the differing berry

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ripeness, fermentation temperatures as well as the use of different yeast strains for fermentation, could perhaps result in the differing ester levels in the various styles, which could aid in their classification and discrimination. Further research is needed to verify this.

2.4.2.3. Fusel alcohols

Higher alcohols, also known as fusel alcohols, have more than two carbon atoms, as well as higher molecular weights and boiling points than ethanol (Moreno-Arribas and Polo, 2009). Fusel alcohols are quantitatively the most important group of volatile compounds released into wine as secondary products of yeast metabolism (Perestrelo *et al.,* 2006). They can be produced via two pathways of yeast action, namely the anabolic pathway from glucose, or the catabolic pathway (Ehrlich mechanism). In the Erhlich reaction (Figure 2.13) the branched amino acids leucine, valine, phenylalanine, iso-leucine and threonine (among others) are metabolised via (A) deamination, where the amino group is transferred to α-ketoglutarate, to form an α-keto acid. Next the α-keto acid undergoes decarboxylation (B) to form an aldehyde which is reduced (C) to form a higher alcohol (Ortega, 2001). Isoamyl alcohol for example is formed from leucine and isobutanol from valine (Ortega, 2001). However, depending on the redox status of the yeast cell, in step (C) of Figure 2.13, the aldehyde can also undergo oxidation, which would form a volatile fatty acid (Vuralhan *et al.,* 2003; Hazelwood *et al.,* 2008; Moreno-Arribas and Polo, 2009).

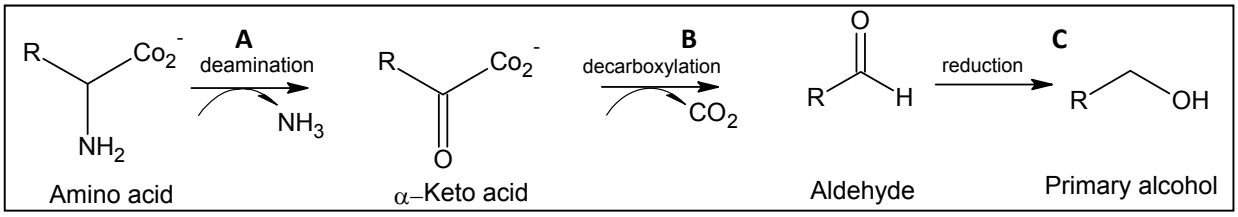

Figure 2.13 The Ehrlich reaction mechanism (catabolic pathway) of higher alcohol formation from yeast (adaptation from Moreno-Arribas and Polo, 2009).

At higher concentration levels (above 400 mg L^{-1}), fusel alcohols have a negative effect on wine, with harsh solvent like odours, but at lower concentrations (below 300 mg L^{-1}) they impart aromas of for example rose, grassy, honey and herb-like (Ferreira *et al.,* 2002; Gil *et al.,* 2006; Campo *et al.,* 2006). The volatile fraction of wine is mainly composed of alcohols of 6 carbon chain length, and aromatic compounds that have conjugated planar ring systems with delocalised π-electrons (e.g., 2-phenyl ethanol). These types of compounds contribute towards the positive sensory characteristics of sweet, floral and roses (Perestrelo *et al.,* 2006; Gil *et al*., 2006). Work done by several researchers suggests that 2-phenyl ethanol, *n*-hexanol and *n*-butanol would in fact be influential in varietal classification (Falque *et al.,* 2001; Camara *et al.,* 2006). Marais and Pool (1982) observed that amyl alcohols and 2-

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phenyl ethanol decreased significantly during the ageing of Chenin blanc wines, possibly due to alcohol oxidation to aldehydes, while isobutanol and *n*-hexanol levels increased (Marais and Pool, 1982). More recently, Roussis *et al.,* (2007) reported higher alcohols remained stable during ageing of Debina and Muscat wines. However no further studies have been undertaken on higher alcohols or their contribution to, or discrepancies between, the styles of dry Chenin blanc table wines.

White wine generally contains lower levels of fusel alcohols than rosé and red wine (Gil *et al.,* (2006) and particularly for the white and rosé styles, fusel alcohols could contribute to the aroma of the wine. The production of fusel alcohols is favored by higher fermentation temperatures levels (Gil *et al.,* 2006), while pre-fermentative clarification often employed during white winemaking suppresses the formation of higher alcohols (Moreno-Arribas and Polo, 2009).

2.4.2.4. Volatile aldehydes

Volatile aldehydes are important to wine aroma as they have low detection thresholds (low µg L^{-1} range) (Schreier *et al.,* 1979; de Revel and Bertrand, 1994). Most aldehydes found in wine, shown in Table 2.5, are produced during alcoholic fermentation; however some are derived from the grapes, while others are extracted from oak during fermentation and maturation (Jackson, 2008). Studies have shown that shorter chained aliphatic aldehydes lend aromas of green, while the longer, 8-12 carbon length aldehydes impart aromas of fruity, nutty and citrus, depending on chemical structure (de Revel and Bertrand, 1994).

Acetaldehyde constitutes more than 90 % of the total aldehyde content of wine, of which only free acetaldehyde has any significance with regards to wine aroma (Gil *et al.,* 2006). At high concentrations (> 200 mg L^{-1}) it can be quite detrimental with pungent and irritating odours of overpowering green and grassy and so called “flatness”; however at lower levels it lends wine a fruity, nutty and pleasant aroma (Ferreira *et al*., 2000; Gil *et al.,* 2006). It is an intermediary of alcoholic fermentation, obtained by decarboxylation of pyruvate (Romano *et al*., 1996), and various fermentation factors, such as enzymes arising from different yeast strains, the amount of sulphur dioxide added to the must as well as catechin levels (Bourden *et al*., 2008) can impact on the acetaldehyde content of a wine (Herraiz *et al.,* 1989; Boulton *et al.,* 1996; Osborne *et al.,* 2006). Acetaldehyde seldom accumulates to detectable levels in table wines, other than occasionally generating a temporary stale aroma in newly bottled wines (Jackson, 2009). No research has been published regarding acetaldehyde levels of

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Chenin blanc wine styles, and more research is needed with regards to sensory impact it may have on the styles.

Table 2.5 Examples of volatile aldehydes in wine; their odour quality and concentrations reported in wine.

Aldehyde	Odour	Concentrations in wine (µg L^{-1})	Odour threshold (µg L^{-1})
trans-2-Heptenal	Herbaceous[1]	Trace	4.6
3-Methylbutanal	Malty, chocolate[1]	3.3-105	16
Phenylacetaldehyde	Honey, sweet[2]	2.4-130	1
trans-2-Decanal	Herbaceous, green[3]	Trace-20	2
trans-2-Hexenal	Green[1]	0.02-1.6	4
trans-2-Octenal	Herbaceous, lemon[1]	0.04-4.1	3
Acetaldehyde	Nutty, fruity, pungent[4]	10000-200000	500

[1]de Revel and Bertrand, 1994; [2]Cullere *et al.,* 2007; [3]Beuttner, 2004; [4]Ferreira *et al.,* 2000.

Acetoin is a by-product of carbohydrate metabolism in the presence of either fermentable carbohydrates or pyruvic acid (Romano and Suzzi, 1996). Winemaking variables, such as temperature and aeration during fermentation, greatly affect the production of acetoin (Romano and Suzzi, 1992; 1996). As illustrated in Figure 2.14, there are three pathways in which acetoin is produced, depending on the substrate with which the acetaldehyde reacts (Romano and Suzzi, 1996). In all three pathways, acetoin gets further reduced to 2,3-butanediol, in a reversible reaction (Romano and Suzzi, 1996). However, the reduction of diacetyl to acetoin in pathway B is irreversible. The contribution of acetoin and 2,3-butanediol to the aroma of wine are generally not perceived as particularly important, with threshold values of approximately 150 mg L^{-1} and 600 mg L^{-1}, respectively (Romano and Suzzi, 1996; Bartowsky and Henschke, 2004). However, in contrast, Varnam and Sutherland reported that acetoin levels as low as 30 mg L^{-1} in specifically fruity wines, contributed favourably to the overall aroma, but levels of over 100 mg L^{-1} in the same wines brought about an undesirable musty flavour (Varnam and Sutherland, 1994).

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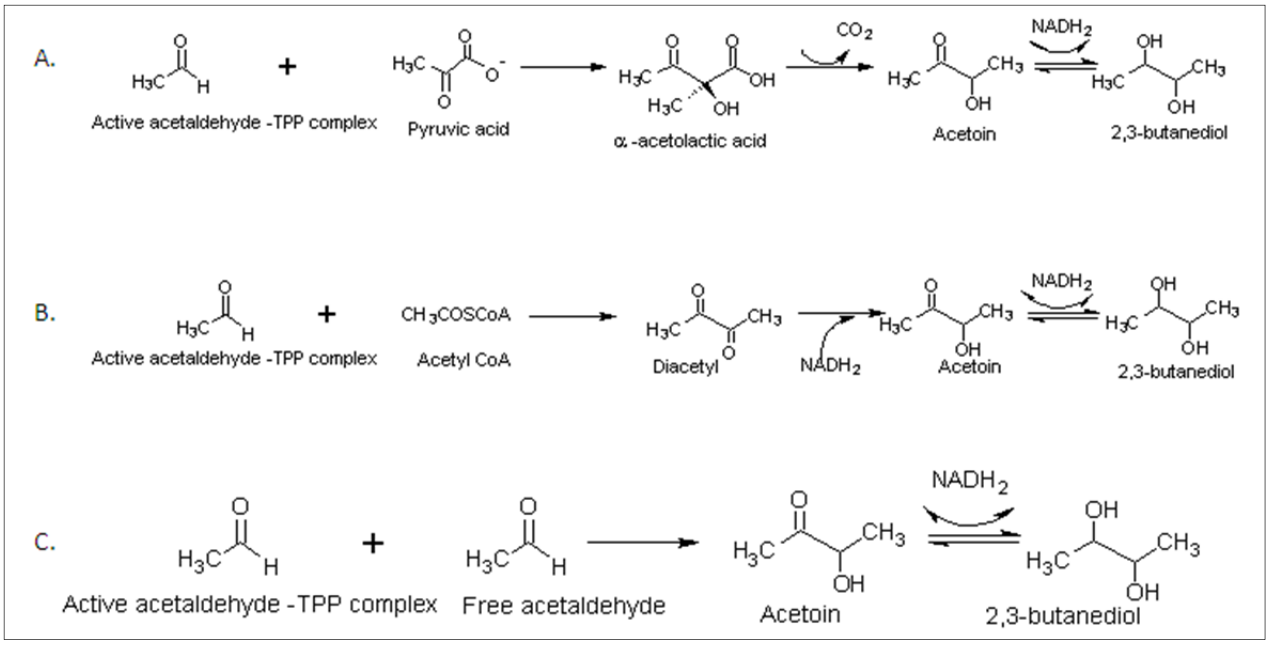

Figure 2.14 The three pathways of acetoin production (adapted from Romano and Suzzi, 1996)

Acetoin was previously identified in Narince wines, which are neutral dry white wine styles from Turkey. However, the content of acetoin in all the wines was below the aroma threshold and thus made no contribution to the overall aroma profile of the styles (Selli *et al.,* 2008). As Chenin blanc too, is a neutral variety, this would indicate that acetoin probably would not have too much of an effect on the aroma profile of the various Chenin blanc styles.

2.4.3. Malolactic fermentation derived compounds

During MLF, bacteria provide de-acidification and stabilisation of the wine; however, the aroma profile of wine is significantly altered as well (Henick-Kling, 1993). Usually MLF of Chenin blanc is discouraged and hence avoided, although the wooded styles are allowed to undergo some (30-70 %) MLF, especially as it can be difficult to hinder natural MLF from commencing during barrel fermentation (O'Kennedy, 2009).

2.4.3.1. Diacetyl

Diacetyl (2,3-butanedione) is a diketone primarily formed from acetaldehyde during malolactic fermentation and is therefore more prominent in red than white wine. A small amount of diacetyl is however also produced by yeast during alcoholic fermentation (Martineau and Henick-Kling, 1995; Bartowsky and Henschke, 2004). Diacetyl is chemically unstable and mainly gets reduced to acetoin, which in turn gets further reduced to 2,3-butanediol (Romano and Suzzi, 1996; Bartowsky *et al.,* 2004). Winemaking variables, such

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as temperature and aeration during fermentation as well as yeast strain, greatly affect the production of diacetyl (Romano and Suzzi, 1992; 1996).

When present in wine at low concentrations (threshold values of 8 mg L^{-1}) diacetyl positively contributes to wine aroma with nutty, buttery and toasty attributes and contributes to the complexity of the wine, while at higher concentrations the buttery aroma, which is negatively associated with a lactic character of wine, becomes overpowering (Martineau *et al.,* 1995; Romano and Suzzi, 1996; Bartowsky and Henschke, 2004). The sensory perception of diacetyl is extremely dependent on factors such as the age and wine style as well as on the presence of other compounds in the wine (Bartowsky and Henschke, 2004; Swiegers *et al*., 2005). Martineau and co-workers illustrated that concentrations of diacetyl of up to ten times lower than the threshold could be perceived in certain white wine styles (Martineau *et al.,* 1995). In fact, Varnam and Sutherland found that diacetyl levels of 0.7 mg L^{-1} made a positive contribution to the aroma of white wine (Varnam and Sutherland, 1994). Nothing has been reported on the levels of diacetyl in Chenin blanc styles, but low levels of diacetyl may be present in some of the wooded styles that have been allowed to undergo MLF, which could contribute to the aroma of the styles.

2.4.3.2. Ethyl esters of diprotic acids

Ethyl esters of diprotic acids increase significantly during maturation, as a result of chemical esterification (Camara *et al.,* 2006). The esters diethyl succinate and ethyl lactate are also formed during malolactic fermentation, and concentrations of these volatiles in white wine can vary considerably (Gil *et al.,* 2006). Diethyl succinate lends wine a berry, fruity and melon aroma at levels below 200 mg L^{-1} while ethyl lactate contributes aromas of butter, cream and fruit (Francis and Newton, 2005) and has an odour threshold of 150 mg L^{-1} (Mendes Ferreira *et al.,* 2001).

As the South African Chenin blanc wooded styles are allowed to undergo some malolactic activity (O'Kennedy, 2009), there may be a possibility that ethyl esters of diprotic acids may have an influence on the classification of the wooded style wines.

2.4.4. Maturation derived compounds

The maturation bouquet is formed during ageing, by various physciochemical and/or enzymatic actions such as esterification reactions, hydrolysis reactions, reduction and oxidation reactions as well as transfer of aromatic components into the wine from the wood (Rapp, 1995; Boulton *et al.*, 1996; Marais and Pool, 1980; Camara *et al.,* 2006). Various factors such as vessel type and size, age and toasting level of the wood, lees contact and

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length of storage all influence the final aroma profile. During ageing, wine slowly gains aromatic complexity which changes the flavour of the wine, with new aroma compounds being formed which also add to the maturation bouquet (du Toit *et al.,* 2006).

2.4.4.1 Wood related compounds

Fermentation and/or ageing of wine in oak barrels is an essential part of winemaking, imparting aroma characteristics such as spicy, woody, smokey, vanilla and coffee to the wine (de Revel *et al*., 2005). The length of time that a wine is aged in the barrel is dependent upon the variety and style of wine that the wine maker wishes to produce. Through thermal degradation of lignin during the oak toasting process, compounds such as furfural, 5-methyl-2-furfural, whiskey lactone, and the volatile phenols eugenol, guaiacol, vanillin are produced (Wilkinson *et al.*, 2004; Camara *et al.,* 2006). These compounds may be transferred to the wine during barrel ageing, as a result of wood and bacterial interactions, changing the composition of the wine and adding a richness and complexity to the aroma of the wine (Ferreira *et al.,* 2006; Jarauta *et al.,* 2005).

Whiskey lactone imparts aromas of coconut, citrus, dark chocolate, berries and vanilla to wine, with a sensory threshold of 67-87 µg L^{-1}. Sensorial analysis has shown that the coconut aroma associated with specifically high quality Chardonnay is due to the *cis*-isomer of whiskey lactone (Alves *et al.,* 2005; Francis and Newton, 2005; Spillman *et al.,* 2004). Eugenol and guaiacol impart aromas of spice, cloves and honey; and smokey, sweet and medicinal, respectively, at levels over their thresholds of 6 µg L^{-1} and 9.5 µg L^{-1}, correspondingly (Francis and Newton, 2005). Levels of eugenol in young white wine have been reported as being between 1.6-9 µg L^{-1} (Francis and Newton. 2005). The sensory contribution of vanillin (vanilla) has been reported at levels above its 200 µg L^{-1} odour detection threshold in wine (Francis and Newton, 2005).

The concentration levels of especially furfural and 5-methyl-2-furfural in the wine depends to a large extent on the age of the barrel used as well as the degree of barrel toasting (Camara *et al.,* 2006). They are the two wood derived volatiles which have been noted to have a relatively low impact on the aroma of wine (Camara *et al.,* 2006). Furfural imparts aromas of caramel, bread, almonds and sweet to wine, at levels above 14.1 mg L^{-1} (Chatonnet *et al.,* 1992). It has been identified in various wine styles below its detection threshold of between 100 µg L^{-1} for Chardonnay, and 0.2-23.3 µg L^{-1} for Madeira wines (Camara *et al.,* 2006).

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2.4.4.2. Acetic acid

The generation of additional acetic acid during ageing has been reported, and possible sources were noted as oxidation of ethanol via acetaldehyde as well as extraction from the wood itself (Singleton, 1995). Optimal concentration levels of acetic acid in wine have been reported as 0.2-0.7 g L^{-1} depending on the wine style; while concentrations of 0.7-1.1 g L^{-1} (style dependant) imparts a negative vinegar-like character to the wine (Swiegers *et al*., 2005).

2.4.4.3. Ethyl esters

During ageing, the acidity from acetic acid and the tannins in the wine catalytically protonate other organic acids, and eventually the acetic acid itself, encouraging ethanol to react as a neucleophile in acid-catalysed esterification reactions, and causing the content of ethyl esters to increase. As a result of this process, ethyl acetate, the ester of ethanol and acetic acid, is the most abundant ester in wine (Campo *et al.,* 2007). At low concentrations (< 150 mg L^{-1}) ethyl acetate positively contributes pleasant fruity and pineapple aromas to wine (Gil *et al.,* 2006; Francis and Newton, 2005), although at high concentrations it is detrimental to the wine as it imparts the unmistakable aroma of nail polish and sour vinegar off-flavour (Gil *et al.,* 2006; Campo *et al.,* 2007).

Marais *et al.,* (1981) observed that the levels of ethyl acetate in South African Chenin blanc and Colombar are quite low (39.3–48.3 mg L^{-1}). However, more recently ethyl acetate was quantified at much higher levels of 112.1 mg L^{-1} in Chenin blanc wines (Malherbe, 2011). Wooded style Chenin blanc wines have been shown to have great ageing potential, and therefore the levels of ethyl esters in these styles may be of significance in distinguishing the wooded from the unwooded styles.

2.4.5. Wine spoilage compounds

Yeasts and bacteria can also produce off-flavours in wine as a result of microbial spoilage. Classes of compounds that can have extremely negative effects on wine arise during any of the before mentioned processes, but for the purpose of this review have been placed in the category of wine spoilage compounds.

2.4.5.1. Sulphur compounds

A class of compounds that have a very important influence on the aroma and flavour of wine are the yeast derived sulphur containing compounds. They are extremely abundant in wine and have a particularly low sensory threshold, and therefore make a big contribution to the sensory properties of wine (Swiegers *et al.,* 2005). Most sulphur compounds (e.g. hydrogen

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sulphide (H_2S) and ethanethiol (C_2H_5SH)) impart negative aromas of rotten eggs, cabbage and skunk which reduces the quality of wine (Henschke and Jiranek, 1993). Due to low redox potential of the wine, volatile sulphur containing compounds such as thioacetic acid esters form, which can also contribute to negative odours in wine, described as cooked vegetables, cabbage and onions (Rauhut, 1993). However, as before mentioned, some sulphur containing compounds, such as the thiols 4MMP and 3MHA, are known to positively contribute to the aroma of wine (section 2.4.1.1).

2.4.5.2. Volatile phenols

Volatile phenols such as 4-vinylphenol and 4-vinylguaiacol are formed by decarboxylation of hydroxycinnamic acids in grape must during fermentation (Cheynier *et al.,* 2010). They are mainly associated with the off-flavours of “horsey”, “stable” and “leathery” in wine, which are the result of high concentrations of ethylphenols (Dubois, 1983).

If the wine has been contaminated by yeasts of the *Brettanomyces* genus, which contains vinylphenol reductase, higher concentrations of volatile phenols often occur. These higher levels of vinylphenols are negatively associated with a phenolic aroma and are known to mask the fruity notes of wine (Chatonnet *et al.,* 1993). Lower concentrations of these compounds are formed in red wines than in white and rosé wine, due to the fact that red wines have higher levels of catechic tannins, which are caboxylase inhibitors (Chatonnet *et al.,* 1993). In white and rosé wines the concentration ranges of these compounds seldom reach significant levels as the addition of ethanol to the vinyl groups degrades them rapidly (Dugelay *et al.,* 1993). Odour threshold values for 4-ethylphenol in neutral wine were found to be 368 µg L^{-1}, while wooded wines tended to have a higher detection threshold of 569 µg L^{-1} (Curtin *et al.,* 2008).

Once again, nothing has been reported on the presence of volatile phenols in South African Chenin blanc wines, or their possible contribution to the aroma profile.

2.5. PART 4: WINE VOLATILE ANALYSIS

The knowledge of wine flavour progressed in parallel to continuous developments within the area of analytical chemistry. During the 19th century the focus was mainly directed at determining major metabolites such as organic acids, ethanol and sugars (Ebeler, 2001). Current trends in wine analysis have involved applying more advanced analytical instrumentation for more rapid and in-depth research. Metabolic profiling of wine refers to the comprehensive qualitative and quantitative analysis of the unique chemical fingerprint of

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components present in a wine; with the main focus on intermediary metabolites and other small molecules participating in or due to chemical and biochemical processes (Oliver *et al.,* 1998; Bennett, 2005).

The volatile composition of a wine is the principal determining factor of its aroma; while volatile and non-volatile composition influence the wine flavour (Swiegers *et al.,* 2005) and therefore the volatile make-up of a wine directly influences the organoleptic characteristics of the wine. The application of separation science and sensory science have significantly advanced our knowledge of how volatile and semi-volatile compounds contribute to wine aroma (Ferreira *et al*., 2000; Francis and Newton, 2005) and extensive research is continuously being carried out on the chemical profiling of wine. This endeavour requires accurate quantification methods for large numbers of wine samples as rapidly as possible. For researchers to keep their competitive advantage and reduce costs while increasing productivity, high throughput analysis is of utmost importance. The abovementioned analytical procedures consist of several equally important steps; i.e. sample preparation, separation, detection and data analysis; and these steps are discussed in more detail below.

2.5.1. Sample preparation

Due to the inherent complexity of wine, it can be extremely difficult to accurately analyse a variety of different compounds in one analysis. Volatiles compounds occur at a wide range of concentrations in wine, from g L^{-1} to as low as sub-ng L^{-1}. Therefore sample preparation, especially extraction and concentration of aroma compounds, is of critical importance in volatile compound analysis (Pino and Queris, 2010; Yanase *et al.,* 2011). Sample preparation is performed to selectively isolate the compounds of interest from the sample matrix (including non-volatile components present if GC is used), as well as to ensure that the sample is in a matrix suitable for introduction into the analytical instrumentation to be used. A large number of different sample preparation techniques have been developed over the years and the ones used most frequently during wine volatile analysis will be discussed here. These include liquid-liquid extraction (LLE), solid phase extraction (SPE) as well as a number of sorptive extraction techniques.

2.5.1.1. Liquid-liquid extraction

Liquid-liquid extraction (LLE) is the most common and well-known sample preparation technique for the extraction of volatiles from aqueous samples before GC analysis (Ortega *et al*., 2001; Gu *et al.,* 2009). The technique is based on the solubility of a compound in two immiscible solvents at an appropriate pH (Raikos *et al.,* 2009). This is achieved by using a suitable solvent which is immiscible in water. While LLE was developed many years ago, it is

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still the preferred extraction method for analysis of volatile wine aroma components (Castro *et al.,* 2004). Requirements for a good LLE method are that it should be robust, cost effective, easy to perform and compatible with a range of analytical instruments. The reason that LLE is so commonly the preferred method of extraction, is that volatile compounds have high partition coefficients in the organic phases commonly used, such as *n*-pentane (Webster *et al.,* 1993), diethyl ether (Louw *et al.,* 2010) and dichloromethane (DCM) (Ortega *et al.,* 2001; Perestrelo *et al.,* 2006) and (2:1) *n*-pentane-DCM (Selli *et al.,* 2003).

In 1988 Chatonnet and Boidron described a LLE technique using DCM for the analysis of volatile phenols in wine (Chatonnet and Boidron, 1988). Ferreira *et al.,* (2000) performed studies on the ability of different solvent and sorbent systems to extract volatile compounds from alcoholic solutions, which illustrated that DCM was the most efficient solvent. DCM has, in fact, been observed as having high extraction efficiency for a wide range of polar to non-polar compounds. Positive attributes are that DCM is immiscibile with water and is highly compatible for use with GC instrumentation. Futhermore DCM is volatile so that it can easily be evaporated from the metabolites for further increase in sensitivity (Lichtfouse *et al.,* 2011).

The main disadvantages of LLE are that the technique often involves using large volumes of toxic solvents, and can be time consuming. It is furthermore not suitable for the extraction of compounds present at very low levels in wine. Additionally, the formation of emulsions, which are a suspension of small droplets of the one solvent, mixed in the other, readily occurs when using certain solvents such as DCM. They can form spontaneously at room temperature, when two solvents are partly miscible. As water and dichloromethane are miscible to a small degree, emulsions are readily formed, which leads to poor separation of the layers and potentially lower extraction yields. Many approaches have been described for removing the emulsion, for example by slow shaking or stirring; however, this approach can be extremely time-consuming. Other techniques are filtration through a glass wool plug or by drop-wise addition of methanol but this also adds to the extraction time (Wells, 2003). However, the addition of salt to the extraction mixture aids in rapidly removing emulsion problems. Futhermore, salt also increases the extraction efficiency, as it increases the ionic strength of the mixture. Increasing the ionic strength reduces the solubility of the organic compounds in the aqueous phase, which in turn increases the partitioning coefficient and thereby the amount of compound extracted by the organic solvent (McNair, 2011).

In a study by Ortega *et al.,* (2001) they observed that using ammonium sulphate salt increased the extraction potential for certain compounds such as acids and alcohols which

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exhibited Lewis acid properties. The exact amount of salt was important, as the aqueous phase's specific gravity could be rendered equal to that of DCM, which would hinder separation of the phases (Ortega *et al.,* 2001).

An investigation of the effect of adding sodium chloride and mixtures of ammonium sulphate and sodium phosphate on the extraction efficiency of wine volatiles was undertaken by Cabredo-Pinillos *et al.,* (2006). By employing ultrasound-assisted LLE they observed that 4 g of sodium chloride gave optimal results when using DCM as the extraction solvent. Still extraction yields of wine volatiles were in some instances very low with an overall recovery of only 39 %. Despite these low extraction yields and the fact that LLE is accepted as the reference method of wine sample preparation, no further or more recent work has been published using salts to improve the extraction efficiency.

2.5.1.2. Solid phase extraction

Solid phase extraction (SPE) is a technique where analytes are temporarily retained on a stationary phase and later selectively eluted off. The sorbent phase, of which many are available, is generally packed in a cartridge. These cartridges can be packed in-house, although commercially available pre-packed cartridges are widely available. SPE can be used in two different ways. In the one instance, if the undesired compounds in the sample have a high affinity for the stationary phase the SPE cartridge can act as a type of filter, with only the compounds of interest eluting. In the second instance, when the analytes of interest have a high affinity for the stationary phase, they would be retained on the stationary phase, while the undesired compounds are washed off. Thereafter, the samples can be washed and dried and eventually eluted from the cartridge using a stronger solvent.

SPE is extremely versatile and can be completely automated. However, if automated instrumentation is not available, then SPE can be a lengthy process. Furthermore SPE cartridges can be expensive and are generally not cleaned for re-use. However, SPE has been widely used in the analysis of wine volatile compounds. For example Pinheiro *et al.,* (2008) successfully applied an SPE technique for the extraction of terpenes from, wine samples. The extraction included a drying step evaporation, which concentrated the samples, whereby the levels of terpenes were increased. This aided in the detection and quantification of the terpenes in the wine.

2.5.1.3. Sorptive extraction techniques

In sorptive extraction techniques, the analytes are not retained in any material as in SPE, but rather based on the dissolution (partitioning) of the analytes in a liquid polymeric material,

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which is an inert means of solute retention. There are several sorptive extraction techniques, which according to a number of authors can be beneficial for the extraction of volatiles from wine (Tredoux *et al.,* 2008; Alves *et al.,* 2005). Some of these techniques include solid phase micro-extraction (SPME), stir bar sorptive extraction (SBSE) and open-tubular traps (OTT).

2.5.1.3.1. Solid phase micro-extraction

Solid phase micro-extraction (SPME), developed in the early 1990's by Arthur and Pawliszyn (1990), is a simple and environmentally friendly extraction technique, as it does not require the use of organic solvents. In this technique the solutes are retained by a specific layer coated onto a fused-silica fibre, due to partitioning between the sample matrix or headspace and the polymeric stationary phase. An additional advantage of SPME is the small amounts of sample needed for extraction. However, the main disadvantages are that SPME fibres can be very expensive and the performance of the extraction can be affected by, for example, the ethanol content of the sample, the sample viscosity, as well as the fibre coating of the SPME fibre (Rodrıguez-Cabo *et al.,* 2011).

There are three modes in which SPME is performed: headspace extraction, membrane protected extraction and direct extraction (Lord *et al.,* 2000). In headspace extraction, the fibre is suspended in the sample vial headspace and the analytes are transferred to the fibre once they are out of the aqueous phase. In the case of extremely dirty samples, membrane protected extraction is employed, which aids in protecting the SPME fibre from being damaged. In direct extraction, the SPME fibre is directly inserted into the sample, with the analytes being distributed between the fibre coating and the sample matrix. At equilibrium the quantity of analyte extracted by the fibre is proportional to its concentration in the sample matrix (Rodrıguez-Cabo *et al.,* 2011). For all three modes, thermal desorption of the extracted analytes is subsequently done prior to GC analysis.

Dziadas and Jelen, (2010) illustrated that extraction by SPE and subsequently SPME, followed by GC mass spectrometry (GC-MS) was successful for the analysis of free and glycosidically bound terpenes in white wine. Using the two techniques in conjunction enabled quantification of terpenes at lower levels than had been observed using SPE only.

A comparison of different extraction techniques for the analysis of wine esters was undertaken by Antalick *et al.,* (2010). Seven different SPME fibres were investigated and compared to one another, as well as to LLE using DCM and a mixture of ether and isohexane. Of the SPME fibres, it was found that polydimethylsiloxane 100 µm (PDMS -100) SPME fibres were overall the most suited for the analysis of esters in wine. However,

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employing LLE with DCM was shown to give a much higher extraction yield of all the polar esters.

2.5.1.3.2. Stir bar sorptive extraction

Stir bar sorptive extraction (SBSE), which was developed in 1999 by Baltussen *et al.,* (1999), is also a solventless extraction technique, in which a magnetic stir bar is encapsulated in a glass sleeve and covered with sorptive stationary phase. The stir bar is inserted into the sample and stirred, so that sorptive extraction of the analytes can occur. As in the case with SPME, extracted analytes are desorbed thermally for GC analysis. This technique is similar to SPME in that the mode of extraction is the same, but, as initially noted by Baltussen *et al.,* (1999) and recently reviewed by de Villiers *et al.,* (2012), typically the sensitivity is much higher for SBSE compared to SPME, due to greater amount of stationary phase.

For the analysis of 38 volatile compounds including lactones, alcohols, esters, acids, phenols and carbonyl compounds in an array of South African red and white wines, Tredoux *et al.,* (2008) illustrated that SBSE was a very sensitive extraction technique, with the resulting data successfully used to classify the different wine cultivars.

SBSE was used in combination with GC-MS for the analysis of volatile phenols in red wine. With high recoveries of all four volatile phenols, it was found to be a very sensitive extraction technique for the analysis of complex samples (Diez *et al*., 2004).

2.5.1.3.3. Open tubular traps

Open tubular traps (OTT) are the oldest of the sorbent techniques (Golay, 1957) and the technique employs a thick film (up to 12 µm) of PDMS coated on the inside of a capillary column for sampling. Typically, traps of 1–3 metres in length are used for the retention of volatiles. The technique involves sucking or pumping the sample through the trap until the analytes start to break through. Elution can either be obtained using an organic solvent or by thermal desorption prior to GC.

Burger and Munro (1986) illustrated the versatility of OTT's by applying the technique on various food products, including Gewürztraminer and Crouchen blanc white wine. Although they identified alcohols from the wine extracts, they stated that the technique could possibly be used for the analysis of wine aroma compounds, as the analytes could be concentrated.

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2.5.1.4. Separation

2.5.1.4.1. Gas chromatography

Due to the advancements within analytical chemistry, particularly the development of gas chromatography (GC) in the 1950's, a new era of chemical analysis began (Ebeler, 2001). GC is a separation technique where analytes travel at different rates through a column. The rate at which they travel is primarily determined by their respective boiling points, the polarity of the solutes as well as the stationary phase composition. Separation of the sample components is based on how they interact with the stationary phase as well as their boiling points. For any given GC analysis, the sequence of events follows the same progression: sample introduction, separation and detection.

Splitless and split injection modes are techniques that introduce the liquid sample into the heated injection port, where it is rapidly vapourised and transferred to the column. In splitless injection mode most of the vapourised sample is transferred to the column, whereas only a fraction of the sample is transferred to the column in split injection mode. In split injections, the remainder of the vapourised sample is removed through the vent line. Split injections generally offer higher resolution separations. However, trace analysis is limited since only a fraction of the vapourised sample enters the column (Miller, 2005). The greatest advantage of splitless injection mode is the improved sensitivity compared to split mode. However, splitless injection mode is not optimal for volatile compounds (Miller, 2005).

Separation occurs in the column, which is situated in the GC oven. There is a wide variety of capillary columns, with differing stationary phases. The choice of column depends on the analytes present in the sample, but in general for polar analytes a polar column is chosen and for non-polar analytes, a non-polar column would be used. For wine volatile analysis, and especially for profiling applications where a wide range of compounds, with differing physico-chemical properties, a polar phase is commonly used. The most common of these are polyethylene glycol (PEG) based phases. By modifying the PEG phase with nitroterephthalic acid the FFAP (free fatty acid phase) was developed. The most important characteristic of this phase is that, unlike other phases, it guarantees good peak shapes even for very polar compounds (eg. acids and alcohols), making it an ideal choice for broad range wine volatile analysis (Louw *et al.,* 2010 Malherbe *et al.,* 2011).

Chromatographic analysis of wine can provide quantitative data regarding compounds formed during different stages of wine making, and through different chemical pathways. It is considered a very useful tool to understand and ultimately control the winemaking process to a greater extent (Ortega *et al*., 2001).

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2.5.1.5. GC detectors

Detection of the sample components as they elute the GC column is the next fundamental step of the analysis. Several detectors have been developed and applied in combination with GC, which have varying detection limits, specificity and linear ranges. Some detectors are universal, while others are selective. Some commonly used GC detectors include flame ionization detectors (FID), mass spectrometry (MS) and olfatoctometry (GC-O).

2.5.1.5.1. Flame ionization detector

The flame ionization detector (FID) is a universal detector due to a response being obtained for virtually all carbon containing compounds. The response results from the conductivity of ions between two electrodes once the analytes have been ionised in a hydrogen flame (Kealey and Haines, 2002). The FID is characterised by having a wide linear range and high sensitivity, and is by far the most widely used for the analysis of volatile compounds (Louw *et al.,* 2010; Pinot and Queris, 2011a; 2011b).

2.5.1.5.2. Mass spectrometry

Another detector commonly coupled with GC is mass spectrometry (MS) which is able to tentatively identify the analytes by their respective mass spectra (Gil *et al,* 2006). MS detectors function by bombarding individual analytes as they enter the detector with a stream of electrons (normally with energy of 70 eV, causing them to fragment into characteristic fragments. This gives a distinguishing mass spectrum for each analyte entering the detector. In GC-MS, the mass spectrum for any particular compound is always similar, which enables database searches of unknown compounds, provided that standard electron energy of 70 eV is used. MS detectors are one of the most commonly used detectors as they are highly specific and sensitive. Although they are more expensive than FID's, GC-MS has been used widely for the analysis of volatiles in wine (Dziadas and Jelen, 2010; Pinot and Queris, 2011a; 2011b).

2.5.1.5.3. Olfactometry

A technique capable of correlating chemical observations to sensory perceptions is GC-olfactometry (GC-O), which is a combination of human and electronic responses which enables the identification and linking of odourants to human perception (Mayol and Acree, 2001). Wine flavour is the most important consideration for a consumer to repeatedly buy a wine (Yegge and Noble, 2001). As previously mentioned, wine flavour is made up of many hundreds of compounds at widely different concentrations ranges. The particular importance

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of each compound is related to its odor perception threshold value, which is the lowest concentration detectable by the human sense of smell. Many studies in the past decade have focused on utilising GC-O to analyse all of the most important wine odorants in various different wine styles (Ferreira *et al.,* 2002; Gomez-Miguez *et al.,* 2007; Dziadas and Jelen, 2010).

2.5.1.6. Comprehensive GC (GC x GC)

In some instances, due to the inherent complexity of many samples, fully resolved peaks are not always possible. Numerous efforts have been made to resolve problems with coeluting peaks, low separation capacity and low sensitivity. The need for greater separation capacity and higher sensitivity led the initiation of method development of comprehensive or two-dimensional GC (GCxGC) (Pierce *et al*., 2008). In GCxGC, two columns of differing polarities are used in series, creating a two-dimensional, orthogonal plane of separation, based on two different physical properties of the analytes (de Geus *et al*., 1996). In many instances, due to higher separation capacity, resolution problems are resolved. However, data analysis is extremely time consuming and requires expertise in the use of sophisticated software. GCxGC is not encountered in every analytical laboratory and it is generally more expensive than conventional GC, and thus the major disadvantages of the technique are the high costs involved and availability of the technique.

On the other hand, advanced chemometric techniques have been developed for the resolution of complex peaks, which could be an alternative to GCxGC, when the technique is not available.

2.6. PART 5: DATA HANDLING

2.6.1. Chemometric techniques

Chemometrics is a statistical way of interpreting patterns in multivariate data. Advancement in technology has made high throughput analysis possible. However, it is often very difficult to interpret the data and establish a relationship between chemical characteristics and sensory properties, taking into account the diverse factors that affect each volatile compound in wine (Weldegergis *et al.,* 2010).

In analytical chemistry, calibration is used extensively, and usually involves using one type of measurement to predict the value of another parameter. An example in chromatography would be using the area of an analyte peak to predict the concentration of the analyte, as the area is directly related to the concentration. These types of calibrations have been widely

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used by analytical chemists in the past century (Brereton, 2007). However, instead of calibrating a single variable to another variable, several variables can be calibrated to one or several other variables to find underlying parameters, for example when linking wine sensory and chemical data. This is possible using multivariate chemometric techniques, which allow for investigation of the correlation between data blocks (Nobel and Ebeler, 2002; Brereton, 2007).

The simplest of the multivariate techniques is principal component analysis (PCA) (Wold *et al.,* 1987; Esbensen, 2002). It is an unsupervised pattern recognition technique, capable of reducing a complex multidimensional or multivariate dataset to a lower dimension of orthogonal components, with the first principal component describing the maximum variation in the dataset. The following components, orthogonal to the previous principal components, model the remaining variation, progressively. In this manner PCA reveals the internal structure of the dataset that best describes the variance of the data (Vandeginste *et al*., 1997). The results of a PCA can be described by means of plotting the components against each other, in terms of component scores (samples), and loadings (variables) (Wold *et al.,* 1987; Bro, 1997; Esbensen, 2002; Abdi, 2010). Symmetric multivariate techniques, such as canonical correlation analysis (CCA), treat two different datasets equally and are used to find a relationship between the datasets (Dijksterhuis, 1995). Techniques such as partial least squares regression (PLS, PLS2) and principal component regression (PCR) can be used to link one dataset to another dataset (Brereton, 2007; Dijksterhuis, 1995).

Apart from the variety of other multivariate techniques for calibration, PLS is the most widely used modelling technique and has been used extensively to investigate the relationship between sensory wine data and chromatographic data (Nobel and Ebeler, 2002; Martens and Naes, 1989; Cozzolino *et al*., 2009; Frank and Friedman, 1993) as it can aid in identifying compounds that mathematically model the sensory profile. The principles of PLS regression are relatively similar to PCA, and it has been described as a “soft modelling” techniques that extracts a set of components called latent variables (LV). LVs are linear combinations of one set of variables that predict a high degree of variation in another dataset (Word *et al.,* 1987; Nobel and Ebeler, 2002; Abdi, 2010; Tobias, 1995; Helland, 1990). The concept of a LV model is based on the assumption that the true dimensionality of the process is defined by the underlying phenomena driving the process, and not by the number of measured variables (Pereira *et al*., 2011).

A PLS regression model is built using a calibration set of variables and objects, which form the two matrices x and y, from which predictions can be made based on the models x-data

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(Wold *et al.,* 2001). Using an appropriate scaling of the data such as autoscaling, the model can focus on more important y-variables. Autoscaling is done by scaling the data (by dividing each value by their standard deviation), followed by centering it, whereby the averages are subtracted, to give each variable the same weighting or importance in the analysis. Autscaling is not always advantageous however, as the the variables are converted to the same variance. This implies that the variables describing the information are given the same importance as the variables that only describe noise (Höskuldsson, 2004). There are many types of scaling modes available, such as Paareto scaling and logarithmic scaling, with the most adequate type usually selected upon trial and error (Höskuldsson, 2004). The appareance and interpretation of all chemometric techniques rely on the preprocessing of the data. Therefore it is important to ensure correct scaling of the data has been done prior to interpretation of the results (Brereton, 2007).

When analysing chromatographic data, it is common to normalise the objects (using the internal standard), by for example, removing the size of the objects, if they are irrelevant (Wold *et al.,* 2001; Geladi and Kowalski, 1986). PLS regression models need to be validated to check their efficiency, and there are a number of validation methods available. The most reliable is test set validation, whereby an independent set of samples is tested by the calibration model (Wold *et al.,* 2001). In another validation method, the cross validation (CV) (e.g. leave-one-out) technique, all the samples are used in the PLS regression model as well as to validate it. The model sample set is calculated by iteratively leaving one sample out of the model. However, this model can be too optimistic as the validation is based on the original model sample set (Esbensen, 2002).

Chemometric methods, predominantly multivariate techniques, have shown to be particularly useful in extracting meaningful information from large datasets, and have proven successful for wine characterisation and classification evaluations (De Villiers *et al*., 2005; Tredoux *et al*., 2008; Vilanova *et al*., 2010; Louw *et al*., 2010; Bester 2011).

ANOVA, PCA and discriminant analysis (DA) were used successfully to differentiate between Spanish red wines according to variety and region (Arozarena *et al*., 2000). Previous classification studies on South African wine cultivars based on volatile components have been performed using analysis of variance (ANOVA), PCA and cluster analysis (CA) (Tredoux *et al*., 2008; Louw *et al*., 2010). ANOVA, PCA, CA and DA have also been used to classify South African wine cultivars based on non-volatile compounds (de Villiers *et al*., 2004; de Villiers *et al*., 2005).

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In 1981 Marais *et al*. successfully classified South African Colombar wines from two regions and South African Chenin blanc wines from three regions according to origin, using volatile compounds, by means of stepwise discriminant analysis (SDA) (Marais *et al*., 1981).

A recent study, linked to this current work, which involved the classification of South African Chenin blanc wines styles by sensory and consumer analysis, was achieved using PCA, QDA, ANOVA and PLS (Bester, 2011). Bester illustrated, using sensory and consumer data, combined with multivariate techniques, that although the wooded and unwooded styles were able to be discriminated between, human perception of intricate stylistic differences between the two unwooded Chenin blanc styles was not possible. This lead to the conclusion that the unwooded styles should be re-categorised as a form of style continuum (Bester, 2011).

2.6.2. Multi-way Techniques

Due to the need for high throughput analysis, analytical instrumental methods are being developed with shorter chromatographic separation times. In an ideal chromatogram, each chromatographic signal would correspond to one single analyte, which would make accurate analysis possible without error from coeluting or interfering compounds. However, in reality, complex analytical samples such as wine samples do not meet these idealistic conditions, and the resulting chromatograms have baseline drift, coelution of peaks, peak shifting as well as background interferences. Using traditional data analysis for quantification can be very difficult and in some circumstances impossible, especially if there is severe coelution of constituents (Skov and Bro, 2008).

Multi-way data is characterised by a number of sets of variables which are measured for several samples (Bro, 1997). Many types of chemical examples of multi-way data exist, including fluorescence emission spectra at various excitation wavelengths measured for different samples as well as any type of chromatographic seperation combined with spectral measurements, for example GC-MS (Bro, 1997). A lot more information can be extracted from these multidimensional measurements than from the traditional two dimensional measurements and there are methods that make use of the multi-way structure of the data, enabling quantification of analytes in the presence of interfering compounds (Skov and Bro, 2008).

Multidimensional techniques have been used for many years to analyse multi-way data, some of these are Tucker3 (Kroonenberg, 1983), PARAllel FACtor Analysis (PARAFAC) as well as merely unfolding the multi-way array and performing two-way chemometric methods

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such as PCA (Bro, 1997). All three mentioned techniques are multi or bilinear decomposition methods that model the data into loading matrices per mode, such that they are able to describe the data in a more comprehensive and compact way (Bro, 1997). Of these multi-way methods, PARAFAC is the most wide-spread technique used for multidimensional chromatographic data (Harshman, 1970; Amigo *et al*., 2008; Skov and Bro 2008; Bro *et al*., 2010; Vosough, 2010). It is a generalisation of PCA to arrays of higher orders and originates from psychometrics (Bro, 1997).

The main difference between PCA and PARAFAC, besides the fact that PCA is a two dimensional technique and PAFARAC is a multi-way technique, is the way in which the principal components are calculated. In PCA the components are calculated separately with the first principal component lying in the direction of the greatest variance. The remaining components are orthogonal to the principal component and model the remaining variance progressively less (Bro, 1997; Esbensen, 2002). In PARAFAC however, the components are not constrained due to orthogonality and are calculated simultaneously so that collectively they model the maximum variance of the data, as long as the data does not deviate from trilinear structure of the model (Skov and Bro, 2008). The manner in which the simultaneous calculations are performed is by first estimating two components and then calculating the remaining components by alternating least squares (ALS) regression. The components are then recalculated iteratively until the model does not change with further calculations. The number of components affects the PARAFAC model significantly and thus the number of components is of great importance (Bro, 1997).

In PARAFAC the loading matrix in the sample mode is usually referred to as the score matrix, and contains the relative concentrations of each analyte in the samples. The elution mode loadings contains the relative retention profile of each chemical compound in the sample while the third mode, the spectral loadings, contains the estimated mass or UV spectra of the factors (Amigo *et al*., 2008). One constraint of PARAFAC however, is that all the modes must contain the same number of factors (Bro, 1997). Hyphenated separation techniques, such as GC-MS produce three-way data, where the retention time is the first dimension, mass spectrum the second, and the sample number is the third. Quantitative and qualitative information can be achieved by decomposing the three-way data and applying an iterative algorithm for second order calibration methodology, such as PARAFAC.

PARAFAC has proven to be a valuable tool for processing and analysing chromatographic data provided the data follow the trilinear structure of the model. However there are some limitations, as it cannot handle non-linear phenomena, such as shifting in any mode.

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PARAFAC2, which is an extended and less restricted version of PARAFAC has been shown to be able to handle shifting in one mode (Skov *et al*., 2009). Tradional calibration has been compared to PARAFAC and PARAFAC2, which illustrated that PARAFAC2 was able to handle phenomena that disturbed the trilinear structure of the data such as chromatographic peak shifting, without prior alignment (Skov and Bro, 2008). Furthermore, they illustrated that both PARAFAC and PARAFAC2 could easily be employed to quantify all analytes in cases where two or more analytes had similar dominating fragments in their mass spectrum. This type of phenomena would pose a problem for traditional methods, even if the coelution of the peaks was not complete (Skov and Bro, 2008).

There is a lot of evidence for the necessity of chemometric multi-way methods with the capability of resolving complex chromatograms, particularly with respect to analyte peak shifting, baseline shifts, peaks with low signal-to-noise, as well as coeluting peaks (Skov and Bro, 2008). Applying hyphenated chromatographic techniques in combination with PARAFAC and PARAFAC2 has been employed increasingly in recent years for complex samples (Skov and Bro, 2008) and has become known as “mathematical chromatography” (Bro *et al*., 2010).

Braga *et al*., (2007) compared the preprocessing capabilities of bilinear least squares (BLLS) to the capabilities of PARAFAC, for the quantification of pesticides in wine by HPLC-DAD. They illustrated that PARAFAC was an extremely useful tool which was capable of enabling analyte quantification in all cases, whereas BLLS was not quite as powerful a technique (Braga *et al*., 2007).

Botha (2010) compared the application of PCA and PARAFAC on raw sensory data of South African red wine affected by brettanomyces spoilage. The overall conclusion was that the results obtained using PARAFAC gave a stronger hierarchy in terms of sensory variables and, unlike with PCA, prevented incorrect conclusions from being made, as more variation and less noise in the data was modeled (Botha, 2010).

Schmidtke (2011) used PARAFAC to model volatile data obtained by GC-MS analysis of Shiraz that had undergone micro-oxygenation. The PARAFAC results suggested that furfural was extracted from oak chips relatively rapidly, and the conversion to furfuryl alcohol occurred, possibly as a result of biological conversion, during the application of oxygen (Schmidtke, 2011).

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However, despite this obvious need for, and interest in, resolving complex chromatographic wine data using PARAFAC and/or PARAFAC2, there has been very little published on the application of these multi-way techniques to wine data.

2.7. CONCLUSIONS

The aroma and flavour of wine is significantly influenced by an array of compounds which are derived by various factor such as grape variety, soil type and viticultural practices, as well as compounds produced through the different biological stages during the oenological practices and bottle ageing and storage. With aromas from woody, spicy, honey, tropical, and floral to fresh and fruity, it is clear that the South African Chenin blanc wine styles display characteristic organoleptic combinations. However, with relatively little research having been undertaken on the accurate chemical profiling of South African Chenin blanc table wines. In fact there is no published work addressing the styles of Chenin blanc and their unique volatile profiles and therefore this work could contribute significantly to shedding some light on the chemical profiles of the three South African Chenin blanc styles.

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Chapter 3

Research results

High throughput quantification of volatile compounds in South African Chenin blanc wine: Method development, validation and application to wine style characterisation

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3. RESEARCH RESULTS

High throughput quantification of volatile compounds in South African Chenin blanc wine: Method development, validation and application to wine style characterisation

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ABSTRACT

There is a wide array of different Chenin blanc wine styles, ranging from fruity and tropical dry and off-dry styles, to sparkling and sweet styles. The objective of this research was to develop an analytical method suitable for the analysis of major as well as minor volatiles in wine, comprising higher alcohols, terpenes, esters, fatty acids and wood derived volatiles. In particular the aim was to determine the volatile profile of the three dry and off-dry South African Chenin blanc wine styles. This study resulted in the development of a simple liquid-liquid extraction (LLE) technique, using dichloromethane, followed by a rapid and robust gas chromatographic flame ionization detection (GC-FID) method, for the quantification of 57 volatiles in wine. One major benefit of this method is that the chromatographic analysis takes less than 30 minutes. The method was validated in terms of accuracy, precision and robustness, and exhibited high accuracy and repeatability (% RSD < 10 %) with limits of detection and quantification suitable for the quantification of these compounds in wine. The sample preparation and analytical method were applied for the high throughput analysis of 48 Chenin blanc wines, representative of the three mentioned styles, and multivariate data analysis methods were used to obtain insight into the metabolic profiles of the respective wine styles.

Keywords: Chenin blanc, volatile metabolic profiling, wine aroma, PCA, PLS, style classification

3.1 INTRODUCTION

Chenin blanc is a white wine grape variety that has its origins in the Loire Valley, France (Kerridge and Antcliff, 1999; LaMar, 2002) and has been cultivated in South Africa since the middle of the 17th century (Robinson, 2006). It is known to be an extremely versatile grape

variety, well suited to be grown in many different terroirs. Chenin blanc grapes have a neutral sensory profile with less pronounced and specific aroma and flavour intensities than other cultivars such as Sauvignon blanc. This gives the producer the opportunity to more freely manipulate winemaking and maturation strategies to produce a wide range of wine styles (Loubser, 2008). South African Chenin blanc table wine styles include dry (< 4 g L^{-1} residual sugar), off-dry (4 to 9 g L^{-1} residual sugar), sweet (> 9 g L^{-1} residual sugar) and sparkling wines, and this variety is also extensively used in the production of brandies and sherries (Marais, 1983; Smith, 2004). Chenin blanc is often referred to as the 'chameleon' among white wines (Decker, 2011) and in South Africa six different wine styles are officially endorsed by the South African Chenin Blanc Association (CBA) (Smith, 2004). These include three dry and off-dry styles, namely fresh and fruity, rich and ripe unwooded, and rich and ripe wooded, that will be the focus of this study.

The systematic investigation of the unique chemical fingerprint of compounds present in a wine, as a result of numerous chemical and biochemical processes is commonly referred to as 'metabolic profiling' (Olivier *et al.,* 1998). The volatile composition of a wine is the primary determinant of the aroma of the wine, however, both volatile and non-volatile composition influence the wine flavour (Swiegers *et al*., 2005). The volatile compounds therefore directly influence the organoleptic characteristics of the wine. The fields of separation science and sensory science have advanced our knowledge and understanding of how volatile and semi-volatile compounds contribute to wine aroma (Ferreira *et al.,* 2000; Francis *et al.,* 2005) and extensive research is continuously being carried out on the profiling of wine volatiles. However, a very limited number of studies have been conducted specifically with regards to the volatile composition of South African wines. The most comprehensive study to date was performed by Louw *et al.,* (2010) who determined the levels of several major fermentation-derived volatile metabolites in more than 900 South African single cultivar young wines. The primary aim was to establish the unique fingerprints of the red wine styles Cabernet Sauvignion, Merlot, Pinotage and Shiraz, as well as the white varieties Chardonnay and Sauvignon blanc.

Vestner *et al.,* (2011) carried out volatile metabolic profiling of South African Pinotage wines which had undergone malolactic fermentation mediated by different lactic acid bacterial strains by using comprehensive two-dimensional gas chromatography (GCxGC). Some volatile compounds, uniquely associated with wines produced with the respective strains, were identified. Similarly, Malherbe *et al.,* (2011) observed significant bacterial strain related variations in the chemical profiles of South African Shiraz and Pinotage wines which had undergone malolactic fermentation mediated by four different bacterial starter cultures.

The volatile compounds responsible for the aroma of wine comprise various different chemical classes, such as alcohols, volatile acids, esters and terpenes, amongst others. The concentrations and presence of these groups are influenced by many factors, including grape variety, ripeness of the grapes at harvest, *terroir*, winemaking and maturation processes (Francis and Newton, 2005).

There have been a limited number of research reports which address the chemical analysis of South African Chenin blanc wine over the past four decades, (Marais and Pool, 1980; Augustyn and Rapp, 1982; Marais, 1983; Malherbe, 2011) indicating that very few studies have been undertaken with respect to the chemical profiling of this specific cultivar. In fact, there are significant gaps in our current knowledge of the chemical profiles of this versatile grape variety, as well as the chemical variation between the different styles.

Marais and Pool (1980) observed that the pleasant fresh (acidic and crisp) and fruity characters of South African Chenin blanc was derived to a large extent from higher alcohol acetate esters, such as isoamyl acetate and 2-phenylethyl acetate. They also observed a significant positive correlation between the concentration of hexanoic, octanoic and decanoic acids and their aroma contribution to Chenin blanc wine. Nevertheless, despite substantial advances considering analytical instrumentation and analysis techniques, no recent work has been carried out on the contributions of volatile fatty acids to the aroma and flavour profile of South African Chenin blanc. Based on gas chromatographic mass spectrometric (GC-MS) analysis, Augustyn and Rapp (1982) reported that South African Chenin blanc wine contained no measureable amounts of terpenes; however, this may largely be as a result of analysis techniques that lacked sufficient sensitivity to detect these compounds. No recent work has been published on the presence or lack of monoterpenes in Chenin blanc wines.

Acids are mainly formed as a result of fatty acid metabolism during fermentation, and at low concentrations they can add complexity to the wine. However, at high concentration they are detrimental to wine aroma (Francis and Newton, 2005). Fatty acids are able to undergo various chemical reactions in wine to form esters, aldehydes or ketones, which could positively contribute towards the aroma of wine (Matthews *et al.*, 2004).

Esters are also mainly produced during alcoholic fermentation, by esterification of an acid and an alcohol. Esters of saturated carboxylic acids and acetate esters of fusel alcohols are generally associated with the fresh and tropical characteristics of young wine (Francis and Newton, 2005). Furthermore, Mateo *et al.,* (2001) stated that esters of saturated carboxylic acids (e.g., ethyl hexanoate and octanoate), and higher alcohols (e.g., isoamyl acetate and 2-phenylethyl acetate) are of great importance to the aroma of specifically white wine.

Besides ethanol (8-16 %), the most abundant alcohols in wine are the higher (fusel) alcohols 2-phenylethanol, *n*-propanol, isobutanol and isoamyl alcohol (Swiegers *et al.,* 2005). Higher alcohols are released into wine as secondary metabolites of yeast metabolism, either by anabolic or catabolic pathways (Perestrelo *et al.,* 2006). Alcohols add to the complexity of wine, although at higher concentrations they induce an overpowering and pungent aroma (Swiegers *et al.,* 2005). Higher fermentation temperatures promote the release of fusel alcohols (Gil *et al.,* 2006) while pre-fermentative clarification, often employed during white winemaking, in turn suppresses the formation of higher alcohols (Moreno-Arribas and Polo, 2009). These winemaking regimes also influence the resulting wine style being produced. This could indicate that fusel alcohol levels may differ significantly in the three South African Chenin blanc dry and off-dry styles.

Another important group of aromatic volatiles occurring in wine are terpenes and related compounds such as terpenoids and norisoprenoids. Especially linalool, nerol, citronellol, geraniol and α-terpenol have been found to be dominant terpenes present in some Muscat varieties (Shimizu and Watanabe, 1981; Marais, 1983). These compounds occur in the free form but are also glycosidically bound to a significant degree to sugars located in the skin of grapes, and play a significant role in the varietal flavour of especially white wine (Gamero *et al.,* 2011). For example Cabrita *et al.,* (2006) regarded β-ionone to be one of the aromatic compounds in this group which contribute to a large extent to the aroma of wine made from neutral grape varieties.

Different winemaking regimes, such as fermentation temperature, must clarification and yeast strain have an impact on the levels of esters present in wine. The formation of acetate esters is promoted by low fermentation temperatures (approximately 10 ºC), while the formation of higher molecular weight esters are promoted by higher temperatures (15-24 ºC). Fresh and fruity style Chenin blanc wines are produced using lower fermentation temperatures (12-13 ºC), while the rich and ripe styles are fermented at higher temperatures (12-24 ºC) (O'Kennedy, 2009). The author speculated that this could point towards the fact that the aroma of the fresh and fruity style has a higher content of low molecular weight esters, while the rich and ripe styles would be characteristic of higher molecular weight esters; this however, is not substantiated by scientific evidence.

While liquid-liquid extraction (LLE) is probably one of the oldest and well-known sample preparation techniques, it remains the most widely used, despite some limitations of the technique (Castro *et al.,* 2004). Advantages of this technique are ease of use, high repeatability and the possibility of carrying out several simultaneous extractions (Andujar-Ortiz *et al.,* 2009). Several solvents have reportedly been used for LLE of wine and one of the most popular

solvents is dichloromethane (DCM). In fact, it has been noted that DCM has high extraction efficiency for a wide range of non-polar to polar compounds. It is immiscibility with water, stable and volatile so that it can easily be removed from the metabolites by evaporation. DCM is highly compatible for use with analytical instrumentation (Lichtfouse, 2011) and several authors have found DCM to be very well suited for extraction of aroma substances from matrices with high alcohol content, such as wine (Perestrelo *et al.,* 2006; Dominguez and Agosin 2010). However, a common occurrence when employing DCM as extraction solvent is the possible formation of emulsions, which decreases extraction efficiency and ease of use.

There are a number of aspects that can affect analyte recovery when employing LLE, and the general consensus is that the overall efficiency of the extraction process increases when a salt is added (McNair, 2011). Salt increases the ionic strength of the aqueous sample matrix, thereby reducing the solubility of organic compounds in the aqueous phase (the wine), and increasing the partitioning coefficient and the amount of a specific analyte extracted by the organic phase (McNair, 2011). Previous work by Ortega *et al.,* (2001) illustrated the importance of using ammonium sulphate to improve the extraction efficiency of wine volatiles such as acids and alcohols that have Lewis acid properties. Cabredo-Pinillos *et al.,* (2006) reported the use of sodium chloride, and mixtures of ammonium sulphate and sodium phosphate to aid in improving the extraction efficiency of wine volatiles in combination with ultrasound-assisted LLE. Sodium chloride provided the best results when using DCM as the extraction solvent; however the extraction yields of wine volatiles were generally very low and ranged between 9-81 %. The mean recovery for the 14 analysed volatiles was only 39 %. To the best of our knowledge, no further work has been published on the use of inorganic salts to improve the extraction efficiency.

The method of choice for analysis of volatiles, including those present in wine is GC, most frequently using FID or MS. As mentioned previously the aroma of wine is extremely complex and the analysis thereof is very challenging. Wine consists of many compounds of highly varying concentration levels (from sub-ng L^{-1} to g L^{-1}), and in addition widely varying physico-chemical properties such as polarity, volatility and solubility (Ebeler, 2001).

Major advantages of using an FID are; the universal nature of the detector that gives a response for virtually all carbon containing volatiles, as well as the high sensitivity. When compared to GC-MS, GC-FID is significantly less expensive and easier to operate. A disadvantage is however that information regarding the analytes' structure, and hence identity, is not obtained. Despite this drawback, GC-FID in combination with the use of reference standards still remains a viable option for routine analysis using well developed and validated methods (Gil *et al.,* 2006).

Chemometric techniques, which allow the extraction of information from chemical measurements by data-analytical driven techniques, play a vital role in wine metabolic profiling and there have been some major achievements in wine characterisation by chemometric analysis of chemical data in the past decade (Saurina, 2010). In particular, principal component analysis (PCA), originally described by Wold *et al.,* (1987) allows for finding latent structures in data, while partial least squares regression (PLS), described by Martens and Naes (1989), has been applied extensively to model the relationship between wine sensory and chromatographic data (Noble and Ebeler, 2002; Frank and Friedman, 1993; Yeniay and Goktas, 2002; Helland, 2000), as it can aid in identifying compounds that mathematically model the sensory profile.

In depth parallel studies on chemical, sensory and consumer profiling of South African Chenin blanc wine styles are currently being undertaken (Bester, 2011; Nieuwoudt, 2011). Together with those studies, this current work on the volatile fingerprinting of Chenin blanc wine styles forms part of a consumer check project. The chemical profiling of South African Chenin blanc also forms part of an aroma project, which involves analysing South African Chenin blanc table wines and adding the data to an existing South African wine database.

3.2. MATERIALS AND METHODS

3.2.1. Chemicals

Dichloromethane, absolute ethanol, as well as sodium sulphate (anhydrous), ammonium sulphate and sodium hydroxide were purchased from Merck (Darmstadt, Germany). Tartaric acid was purchased from Sigma-Aldrich (St. Louis, MO, USA). Water was purified by a Milli-Q purification system (Millipore, Bedford, MA, USA). The chemicals, all of which were of analytical grade, were purchased from Riedel de Haën (Seelze, Germany), Sigma-Aldrich (St. Louis, MO, USA), Fluka (Buchs, Switzerland), and Aldrich (Steinheim, Germany), as shown in Table 3.1.
The internal standards, 4-methyl-2-pentanol (0.5 mg L^{-1}) and 2,6-dimethyl-6-hepten-2-ol (0.1 mg L^{-1}) were dissolved in synthetic base wine simulant.

3.2.2. Synthetic base wine simulant

A synthetic base wine simulant was prepared as described by Louw *et al*., (2009) and consisted of 2.5 g L^{-1} tartaric acid and 12 % (v/v) ethanol dissolved in purified water. The pH was adjusted to 3.5 with 0.1 M sodium hydroxide.

3.2.3. Liquid-liquid extraction procedure

2.5 grams of ammonium sulphate was added to 15 mL culture tubes (Pyrex, Milian, USA). Five milliliters of wine and 100 µL of the internal standard solution (0.5 mg L^{-1} 4-methyl-2-pentanol and 0.1 mg L^{-1} 2,6-dimethyl-6-hepten-2-ol in synthetic base wine) were extracted using 1 mL of dichloromethane by sonicating for one hour with shaking every 10 minutes. The extract was then centrifuged at 3000 rpm for 30 minutes and the dichloromethane layer (top layer) removed and dried on anhydrous sodium sulphate. Each wine sample was extracted in triplicate and injected into a Hewlett Packard 6890 GC-FID (Agilent, Little Falls, USA) in duplicate.

3.2.4. Gas chromatographic conditions

A J & W DB-FFAP capillary column (Agilent, Little Falls, USA) with the dimensions 60 m length by 0.32 mm internal diameter and 0.5 µm film thickness was used. The initial oven temperature was set at 40 ºC and held for 5 minutes, after which the temperature was increased by 8 ºC min^{-1} to 225 ºC, and held for 1.2 minutes. 3 µL of the extract was injected at 200 ºC in split mode (18:1, with a split flow rate of 98.9 mL min^{-1}). The carrier gas was hydrogen and the column flow rate was 5.5 mL min^{-1}. A post run of 5 minutes, with oven temperature at 240 ºC and a flow rate 6 mL min^{-1} removed any contaminants of high boiling point from the column, making the total run time 29.3 minutes. The detector temperature was set at 250 ºC, with an air flow of 350 mL Min^{-1}, the make-up gas (nitrogen) flow was 30 mL min^{-1} and the hydrogen flow rate was mL min^{-1}.

3.2.5. Calibration

Calibration curves were constructed using authentic standards of known concentration, and prepared using the LLE procedure described in 3.2.3. Each calibration standard was extracted 3 times and injected in duplicate. The average values were used to construct the calibration curves by plotting normalised peak area (peak area divided by peak area of internal standard) against the concentration. Six calibration points were used to construct the calibration curves for each compound.

Table 3.1 List of chemical standards used in this work, and their purity.

Chemical class	Analyte	Supplier	Purity (%)
Alcohols	1-Propanol	Fluka	>99.8
	Methanol	Sigma-Aldrich	>99.9
	n-Butanol	Fluka	>99.5
	Isobutanol	Fluka	>99.5
	4-Methyl-2-pentanol	Fluka	>99
	Isoamyl Alcohol	Aldrich	>99
	1-Pentanol	Fluka	>99
	2-Phenylethanol	Merck	>99
	n-Hexanol	Merck	>98
	1-Octen-3-ol	Fluka	>98
	2,6-Dimethyl-6-hepten-2-ol	Fluka	>99.5
	3-Methyl-1-pentanol	Sigma-Aldrich	>97
	3-Ethoxy-1-propanol	Sigma-Aldrich	>97
	4-Methyl-1-pentanol	Sigma-Aldrich	>95
Acetate esters	Hexyl Acetate	Fluka	>99
	Ethylphenyl Acetate	Aldrich	≥98
	Ethyl Acetate	Sigma-Aldrich	>99.7
	Isoamyl Acetate	Riedel de Haën	>98
	2-Phenylethyl Acetate	Fluka	>99
	Ethylphenyl Acetate	Fluka	>99
Acids	Propionic Acid	Fluka	>99.5
	Isovaleric Acid	Fluka	>99
	Isobutyric Acid	Fluka	>99.5
	Valeric Acid	Fluka	>99
	Hexanoic Acid	Aldrich	>99.5
	Octanoic Acid	Aldrich	>99.5
	Acetic Acid	Merck	>98
	Decanoic Acid	Sigma	>98
	Butyric Acid	Fluka	>99.5
Esters	Ethyl Butyrate	Fluka	>98
	Ethyl-2-methylbutyrate	Aldrich	≥98
	Ethyl Isovalerate	Aldrich	≥98
	Ethyl Propionate	Aldrich	≥97
	Ethyl Lactate	Fluka	>99
	Diethyl Succinate	Fluka	>98
	Ethyl Ocatanoate	Fluka	>98
	Ethyl Hexanoate	Fluka	>99
	Ethyl Decanoate	Aldrich	>99
	Ethyl-3-hydroxybutanoate	Sigma-Aldrich	≥97
Carbonyls	Acetoin	Fluka	>97
	Diacetyl (2,3-Butanedione)	Fluka	>99.5
	Acetaldehyde	Fluka	>99.5
Terpenes	α-Terpeneol	Sigma-Aldrich	>99
	Citronellol	Fluka	>99
	Fenchone	Aldrich	>98
	β-Ionone	Aldrich	>97
	α-Ionone	Aldrich	>90
	β-Farnesol	Fluka	>99
	Limonene	Fluka	>99
	Linalool	Sigma-Aldrich	>99
	Linalool Oxide	Aldrich	>97
	Linalyl Acetate	Aldrich	≥97
	Geraniol	Fluka	>99
	Nerol	Fluka	>99
Wood derived	Furfuryl Alcohol	Aldrich	>98
	Furfural	Sigma-Aldrich	99
	5-Methyl-2-furfural	Sigma-Aldrich	99
	Guaiacol	Aldrich	>98
	Whiskey Lactone	Aldrich	>98

3.2.6. Method validation

3.2.6.1. Linearity, accuracy and precision

The linearity was evaluated as described in section 3.2.5, while the accuracy of the calibration curves (Addendum A) was tested by injecting standards of all the chemicals, with known concentration, in synthetic base wine simulant, employing Equation 3.1:

$$\%\ \text{Accuracy} = 100 \times \frac{\text{measured amount}}{\text{actual concentration}} \quad \text{.....Equation 3.1}$$

Precision was determined by spiking wine with known concentrations of the volatile compounds. The spiked wine was extracted in triplicate and injected in duplicate.

3.2.6.2. Recovery

The recovery was calculated by comparing six replicate determinations of spiked and unspiked wine for each of the compounds and evaluating the recovery from the calibration curves, using Equation 3.2:

$$\%\ \text{Recovery} = 100 \times \frac{\text{measured amount in spiked wine} - \text{measured amount in wine}}{\text{amount spiked}} \quad \text{.....Equation 3.2}$$

The replicate analysis of spiked and unspiked wine during the recovery study also investigated the possibility of matrix effects.

3.2.6.3. Limit of detection and quantification

The limit of detection (LOD) was determined as the concentration of analyte with a peak height three times higher than that of the baseline noise (signal-to-noise ratio of 3:1), while the limit of quantification (LOQ) was determined at a signal-to-noise ratio of 10:1.

3.2.6.4. Repeatability and intermediate repeatability

The repeatability and intermediate repeatability of the method were determined by duplicate extraction and analysis of wine and synthetic wine containing known amounts of volatile components on three independent days. The intra-day precision gave the repeatability (repeatability during one day), and was evaluated by injecting the same extract six consecutive times. The inter-day precision gave the intermediate repeatability (repeatability over three days) and was evaluated by analysing the same samples over three days. The repeatability and intermediate repeatability were expressed as percentage relative standard deviation (% RSD) for each compound.

3.2.7. Data processing and multivariate data analysis

After quantitation, descriptive statistical measurements including mean, standard deviation, % RSD and recovery were calculated using Microsoft Excel 2007. The data were subjected to one-way analysis of variance (ANOVA) using Statistica (version 10, Statsoft Inc., www.statsoft.com), followed by a post-hoc Fisher LSD analysis, to determine whether there were significant differences in the volatile content between the three Chenin blanc wine styles. Differences between the volatiles with a significance level of 5 % ($p \leq 0.05$) were considered significant. Correcting with respect to the number of tests run was not considered, as this would be too restrictive and would have resulted in very few significant variables. Variables found to be of significance would need to be further investigated.

The quantitative data were exported to the chemometric software, LatentiX (version 2.00, www.latentix.com, Latent5, Copenhagen, Denmark) for performing PCA to evaluate the underlying trends in the data and to see whether the stylistic differences could be detected. Partial least squares regression discriminant analysis (PLS-DA) was performed using the SIMPLS algorithm in the PLS Toolbox (Wise *et al*., 2006) which runs in MATLAB (version 7.9.0.529, R2009b, Mathworks Inc., USA) to observe underlying trends in the data, and to predict the style from the chemical profile.

The PLS-DA models contained a dummy y-variable as a reference value, with the model developed by regression of the volatile data (x-variables) against the assigned reference value (dummy variable). Dummy variables are arbitrary number assignments to a sample belonging to a particular category. Each sample is assigned a dummy variable (signified by 0 for not belonging to a specific group and 1 for belonging to a specific group) to test the ability of the volatile data to discriminate between the styles (Næs *et al.,* 2002).

For PLS-DA and PCA, the principal variables were calculated by LatentiX. With regard to PCA, the principal variables (volatiles) were calculated based on the entire dataset as latent variables (LV). By extracting one variable, the variation in the remaining data would be maximally lowered. For PLS-DA the variable selection was performed taking into account the dependent variables, selecting one variable at a time, which is known as a forward selection method (Höskuldsson, 1994). The selection is based on locating the x variables that best describe y, followed by removing the variation from y and from that locating the variables that best describe the remainder of y.

For PCA auto-scaling transformation, whereby the data were centred and then scaled, which removed the focus from variables (volatiles) having high standard deviations, compared to other volatiles; was used. To check the LatentiX model for efficiency, it was validated by full cross

validation (CV) (e.g. leave-one-out) technique, whereby all the samples are used in the model as well as to validate it. The model sample set is calculated by iteratively leaving one sample out of the model (Esbensen, 2002).

For PLS-DA auto scaling transformation was used. Two thirds of the data (the calibration set) were used to create the model and it was initially validated using full CV. This was followed by validation using a test set to ensure the optimal number of principal components had been selected. The predictability suggested by the cross validation error was evaluated by the prediction of the style assignment of the test set. The class assignment (misclassification rate) of the style in the test set was based on the model parameters of the calibration set, based on the same number of LVs as used for the calibration set. The error rate of the test set data (the % misclassification rate) was used as a measure of the classification power of the model.

Furthermore, the results were submitted for discriminant analysis (DA) best subset, using Statistica to identify variables that contributed most to the distinction between styles. Best subset considers all the possible combinations of variables to find the best set to predict a variable. The models were set up using five variables at a time. Validation in terms of full cross validation (leave-one-out) was performed on the DA models.

3.2.8. Chenin blanc wine samples

A total of 48 different commercial South African Chenin blanc table wines of the three styles fresh and fruity (ff, n=23); rich and ripe wooded (w, n=14); and rich and ripe unwooded (rr, n=11) were analysed with the developed methodology. The sample set contained wines from cellars in the Western Cape region of South Africa, of different vintages, given in Table 3.2. The style designations, verified by correspondence with the respective winemakers, were used as the style references for the analyses. Although the influence of geographic origin was not investigated in this study, the wines were selected to be representative of major wine producing regions in the Western Cape, in order to capture the extent of the chemical variation in Chenin blanc volatile profiles.

Table 3.2 List of the South African Chenin blanc table wines used for this work. The style descriptions indicated on each of the bottle labels, and verified by the respective winemakers, were used.

Style	Wine name	Geographic region	Vintage
Fresh and fruity	McGregor	McGregor	2010
	Tulbagh Winery	Tulbagh	2010
	Ashton	Ashton	2010
	Botha	Worcester	2010
	De Krans	Calitzdorp	2010
	Cape Dreams	Robertson	2010
	Eagle's Cliff	Breede River	2010
	Groot Parys Die Tweede Droom Spontane Gisting	Paarl	2010
	Groot Parys Die Tweede Droom Ongehout	Paarl	2010
	Stellar (Organic)	Klawer	2010
	Bonnievale	Bonnievale	2010
	Wineways Mountain Shadow	Cape Town	2010
	Ormonde Ondine	Darling	2009
	Bovlei	Wellington	2009
	Goudini Range	Rawsonville	2008
	Goudini Umfiki Range	Rawsonville	2010
	Slanghoek	Rawsonville	2009
	Leopards Leap	Stellenbosch	2010
	Kanu	Stellenbosch	2009
	Kleine Zalze (Bush Vine)	Stellenbosch	2010
	Raats	Stellenbosch	2010
	Jasons Hill	Rawsonville	2009
	Ken Forrester Petit Chenin	Stellenbosch	2009
Rich and ripe unwooded	Dornier	Stellenbosch	2010
	Douglas Green - Vineyard Creation	Wellington	2010
	Le Pommier	Stellenbosch	2010
	Bochendal	Franschoek	2010
	Mooiplaas	Stellenbosch	2010
	Conradie	Worcester	2009
	Hawksmoor	Paarl	2008
	Hawksmoor	Paarl	2007
	Landskroon	Paarl	2010
	Pulpit Rock	Riebeek West	2010
	Jacques Smit Wines	Wellington	2006
Rich and ripe wooded	Lammershoek	Malmesbury	2009
	Mullineux Kloof Street	Riebeek-Kasteel	2009
	Kanu KCB	Stellenbosch	2007
	Katbakkies	Stellenbosch	2008
	Andy Mitchell	Greyton	2009
	Nederburg	Paarl	2008
	Rijks Private Cellar	Tulbagh	2007
	Bruere Gold Reserve Bon Courage	Robertson	2008
	Villiera Traditional	Stellenbosch	2009
	Villiera	Stellenbosch	2009
	Simonsig Avec Chene	Stellenbosch	2009
	Perdeberg Rex Equus	Paarl	2008
	Jordan Barrel Fermented	Stellenbosch	2009
	Graham Beck Bowed Head	Franschoek/Stellenbosch	2009

3.3. Results and discussion

3.3.1. Method development and validation

The LLE technique was developed in conjunction with the GC-FID method, so that the best possible extraction and separation techniques could be combined for the optimal analysis of major and minor wine volatiles. The use of no salt as well as different amounts of sodium chloride (1 to 3 g) and ammonium sulphate (1 to 3 g) were tested during the initial stages of LLE development. According to Cabredo-Pinillos *et al.,* (2006) extraction yields were higher using sodium chloride, while Ortega *et al.,* (2001) observed that ammonium sulphate improved the extraction efficiency of wine volatiles such as acids and alcohols that had Lewis acid properties. Figure 3.1 illustrates a comparison of the% recovery observed for each of the wine volatiles, using the experimentally determined optimum amount of each salt, which were 1 g and 2.5 g of sodium chloride and ammonium sulphate, respectively.

Although some of the volatiles were extracted better using sodium chloride as the salt in the LLE procedure, the majority of the compounds were extracted with similar recoveries using either salt. However, only the ammonium sulphate salt altered the specific gravity of the aqueous phase, so that the organic layer was the top layer, unlike what is normally observed for DCM (Chen *et al.,* 2001). As is common in micro-scale LLE, centrifuge vials are used rather than separating funnels, with the organic layer aspirated by Pasteur pipette. The procedure is simplified when the organic layer has a lower specific gravity than the aqueous phase, as less contamination of the organic phase by the aqueous phase will occur during the aspiration. The selected conditions were 5 mL of wine, 1 mL of DCM and 2.5 g ammonium sulphate, with which no emulsion problems were observed in the analyses using the proposed LLE method.

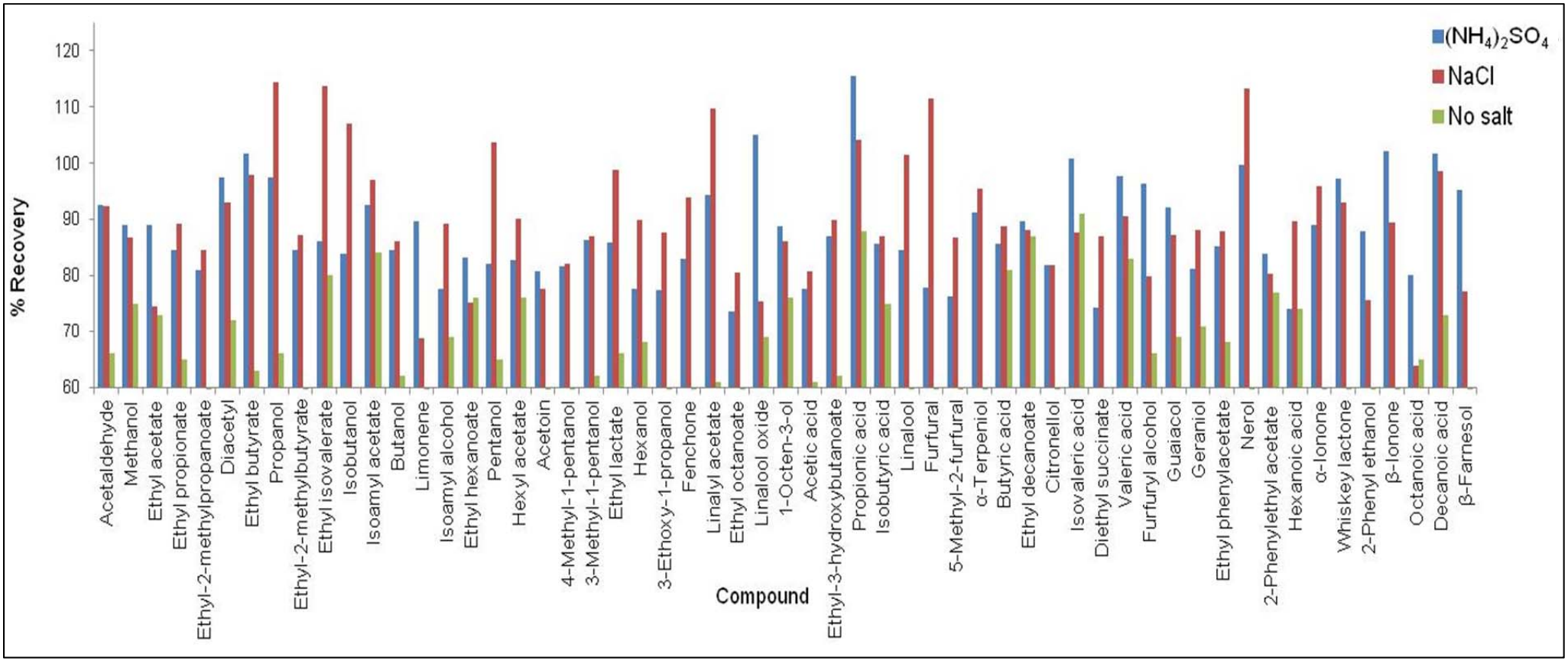

Figure 3.1 Comparison of the effect of using 1 g sodium chloride (NaCl), 2.5 g ammonium sulphate ($(NH_4)_2SO_4$) as well as no salt, on the % recoveries of the aroma compounds.

The GC oven temperature, temperature ramp rate, flow rate, injection mode (split versus split-less) as well as injection temperatures were optimised to obtain a rapid method with well resolved peaks, to achieve the most favorable elution profile in the shortest time possible. Generally the sensitivity of split-less injection mode is higher than that of split mode, however, split injection mode results in better peak shapes compared to split-less mode. A split ratio of 18:1 was chosen and this, in combination with the well-known solvent focusing effect, provided excellent chromatographic performance (no overloading of the column or peak fronting) as well as sensitivity due to sharp peaks obtained for all of the compounds.

The temperature ramp rate was fine-tuned to decrease retention times while still obtaining fully resolved peaks. Ramp rates of 8 to 20 ºC min^{-1} were investigated. Higher ramp rates resulted in sharper peaks and faster analysis times, however some co-elution occurred towards the middle and end of the analysis. A ramp rate of 8 ºC min^{-1} resulted in optimum separation of all peaks in the shortest possible time. The initial oven temperature of 40 ºC was selected from a range of investigated temperatures, of 35 to 45 ºC, due to higher initial temperatures resulting in some of the early eluting compounds co-eluting with ethanol; and decreased initial temperatures giving poorer peak shapes.

Column flow rates of 2 to 8 mL min^{-1} were investigated, as speeding up the carrier gas flow would increase the speed with which the compounds would move through the column and a flow rate of 5.5 mL min^{-1} was found to be optimal for resolution of all compounds. Upon selection of the most optimal conditions; initial oven temperature of 40 ºC, temperature ramp rate of 8 ºC min^{-1} and column flow rate of 5.5 mL min^{-1}, the method was validated in terms of linearity, limits of detection and quantification, recovery, accuracy and precision.

Once the method was fully developed, calibration graphs were constructed as described in section 3.2.5, using the internal standard method. The concentration ranges of the calibration curves covered the expected concentration ranges for the mentioned analytes in white wine, as previously reported in the literature (Ortega *et al.,* 2001; Louw *et al.,* 2009; Malherbe, 2011). Regression coefficients, slopes and intercepts were calculated by linear least squares regression and all the calibration curves were found to have excellent linearity in the given concentration range, with correlation coefficient (R^2) values greater than 0.997 (Table 3.3).

The slope of the straight line calibration curve gives an indication of the sensitivity of the method. The sensitivity depends on detector response, the fewer the carbon/hydrogen bonds present in the compound, the lower the FID response will be. The sensitivity also depends on the extraction efficiency of the analyte. From Table 3.3 the lowest sensitivities were obtained for methanol as well

as for some of the terpenes. These were also some of the analytes that had poorer recoveries from the extraction, as well as some of the smaller molecules (Table 3.3).

The linearity ranges of all analytes, calculated as described in section 3.2.6.1, and the LOD's and LOQ's, calculated as described in section 3.2.6.3, can be seen in Table 3.3. The LOD's ranged between 0.01 mg L^{-1} and 0.2 mg L^{-1}. The LOQ's ranged between 0.03 mg L^{-1} and 0.8 mg L^{-1}, for 2-phenylethyl acetate and methanol, respectively. These limits were seen as acceptable for the purposes of wine analysis. The obtained LOD's and LOQ's were in a similar range to the previously described method validation by Cabredo-Pinillos *et al.,* (2006). The exception was ethyl hexanoate, for which the reported LOD and LOQ were 0.2 mg L^{-1} and 0.7 mg L^{-1}, respectively, which were higher than LOD's and LOQ's determined during this work.

The range of recoveries (Table 3.3) varied between 65-115 %, which was to be expected, considering the different polarities of the compounds. The more polar analytes (such as alcohols) would be highly soluble in water and would therefore be more poorly extracted by DCM than the non-polar compounds. Long carbon chains of 6 carbon atoms or more were very well extracted using the developed methodology. Compounds with carbon chains of less than 6 carbon atoms are more polar and as expected, were more poorly extracted by DCM. These findings corresponded with the observations made by Ortega *et al.,* (2001), that polar compounds were extracted more poorly using DCM.

The repeatability (repeatability within-day), expressed in percentage relative standard deviation (% RSD) using 6 independent analyses of a standard solution and by standard addition to white wine, given in Table 3.4. The average repeatability in wine was 1.9 % with the maximum value being close to 10 % for linalool and the minimum 0.1 % for butyric acid. The intermediate repeatability (between-day) expressed as % RSD were obtained from six independent analyses of standard solution and by standard addition to white wine, over three days (Table 3.4). The intermediate repeatability in wine ranged between 0.1-10.4 % for ethyl decanoate and linalool, respectively. These values were acceptable and no carry-over between different injections was observed.

Separation of the 57 major and minor volatile wine components (as well as 2 internal standards) was successfully achieved with a total run time of 29.3 minutes. This method would serve to increase sample throughput for volatile metabolic profiling of wine. A chromatogram of a sample analysed using the developed method can be seen in Figure 3.2.

Table 3.3 Average recoveries (%) of the volatile compounds in the Chenin blanc samples (N=6) as well as the limits of detection (LOD), limits of quantification (LOQ), linearity data and calibration ranges of the developed GC-FID method.

Compound class	Compound	Recovery in wine (%) ± RSD (%)	LOD[a] mg L^{-1}	LOQ[b] mg L^{-1}	R^2	Slope	Range mg L^{-1}
Alcohols	Methanol	89.1 ± 3.7	0.2	0.8	0.997	0.070	10.0 – 150.0
	n-Propanol	97.4 ± 2.1	0.02	0.08	0.998	0.779	2.0 - 201.0
	Isobutanol	83.8 ± 1.8	0.02	0.07	0.999	0.884	1.3 - 103.8
	n-Butanol	89.6 ± 1.5	0.02	0.07	0.997	0.822	0.3 - 20.30
	Isoamyl Alcohol	83.1 ± 1.4	0.02	0.06	0.998	0.989	5.5 - 477.3
	Pentanol	82.9 ± 2.7	0.02	0.05	0.999	0.926	1.0 - 100.0
	4-Methyl-1-pentanol	86.3 ± 1.7	0.02	0.06	0.999	0.974	1.0 - 100.0
	n-Hexanol	77.4 ± 2.6	0.01	0.05	0.999	1.174	0.3 - 30.9
	3-Ethoxy-1-propanol	83.07 ± 2.2	0.03	0.09	0.999	0.623	1.0 - 100.0
	3-Methyl-1-pentanol	85.8 ± 1.6	0.02	0.06	0.999	0.968	1.0 - 100.0
	1-Octen-3-ol	88.7 ± 2.3	0.02	0.07	0.999	0.851	0.1 - 100.0
	2-Phenyl Ethanol	87.8 ± 1.4	0.02	0.05	0.999	1.070	0.6 - 51.0
Carbonyls	Acetaldehyde	92.7 ± 7.3	0.03	0.05	0.999	0.630	0.1 - 100.0
	Diacetyl	97.4 ± 2.3	0.03	0.04	0.999	0.373	0.50- 100.0
	Acetoin	81.8 ± 3.08	0.04	0.1	0.999	0.465	1.0 - 100.0
Acetate esters	Ethyl Acetate	88.9 ± 1.0	0.03	0.09	0.998	0.623	3.6 - 360.8
	2-Phenylethyl Acetate	83.8 ± 6.8	0.02	0.06	0.999	0.937	0.3 - 20.6
	Isoamyl Acetate	85.3 ± 2.4	0.03	0.1	0.999	0.715	0.2 - 19.2
	Hexyl Acetate	80.7 ± 4.4	0.02	0.08	0.997	0.738	0.3 - 21.9
	Ethyl Phenylacetate	85.2 ± 2.09	0.02	0.06	1.000	0.999	1.0 - 100.0
	Ethyl Propionate	84.7 ± 3.6	0.03	0.1	0.998	0.556	1.0 - 100.0
	Ethyl-2-methylpropanoate	81.06 ± 3.8	0.03	0.1	0.998	0.610	1.0 - 100.0
	Ethyl Butyrate	101.7 ± 7.5	0.03	0.09	0.998	0.654	0.3 - 22.0
Esters	Ethyl-2-methylbutyrate	84.5 ± 2.02	0.02	0.08	0.999	0.685	1.0 - 100.0
	Ethyl Isovalerate	86.2 ± 2.09	0.02	0.08	0.999	0.712	1.0 - 100.0
	Ethyl Hexanoate	82.09 ± 2.8	0.03	0.08	0.999	0.705	0.4 - 30.6
	Ethyl Lactate	77.7 ± 3.2	0.04	0.1	0.999	0.461	6.3 - 500.2
	Ethyl Octanoate	103.0 ± 2.6	0.02	0.06	0.999	0.945	0.07 - 4.0
	Ethyl-3-hydroxybutanoate	84.5 ± 2.9	0.03	0.1	0.999	0.544	0.4 - 100.0
	Ethyl Decanoate	89.6 ± 5.04	0.1	0.5	0.999	1.215	0.5 - 3.5
	Diethyl Succinate	74.3 ± 2.4	0.03	0.09	1.000	0.618	0.4 - 31.4

Table 3.3 continued

Compound class	Compound	Recovery in wine (%) ± RSD (%)	LOD[a] mg L^{-1}	LOQ[b] mg L^{-1}	R^2	Slope	Range mg L^{-1}
Terpenes	Limonene	69.7 ± 1.3	0.04	0.1	0.999	0.125	0.1 - 1.0
	Fenchone	73.7 ± 1.3	0.02	0.04	0.999	0.258	0.04 - 1.0
	Linalool Oxide	73.8 ± 3.2	0.04	0.2	0.999	0.079	0.05 - 1.0
	Linalool	65.02 ± 8.2	0.02	0.08	0.999	0.188	0.08 - 1.0
	Linalyl Acetate	86.0 ± 3.3	0.02	0.06	0.999	0.138	0.06 - 1.0
	Citronellol	71.8 ± 1.3	0.02	0.06	0.999	0.193	0.01 - 1.0
	α-Terpeneol	81.2 ± 5.8	0.02	0.03	0.999	0.383	0.01 - 1.0
	Nerol	99.5 ± 6.6	0.02	0.03	0.999	0.455	0.03 - 1.0
	Geraniol	79.2 ± 1.06	0.02	0.04	1.000	0.229	0.04 - 2.0
	β-Ionone	102.1 ± 1.6	0.03	0.09	1.000	0.137	0.09 - 1.0
	α-Ionone	88.9 ± 1.1	0.03	0.1	0.999	0.086	0.1 - 1.0
	β-Farnesol	95.2 ± 9.7	0.04	0.1	0.998	0.109	0.1 - 5.0
Acids	Acetic Acid	77.7 ± 1.3	0.1	0.4	0.999	0.155	22.5 - 1000.0
	Propionic Acid	115.5 ± 4.8	0.04	0.2	0.999	0.402	0.4 - 100.0
	Isobutyric Acid	76.4 ± 7.2	0.03	0.1	0.999	0.560	0.3 - 20.9
	Butyric Acid	85.6 ± 8.5	0.03	0.1	0.999	0.566	0.3 - 21.2
	Iso-Valeric Acid	100.7 ± 1.1	0.03	0.09	0.999	0.640	0.5 - 39.3
	Hexanoic Acid	74.1 ± 2.2	0.02	0.08	0.999	0.731	0.4 - 29.7
	Valeric Acid	97.9 ± 5.5	0.05	0.2	0.999	0.640	0.3 - 20.7
	Octanoic Acid	81.2 ± 1.0	0.03	0.08	0.999	0.822	0.5 - 40.4
	Decanoic Acid	101.6 ± 1.3	0.03	0.1	0.998	0.650	0.6 - 56.0
Wood derived	Furfuryl Alcohol	95.8 ± 7.1	0.05	0.2	0.999	1.011	0.2 - 25.0
	Furfural	86.7 ± 5.07	0.01	0.03	0.999	0.943	1.0 - 100.0
	5-Methyl-2-furfural	79.7 ± 9.4	0.02	0.05	0.999	0.557	0.04 - 35.0
	Guaiacol	90.2 ± 5.0	0.02	0.08	0.999	0.710	0.1 - 20.0
	Whiskey Lactone	97.3 ± 2.5	0.03	0.09	0.999	1.120	0.1 – 20.0

[a]Limit of detection ; [b]Limit of quantification

Table 3.4 Repeatability and intermediate repeatability (expressed in% RSD) for the developed method in white wine and synthetic wine.

Compound class	Volatile compound	Repeatability		Intermediate repeatability	
		White wine	Synthetic	White wine	Synthetic
Alcohols	Methanol	4.0	5.7	4.2	6.0
	n-Propanol	3.7	2.3	3.9	2.4
	Isobutanol	1.5	0.8	1.5	0.8
	n-Butanol	0.1	0.4	0.1	0.4
	Isoamyl Alcohol	1.5	6.3	1.5	6.6
	Pentanol	0.6	0.8	0.6	0.8
	4-Methyl-1-pentanol	0.4	0.4	0.4	0.4
	3-Methyl-1-pentanol	0.4	0.4	0.4	0.4
	n-Hexanol	0.3	0.2	0.3	0.2
	3-Ethoxy-1-propanol	0.6	0.3	0.7	0.4
	1-Octen-3-ol	0.5	0.2	0.6	0.2
	2-Phenyl Ethanol	0.9	0.5	1.0	0.6
Carbonyl compounds	Acetaldehyde	2.3	2.7	2.4	2.9
	Diacetyl	0.6	0.7	0.6	0.7
	Acetoin	1.0	0.8	1.0	0.9
Acetate esters	Ethyl Acetate	1.5	1.7	1.5	1.7
	Ethyl Phenylacetate	0.5	0.5	0.5	0.5
	2-Phenylethyl Acetate	0.4	0.2	0.4	0.2
	Isoamyl Acetate	0.1	0.4	0.1	0.4
	Hexyl Acetate	0.2	0.2	0.2	0.2
Esters	Ethyl Ocatanoate	0.3	0.1	0.3	0.1
	Ethyl Decanoate	0.1	0.5	0.1	0.5
	Diethyl Succinate	0.7	0.4	0.8	0.4
	Ethyl Butyrate	0.5	0.4	0.5	0.4
	Ethyl Propionate	0.8	0.4	0.9	0.4
	Ethyl-3-hydroxybutanoate	0.5	0.7	0.5	0.7
	Ethyl Isovalerate	0.5	1.0	0.5	1.1
	Ethyl-2-methylbutyrate	0.5	1.4	0.5	1.4
	Ethyl Lactate	0.9	2.4	0.9	2.5
	Ethyl-2-methylpropanoate	0.9	0.6	0.9	0.7
	Ethyl Hexanoate	0.3	0.2	0.3	0.2
Terpenes	Limonene	3.3	9.4	3.4	10.0
	Fenchone	2.4	4.8	2.5	5.0
	Linalool Oxide	7.5	6.2	7.9	6.5
	Linalool	9.9	7.8	10.4	8.7
	α-Terpeniol	6.0	3.0	6.2	3.1
	Linalyl Acetate	9.7	9.9	10.2	10.4
	Citronellol	4.2	9.4	4.4	9.9
	Geraniol	2.5	6.9	2.6	7.2
	Nerol	8.8	7.0	9.3	7.3
	α-Ionone	2.4	8.2	2.6	8.6
	β-Ionone	4.1	4.4	4.3	4.6
	β -Farnesol	4.9	2.6	5.2	2.7
Acids	Acetic Acid	6.6	7.9	6.9	8.2
	Propionic Acid	6.0	0.6	6.3	0.6
	Isobutyric Acid	0.5	0.4	0.5	0.4
	Butyric Acid	0.6	0.3	0.7	0.3
	Iso-Valeric Acid	0.1	0.4	0.1	0.5
	Valeric Acid	0.5	0.1	0.6	0.1
	Hexanoic Acid	0.3	0.3	0.3	0.4
	Octanoic Acid	0.1	0.7	0.1	0.8
	Decanoic Acid	0.2	0.5	0.2	0.6
Wood derived	Furfural	0.5	0.5	0.5	0.6
	Furfuryl Alcohol	0.6	0.4	0.7	0.4
	5-Methyl-2-furfural	0.4	0.2	0.5	0.2
	Guaiacol	0.2	0.4	0.2	0.4
	Whiskey Lactone	0.1	0.3	0.2	0.4

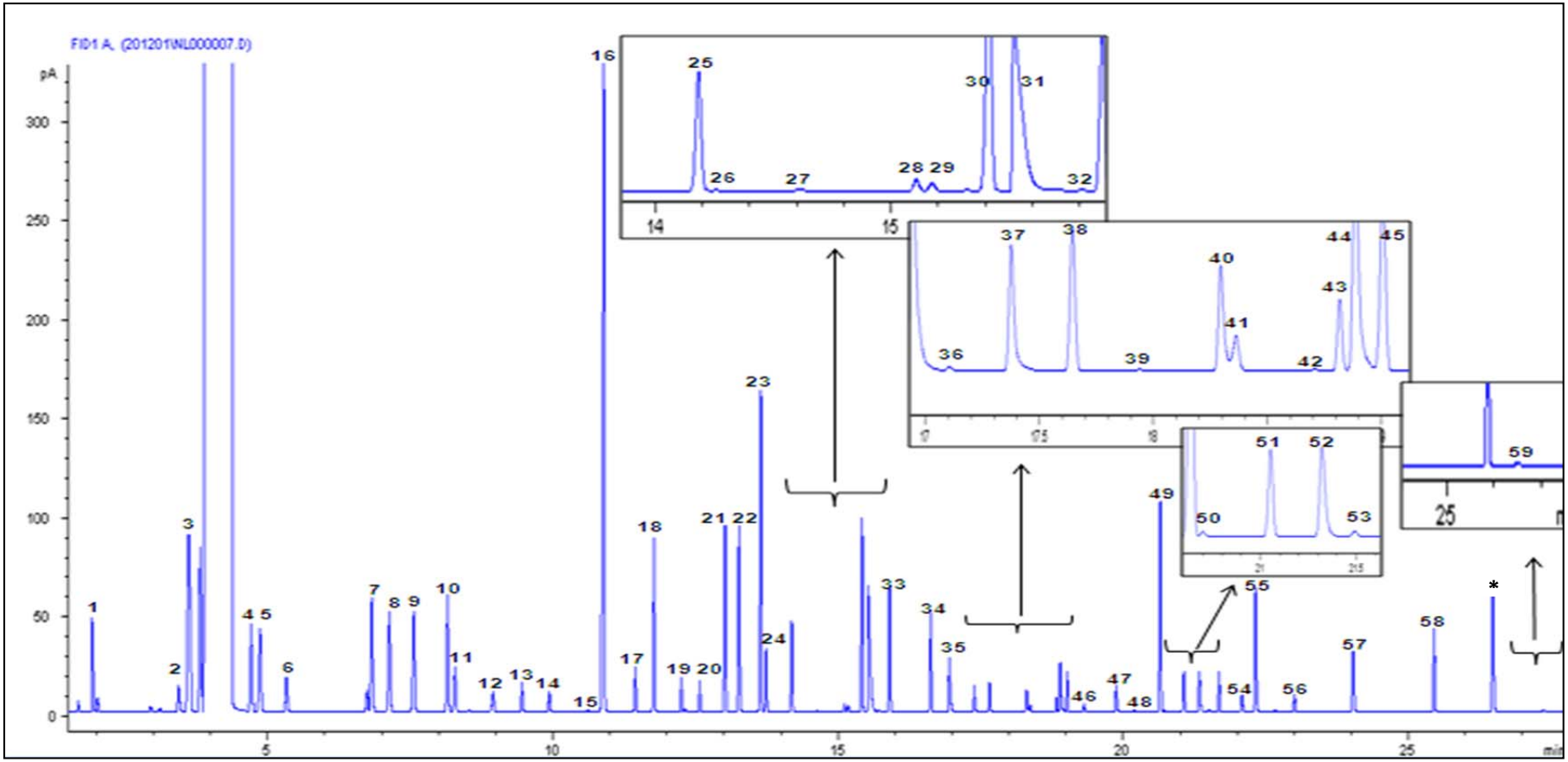

Figure 3.2 A chromatogram of a spiked Chenin blanc wine sample (spiked with 0.5 mL of the calibration solution) analysed using the high throughput method. The analytes are: (1) Acetaldehyde, (2) Methanol, (3) Ethyl Acetate, (4) Ethyl Propionate, (5) Ethyl-2-methylpropanoate, (6) Diacetyl, (7) Ethyl Butyrate, (8) *n*-Propanol, (9) Ethyl-2-methylbutyrate, (10) Ethyl Isovalerate, (11) Isobutanol, (12) Isoamyl Acetate, (13) *n*-Butanol, (14) 4-Methyl-2-pentanol (Internal standard, (IS)), (15) Limonene, (16) Isoamyl Alcohol, (17) Ethyl Hexanoate, (18) Pentanol, (19) Hexyl Acetate, (20) Acetoin, (21) 4-Methyl-1-pentanol, (22) 3-Methyl-1-pentanol, (23) Ethyl Lactate, (24) *n*-Hexanol, (25) 3-Ethoxy-1-propanol, (26) Fenchone, (27) Linalyl Acetate (28) 2,6-dimethylphenol (IS), (29) Ethyl Octanoate, (30) 1-Octen-3-ol, (31) Acetic Acid, (32) Linalool Oxide, (33) Ethyl-3-hydroxybutanoate, (34) Propionic Acid, (35) Isobutyric Acid, (36) Linalool, (37) Furfural, (38) 5-Methyl-2-furfural, (39) α-Terpeniol, (40) Butyric Acid, (41) Ethyl Decanoate, (42) Citronellol, (43) Isovaleric Acid, (44) Diethyl Succinate, (45) Valeric Acid, (46) Furfuryl Alcohol, (47) Guaiacol, (48) Geraniol, (49) Ethyl Phenylacetate, (50) Nerol, (51) 2-Phenylethyl Acetate, (52) Hexanoic Acid, (53) α-Ionone, (54) Whiskey Lactone, (55) 2-Phenyl Ethanol, (56) β-Ionone, (57) Octanoic Acid, (58) Decanoic Acid, (59) β-Farnesol, (*) unknown peak.

3.3.2. Application to Chenin blanc wine samples

3.3.2.1. Quantitative data

The wines selected for this work were a representative sample set of prominent commercial South African Chenin blanc wines, within the three styles. Representative of the entire wine region, they were selected from the top and middle of the range (price and perceived quality), representing a wide range of winemaking procedures. The new analytical method was applied to the 48 commercial Chenin blanc samples, each bottle was extracted in triplicate. Pure standards were used to identify and quantify the volatile components in the samples.

Table 3.5 shows the analyte ranges and standard deviations (in mg L^{-1}) observed for each style as well as between all three styles. Repeatability was excellent between technical repeats with less than 10 % RSD. The high standard deviations observed in Table 3.5 are due to variations between all the samples analysed, both within and between the three Chenin blanc styles. The variation within and between the styles could be explained due to the wines having been obtained from various origins around the Western Cape, and the different vintages between 2006 and 2010. Another possible explanation for this variation could be due to the fact that each wine estate would have their individual winemaking practices.

The concentrations ranges of esters, acids and fusel alcohols obtained were in agreement with levels previously reported for Chenin blanc (Malherbe, 2011). However, in that work, only four wines were analysed and the styles of Chenin blanc were not identified. Additionally, no terpenes, carbonyl compounds or wood derived compounds were analysed in this work. Previous work by Louw *et al.,* (2010) on young South African single cultivar white wines, illustrated that Sauvignon blanc wines contained significantly higher content of decanoic (0.4–5.8 mg L^{-1}) and octanoic (1.7–12.2 mg L^{-1}) acids, as well as hexyl acetate (0.07–2.5 mg L^{-1}), in comparison to the Chenin blanc wines analysed in this study, while the Chardonnay wines contained significantly higher concentrations of ethyl hexanoate (0.7–2.3 mg L^{-1}). In this work, the fresh and fruity style Chenin blanc wines were correlated with higher average content of decanoic (1.6–4.7 mg L^{-1}) and octanoic acids (3.5–12.0 mg L^{-1}), while the rich and ripe styles had a higher average ethyl hexanoate concentration (0.8–1.6 mg L^{-1}).

The wooded styles contained the highest average concentrations of total alcohols, aldehydes and wood derived compounds: 341.8 mg L^{-1}, 10.7 mg L^{-1} and 14.2 mg L^{-1}, respectively. Not surprisingly, the fresh and fruity style Chenin blanc wines contained the lowest average concentration of the before mentioned compound groups, of 292.7 mg L^{-1} 2.7 mg L^{-1} and 7.4 mg L^{-1}, respectively. Furthermore, while the unwooded rich and ripe styles contained the overall highest total concentration of terpenes (108.5 mg L^{-1}), it was surprising that the fresh and fruity style contained the lowest overall concentration of terpenes at 35.5 mg L^{-1}.

ANOVA was used to highlight the significantly different compounds between the different wine styles (Table 3.5), and this information provides a basis for further investigation of the effect of winemaking techniques on the volatile profiles of the styles. Such investigation was however beyond the scope of this study.

Table 3.5 Quantitative data of the three Chenin blanc styles obtained using the high throughput method. The ranges and standard deviation (SD) are given in mg L^{-1}; (n is the total number of wines analysed per group) as well as the significant difference ($p < 0.05$) with values followed by different alphabetic letters row-wise, indicating significant difference between styles (significant p-values are in bold).

Compound class	Analyte	All samples (n=48)		Rich & ripe wooded (n=13)		Rich & ripe un-wooded (n=11)		Fresh & fruity (n=24)		p-value
		Range	± SD	Range	± SD	Range	± SD	Range	± SD	(p < 0.05)
Aldehydes	Acetaldehyde	0.3 - 6.02	1.06	1.9 - 5.3 a	0.9	0.5 - 6.0 b	1.4	0.3 - 2.6 b	0.6	**0.01**
	Diacetyl	0.1 - 0.8	0.1	0.2 - 0.8 a	0.1	0.2 - 0.4 a	0.08	0.1	N/A[1]	0.29
	Acetoin	0.3 - 20.7	4.3	4.3 - 17.9 a	4.3	0.4 - 20.7 b	5.5	0.3 - 5.3 c	1.3	**0.01**
Alcohols	Methanol	40.7 - 86.07	9.2	42.6 - 86.1 a	12.2	40.7 - 58.4 a	5.3	40.8 - 74.7 a	8.5	0.15
	n-Propanol	13.04 - 63.8	13.3	13.0 - 61.6 a	11.9	35.8 - 62.7 b	6.8	16.1 - 63.8 b	10.5	**0.01**
	Isobutanol	12.5 - 46.9	7.9	21.7 - 43.1 a	7.6	16.7 - 46.9 b	7.9	12.5 - 33.1 b	4.9	**0.01**
	n-Butanol	0.4 - 1.7	0.3	0.7 - 1.7 a	0.3	0.5 - 1.3 ab	0.3	0.4 - 1.6 b	0.3	**0.01**
	Isoamyl Alcohol	122.63 - 248.3	27.5	129.9 - 248.3 a	35.2	122.6 - 219.6 b	29.3	140.8 - 211.8 b	17.8	**0.01**
	Pentanol	0.05	N/A[1]	0.05	N/A[1]	0.05	N/A[1]	0.05	N/A[1]	N/C[2]
	4-Methyl-1-pentanol	0.06	N/A[1]	0.06	N/A[1]	0.06	N/A[1]	0.06	N/A[1]	N/C[2]
	3-Methyl-1-pentanol	0.06	N/A[1]	0.06	N/A[1]	0.06	N/A[1]	0.06	N/A[1]	N/C[2]
	n-Hexanol	0.9 - 3.4	0.4	1.3 - 3.4 a	0.5	1.1 - 2.6 a	0.5	0.9 - 2.5 a	0.4	0.07
	3-Ethoxy-1-propanol	0.2 - 4.7	1.3	0.2 - 4.2 a	1.1	1.34- 4.7 b	1.3	0.3 - 4.4 c	1.2	**0.01**
	1-Octen-3-ol	0.07 - 0.2	0.01	0.07 - 0.2	0.03	0.07	N/A [a]	0.07	N/A [a]	N/C[2]
	2-Phenyl ethanol	10.8 - 53.1	9.1	13.0 - 46.7 a	10.8	10.8 - 38.4 b	7.2	11.0 - 53.1 b	7.76	**0.01**
Acetate esters	Ethyl acetate	48.2 - 209.6	35.5	76.6 - 209.6 a	40.5	54.2 - 121.8 b	24.5	48.6 - 143.7 b	19.4	**0.01**
	Isoamyl Acetate	0.2 - 4.8	1.2	0.2 - 1.9 a	0.5	0.2 - 3.4 b	1.1	0.3 - 4.8 c	1.3	**0.01**
	Hexyl Acetate	0.08 - 0.2	0.05	0.08 - 0.1 a	0.01	0.1 - 0.2 b	0.03	0.08 - 0.2 b	0.05	**0.01**
	2-Phenylethyl acetate	0.07 - 0.8	0.1	0.1 - 0.5 a	0.1	0.1 - 0.3 a	0.07	0.07 - 0.8 b	0.2	**0.04**
	Ethyl Phenylacetate	0.2 - 0.6	0.05	0.2 - 0.6 a	0.06	0.3 - 0.4 a	0.03	0.3 - 0.6 b	0.05	0.38
Esters	Ethyl Propionate	0.2 - 0.7	0.1	0.3 - 0.7 a	0.1	0.2 - 0.5 b	0.07	0.2 - 0.6 b	0.09	**0.01**
	Ethyl-2-Methylpropanoate	0.1 - 0.6	0.08	0.1- 0.6 b	0.1	0.1 - 0.5 a	0.07	0.2 - 0.4 b	0.05	**0.01**
	Ethyl Butyrate	0.2 - 0.8	0.09	0.3 - 0.6 a	0.09	0.3 - 0.6 b	0.08	0.3 - 0.8 b	0.09	**0.02**
	Ethyl-2-methylbutyrate	0.08 - 2.2	0.4	0.2 - 0.3 a	0.05	0.3 - 2.2 b	0.6	0.08 - 0.6 c	0.1	**0.01**
	Ethyl Isovalerate	n.d - 0.6	0.2	0.08 b	N/A[1]	0.4 - 0.5 a	0.03	nd - 0.6 a	0.2	**0.01**
	Ethyl Lactate	5.0 - 287.2	55.9	58.9 - 287.2 a	60.5	7.6 - 79.9 b	19.0	5.0 - 103.7 b	19.7	**0.01**
	Ethyl Hexanoate	0.6 - 1.6	0.2	0.8 - 1.5 a	0.2	1.1 - 1.5 b	0.1	0.6 - 1.5 b	0.2	**0.01**
	Ethyl Octanoate	0.1 - 3.4	0.7	0.1 - 3.1 a	0.7	1.5 - 3.1 b	0.5	0.3 - 3.4 b	0.7	**0.01**
	Ethyl-3-hydroxybutanoate	0.04 - 1.2	0.2	0.8 - 1.2 a	0.1	0.04 - 1.0 b	0.3	0.1 - 1.1 b	0.1	**0.01**
	Ethyl Decanoate	0.1 - 1.2	0.2	0.1 - 1.2 a	0.3	0.1 - 0.7 a	0.2	0.1 - 0.8 a	0.2	0.21
	Diethyl Succinate	0.7 - 22.3	5.6	9.4 - 22.3 a	3.1	1.52- 10.8 b	3.6	0.7 - 12.1 c	2.4	**0.01**
Terpenes	Limonene	0.01	N/A[1]	0.01	N/A[1]	0.01	N/A[1]	0.01	N/A[1]	N/C[2]
	Fenchone	0.04 - 0.1	0.01	0.04 - 0.05	1.8	0.04	N/A[1]	0.04 - 0.1	0.01	N/C[2]

Table 3.5 continued

Compound class	Analyte	All samples (n=48)		Rich & ripe wooded (n=13)		Rich & ripe un-wooded (n=11)		Fresh & fruity (n=24)		p-value
		Range	± SD	Range	± SD	Range	± SD	Range	± SD	(p < 0.05)
	Linalool Oxide	0.12- 0.8	0.1	0.01	N/A[1]	0.01 - 0.8	0.2	0.2 - 0.7	0.1	N/C[2]
	Geraniol	0.02 - 0.3	0.06	0.04 - 0.2 a	0.01	0.05 - 0.3 b	0.08	0.02 - 0.2 b	0.04	**0.01**
	Nerol	0.03 - 0.06	0.01	0.03 - 0.06	0.01	25.5	N/A[1]	0.04	N/A[1]	N/A[2]
	α-Terpeniol	0.03 - 0.08	0.01	0.03 - 0.05 a	4.6	0.03 - 0.08 b	0.01	0.04 a	N/A[1]	**0.01**
	α-Ionone	0.02 - 4.0	0.8	0.3 - 4.00 a	1.0	0.3 - 2.0 ab	0.6	0.02 - 2.5 b	0.7	**0.03**
	Linalool	0.08 - 6.4	1.3	0.01 - 5.6 a	1.4	0.08 - 6.4 b	2.5	0.08 - 1.5 ab	0.3	**0.01**
	Citronellol	0.06 - 0.2	0.02	57.7	N/A[1]	0.06 - 0.1	0.02	0.06 - 0.2	0.02	N/C[2]
	Linalyl Acetate	0.06 - 0.7	0.1	0.01 - 0.3 a	0.01	0.08 - 0.5 a	0.1	0.06 - 0.7 a	0.1	0.56
	β-Ionone	0.01 – 0.5	0.07	0.09 - 0.1 a	0.02	0.07 - 0.5 b	0.1	0.01 - 0.2 c	0.03	**0.01**
	β-Farnesol	0.3 - 4.1	0.7	0.6 - 4.1 a	1.0	0.6 - 2.1 a	0.5	0.26 - 2.1 b	0.5	**0.01**
Acids	Acetic Acid	93.9 - 778.3 [a]	148.0	213.4 - 778.3	151.4	119.6 - 330.7 b	64.0	93.9 - 506.7 c	100.6	**0.04**
	Propionic Acid	1.4 - 5.5	1.1	2.0 - 5.5 a	1.1	1.4 - 3.3 b	0.5	1.4 - 5.5 b	0.9	**0.01**
	Isobutyric Acid	0.3 - 1.71	0.3	0.7 - 1.7 a	0.2	0.04 - 1.1 b	0.2	0.3 – 1.0 b	0.2	**0.01**
	Butyric Acid	0.8 - 2.4	0.3	0.9 - 1.6 a	0.2	1.0 - 2.0 b	0.4	0.8 - 2.43 c	0.4	0.05
	Isovaleric Acid	0.2 - 2.0	0.2	0.3 – 1.0 a	0.3	0.3 - 1.1 b	0.2	0.2 - 2.0 c	0.3	0.92
	Valeric Acid	0.03 - 2.3	0.5	0.1 - 1.2 a	0.3	0.03 - 2.3 a	0.7	0.2 - 2.1 a	0.5	0.05
	Hexanoic Acid	2.5 - 7.9	0.9	2.5 - 5.2 a	0.8	3.4 - 6.0 b	0.8	3.6 - 7.9 b	0.9	**0.01**
	Octanoic Acid	3.2 – 12.0	1.8	3.2 - 9.0 a	1.5	4.1 - 9.9 a	1.4	3.5 – 12.0 b	1.6	**0.01**
	Decanoic Acid	0.7 - 4.7	1.0	1.1 - 2.7 a	0.5	0.7 - 4.2 a	1.3	1.6 - 4.7 b	0.9	**0.01**
Wood derived	5-Methyl-2-furfural	1.5 - 17.8	3.6	6.9 - 17.8 a	3.4	4.3 - 14.6 b	2.8	1.5 - 11.7 c	2.5	**0.01**
	Whiskey Lactone	0.09 - 1.4	0.3	0.1 - 0.3	0.06	0.09 - 1.4	0.5	0.09 - 0.5	0.09	N/C[2]
	Furfuryl Alcohol	0.2 - 2.2	0.6	0.9 - 2.2 a	0.4	0.21- 0.3 b	0.04	0.19 - 0.8 b	0.2	**0.01**
	Furfural	0.06 - 0.8	0.2	0.4 - 0.8 a	0.06	0.07 - 0.3 b	0.06	0.06 - 0.2 b	0.04	**0.01**
	Guaiacol	0.08 - 1.2	0.2	0.08 - 1.2	0.3	0.08	N/A[1]	0.08 - 0.09	N/A[1]	N/C[2]

[1]N/A not applicable; [2]N/C not calculated; nd not detected;

3.3.2.2. Style classification

The principal variables (volatiles) were calculated in LatentiX, based on the entire dataset as latent variables (Höskuldsson, 1994). The 24 volatiles that were most descriptive of the styles and explained more than 90 % of the total variation (Table 3.6) were used to calculate the PCA model. The data were subjected to auto-scaling transformation and validated by CV. CV is not as reliable as using a test set for validation, and can be too optimistic as the validation is based on the original model sample set; however the sample set was extremely small, and it was therefore decided to use CV to maximize the number of samples used for the classification model.

Table 3.6 Volatiles that described > 90 % of the total variance and were most descriptive of the three styles and the numbers associated with each volatile for the PCA loadings plot.

Compound class	Number on PCA loadings plot	Volatiles	Explained variance (%)
Alcohols	27	1-Octen-3-ol	90.0
	53	2-Phenyl ethanol	98.5
Carbonyl compounds	19	Acetoin	97.9
	6	Diacetyl	90.3
	1	Acetaldehyde	98.5
Esters	18	Hexyl Acetate	98.0
	12	Isoamyl Acetate	98.3
	22	Ethyl Lactate	98.4
	41	Diethyl Succinate	98.5
	47	2-Phenylethyl Acetate	98.5
	16	Ethyl Hexanoate	98.5
	10	Ethyl Isovalerate	98.5
Acids	33	Propionic Acid	98.1
	43	Hexanoic Acid	98.2
	28	Acetic Acid	98.3
	40	Isovaleric Acid	98.4
	37	Butyric Acid	98.5
	55	Octanoic Acid	98.6
Terpenes	29	Linalool Oxide	98.3
	49	Geraniol	95.0
	54	β-Ionone	93.4
Wood derived	51	Whiskey Lactone	98.3
	36	Guaiacol	91.0
	30	Furfural	95.0

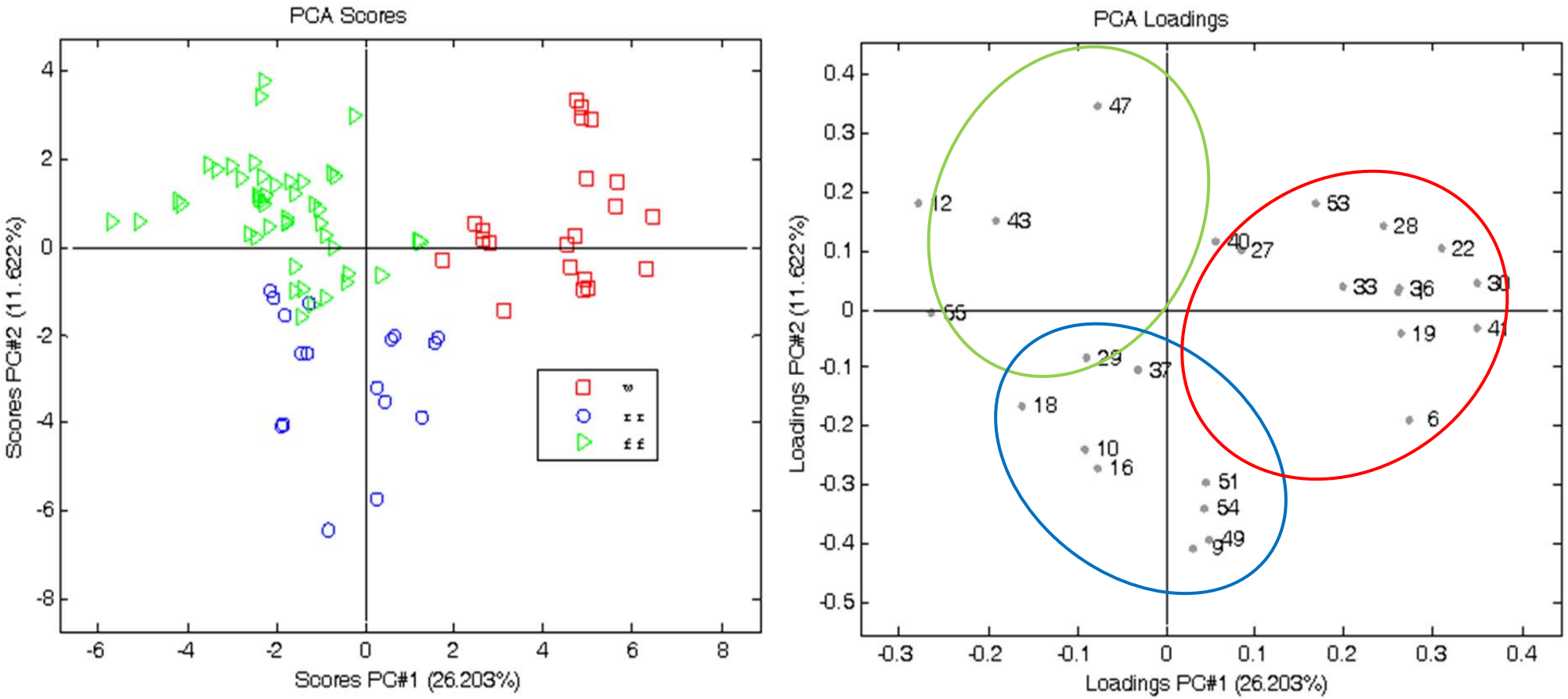

Figure 3.3 The PCA scores plot (left frame) shows clear differentiation between the wooded and non wooded Chenin blanc styles, where w (red, squares) is rich and ripe wooded, rr (blue, circles) is rich and ripe unwooded and ff (green, triangles) is fresh and fruity. The PCA loadings plot (right frame) indicated that higher content of hexyl acetate and ethyl hexanoate were associated with the rr style, and high content of isoamyl acetate and 2-phenylethyl acetate were correlated with the ff styles, whereas the w styles were associated with higher content of 2-phenyl ethanol and ethyl lactate (numbering for the volatiles is given in Table 3.6)

PCA was done using the volatile data, and some differentiation of the three styles was achieved. As illustrated by the PCA scores plot (Figure 3.3 (left frame), there was a distinction between the wooded and unwooded styles. Differentiation between the two unwooded styles (rr and ff in the PCA scores plot, Figure 3.3 (left frame), was also observed, but to a somewhat lesser extent, with the distinction not being as clear as between wooded and unwooded styles.

Volatiles associated with the fresh and fruity Chenin blanc style were in the top left quadrant of PC1 of the loadings plot (Figure 3.3, right frame, green, circles). These included acetate esters such as isoamyl acetate and 2-phenylethyl acetate. This result for the fresh and fruity style corresponds very well with literature regarding the sensory attributes associated with these compounds. Fruity aromas of banana and pear are associated with isoamyl acetate, while 2-phenylethyl acetate imparts rose, honey, fruity and flowery aromas to wine (Francis and Newton, 2005).

Ethyl butyrate, ethyl hexanoate and the terpenes geraniol and β-ionone, were positively correlated with the unwooded rich and ripe Chenin blanc style (rr), (blue, circles in the loadings plot Figure 3.2 (right frame, the numbers in the loadings plot are associated with the volatiles as shown in Table 3.6). Ethyl butyrate lends an apple aroma to wine, while ethyl hexanoate provides wine with fruity, floral, apple peel and strawberry notes (Francis and Newton, 2005). β-Ionone is known to lend aromas of violet, floral, raspberries and seaweed to wine (Francis and Newton, 2005) while geraniol is associated with aromas of rose and geranium above the odour detection value of 30-130 μg L^{-1} (Swiegers *et al.,* 2005).

The volatiles situated towards the positive side of PC1 of the loadings plot (circled in red, Figure 3.3 (right frame), with numbering associated with the volatiles given in Table 3.6), were associated with the wooded style Chenin blanc wines. Of these, ethyl lactate, acetic acid, diacetyl and acetoin are volatiles known to increase when wine undergoes malolactic fermentation (Bartowsky and Henschke, 1995; Gil *et al.,* 2006). Although malolactic fermentation of Chenin blanc wine is usually not encouraged by winemakers, the wooded styles are the only South African Chenin blanc wines which are allowed to undergo between 30-70 % malolactic fermentation (O'Kennedy, 2009). Ethyl lactate contributes buttery, coconut and creamy aromas (Swiegers *et al.,* 2005) while acetoin and diacetyl have been known to lend white wine nutty, toasty aromas and add complexity to the wine when below 30 mg L^{-1} and 0.2-8 mg L^{-1}, respectively (Varnam and Sutherland, 1994; Martineau *et al.,* 1995; Gil *et al.,* 2006).

The rich and ripe wooded Chenin blanc wines were also positively correlated with fusel alcohols (e.g., 2-phenyl ethanol), furfural, guaiacol and furfuryl alcohol (circled in red, Figure 3.3, right frame). Furfural and guaiacol exhibit aromas of caramel, sweet, bread and almond, and smokey

and sweet, respectively (Prida and Chatonnet, 2010). 2-Phenyl ethanol confers attributes of honey, spice and floral (Francis and Newton, 2005).

Subsequently the chemical data was investigated as to whether or not the wines could be classified according to their vintages. The results were much less obvious than classification by styles (data not shown). However, some of the distinctive trends were that the younger Chenin blanc wines tended to exhibit higher content of isoamyl acetate and 2-phenylethyl acetate than the older wines. This corresponds with reported findings that as white wine ages, the concentration of isoamyl acetate and 2-phenylethyl acetate decrease significantly (Camara *et al.,* 2006). This decrease in acetate esters as wines age could be a reason that white wines, during the first period of maturation, have a higher degree of fruitiness than in the following stages of maturation (Camara *et al.,* 2006).

The more mature wines were correlated with higher content of acetic acid and furfural. The increase in acetic acid in ageing wine is due to ester hydrolysis, as initially noted by Simpson and Miller (1983) and more recently addressed by Sumby *et al.,* (2010). Furthermore, furfural has been used successfully in the past to predict age of wine, as it has been reported to increase in a linear manner during maturation (Camara *et al.,* 2006; Silva Ferreira *et al.,* 2003).

3.3.2.3. PLS-DA prediction model

PLS is a flexible tool that can be used to describe interdependencies between a set of x-variables and a set of y-variables, which, if the correlation is sufficiently strong, can be used to predict the y-values for another set of x-values of unknown samples. In this case, the model could be used to predict the styles of unknown wine samples by using chromatographic data. A Chenin blanc style “dummy variable” was selected as the y-vector, and due to that the analysis performed was partial least squares discriminant analysis (PLS-DA), a variant of PLS.

The data was auto scaled and two thirds of the samples from each style were randomly selected to make up the model calibration set. The x-matrix was set up using the calibration set and all the variables (volatile compounds) that were present above their LOQs in at least two styles. The principal variables based on the calibration set were calculated, and only the twenty two volatiles (Figure 3.7) that were most descriptive of the styles and explained more than 90 % of the total variation in the data were used for the PLS-DA model. This could in future reduce the number of compounds needed to be quantified for the purpose of style classification and prediction, reducing the need for integration of all fifty seven volatiles.

Table 3.7 Volatiles that described > 90 % of the total variance and were most descriptive of the three styles, used for the PLS model and the numbers associated with the volatiles in the loadings plot

	Number on PLS loadings plot	Volatiles	Explained variance (%)
Esters	7	Ethyl butyrate	91.3
	16	Ethyl Hexanoate	96.4
	12	Isoamyl Acetate	97.2
	18	Hexyl Acetate	94.7
	41	Diethyl succinate	98.4
	47	2-Phenylethyl Acetate	98.8
	22	Ethyl Lactate	96.6
	9	Ethyl-2-methylbutyrate	93.8
Acids	33	Propionic Acid	89.4
	37	Butyric Acid	93.4
	55	Octanoic Acid	93.3
	40	Isovaleric Acid	98.3
	48	Hexanoic Acid	95.8
Wood derived	30	Furfural	93.5
Alcohols	15	Isoamyl Alcohol	91.5
	11	Isobutanol	95.7
	53	2-Phenyl Ethanol	94.4
Carbonyl compounds	1	Acetaldehyde	96.3
	6	Diacetyl	93.2
Terpenes	34	Linalyl Acetate	91.7
	49	Geraniol	90.6
	54	B-Ionone	93.7

The PLS-DA model based on the regression of the twenty two significant volatiles against the Chenin blanc style (Table 3.7) was calculated using the calibration set and validated using full CV. The average misclassification rate of the PLS-DA model was found to be 10 % based on 3 LVs (Figure 3.4), which is quite a significant error, but not surprising for such a small sample set.

The test set was used to determine the classification powers of the PLS-DA model. The number of LVs used was based on the number of LVs used for modelling the calibration data set. The model was used to classify the remaining samples, which is identical to validating the model with a test set. The classification results showed that no misclassifications had been made for both the fresh and fruity and the wooded rich and ripe styles. However, there was a 5 % error rate for the rich and ripe unwooded Chenin blanc wines (Figure 3.5).

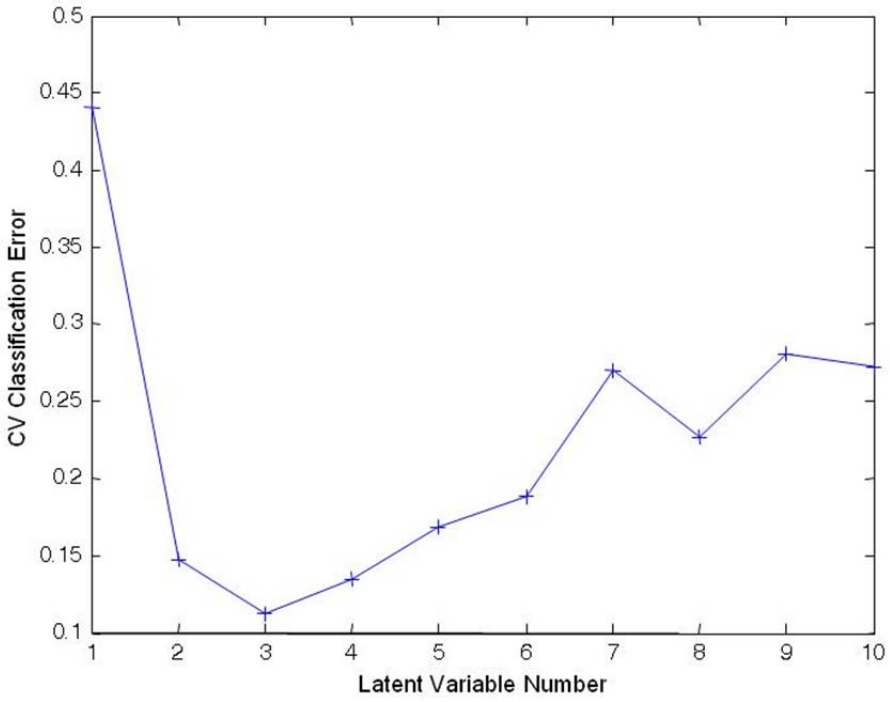

Figure 3.4 The PLS-DA classification results, illustrating that the CV error rate of the model is 10 % (0.10 on the plot) based on 3 Latent variables.

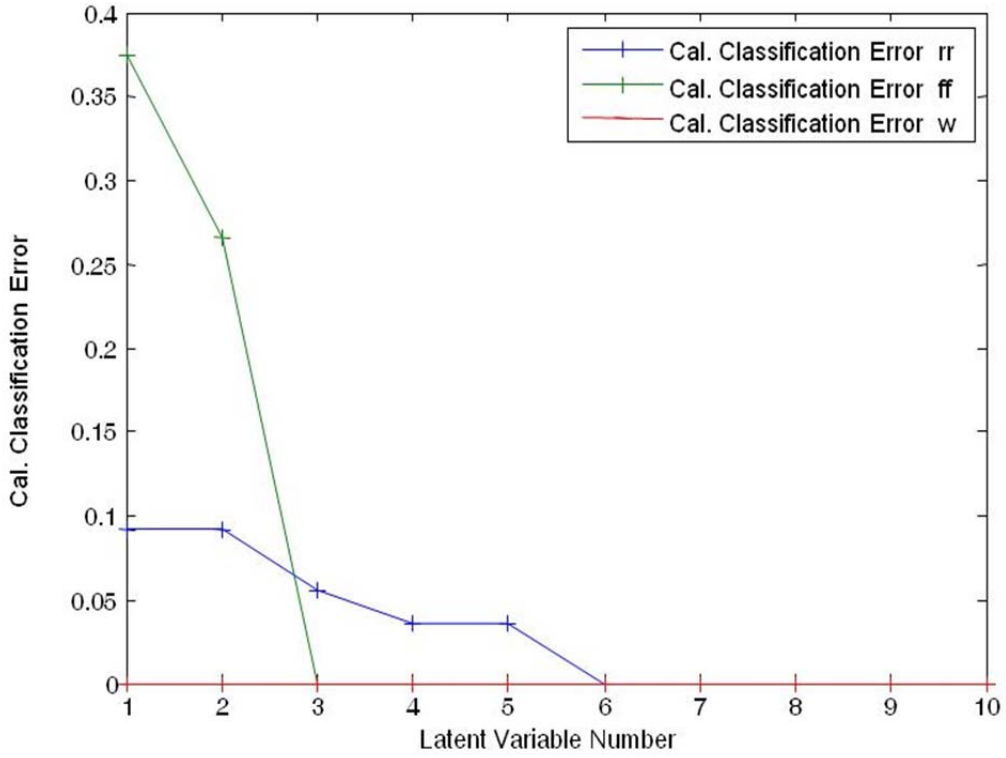

Figure 3.5 The PLS-DA classification results, illustrating that based on 3 LVs no fresh and fruity or wooded samples were misclassified. 5 % of the rich and ripe unwooded samples were misclassified.

The model was mainly able to predict the style of the remaining Chenin blanc samples, illustrated in Figure 3.6, showing good specificity in the model's ability to discriminate between the three styles. A slight overlap of the two unwooded styles is seen in Figure 3.6, which corresponds to the misclassification error. However, the model was clearly able to discriminate between the three styles. These results were seen as acceptable for further investigation of the scores and loadings.

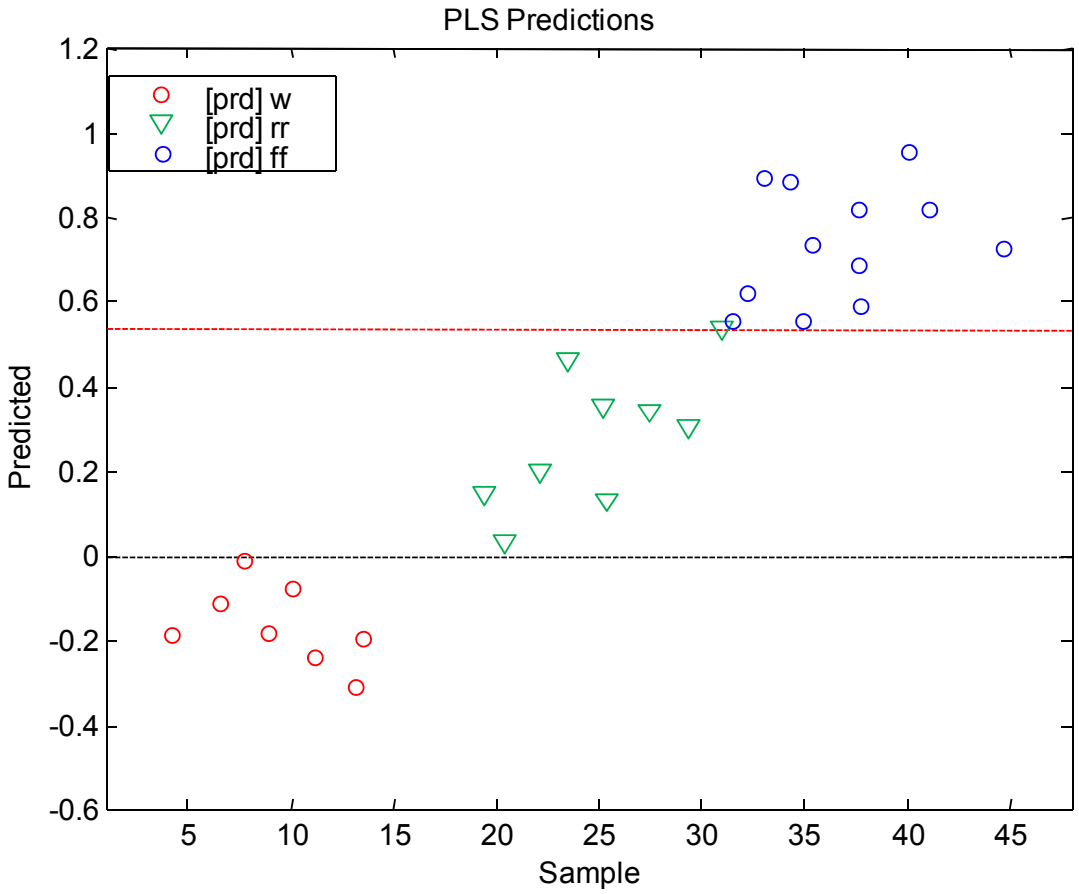

Figure 3.6 The predicted values for the styles of the remaining Chenin blanc wine samples (test set) showed that the model had good specificity in discriminating between the styles, with the small misclassification error seen by the overlap of the two unwooded styles. Fresh and fruity (top right-hand side, blue circles), rich and ripe wooded (lower left-hand side, red circles) and rich and ripe unwooded (green triangles)

PC1 and PC2 of the PLS-DA model explained slightly more of the variance in the data (49.8 %) than PC1 and PC2 of the PCA analysis (37.8 %) observed earlier. As illustrated on the scores plot, Figure 3.7 (a), discrimination between the three Chenin blanc styles was obtained, however as the sample set was very small, this is only an indication of the possibilities achievable using larger datasets.

Illustrated on loadings plot, Figure 3.7 (b) discrimination of the three styles was possible based on the twenty two most significant volatiles (numbering of the volatiles stated in Table 3.7). The two unwooded styles were mainly discriminated by the variables β-ionone and 2-phenylethyl acetate. The unwooded rich and ripe Chenin blanc wines were associated with higher content of β-ionone, while the fresh and fruity style were associated with higher content of 2-phenylethyl acetate. Higher content of ethyl hexanoate, ethyl butyrate and hexyl acetate was also associated with the unwooded rich and ripe Chenin blanc wines. These findings were in agreement with the earlier PCA results

The fresh and fruity wines were also correlated with higher content of isoamyl acetate, which agreed with the PCA observations in section 3.3.2.2. Furthermore, they were also associated with lower levels of acetaldehyde, ethyl lactate and furfural, which were the volatiles responsible for the discrimination of the fresh and fruity styles from the wooded rich and ripe wines. The wooded styles were associated with higher levels isoamyl alcohol and 2-phenyl ethanol, which were two of the volatiles responsible for the discrimination between the two rich and ripe wine styles. These findings agreed with the PCA observations that the wooded styles were associated with higher levels of ethyl lactate and fusel alcohols.

As the sample set used for the PLS-DA model was very small, this only gives an indication of the possibilities achievable using a larger dataset. The significant variables chosen for the model could be of interest for the future predictions of South African Chenin blanc styles.

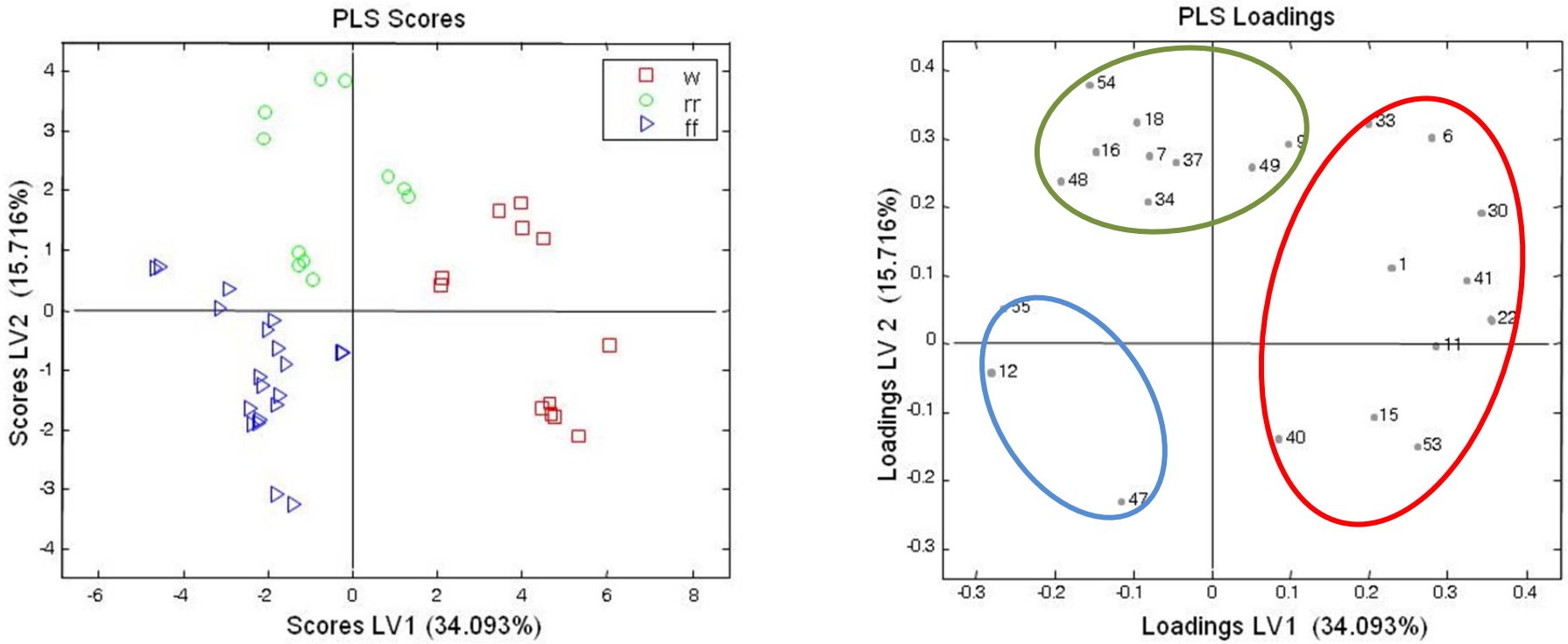

Figure 3.7 PLS scores plot (left frame) for classification of the Chenin blanc wine styles, fresh and fruity (ff), rich and ripe wooded (rr) and rich and ripe unwooded (rr). The loadings plot (right frame) illustrates that some of the main volatiles responsible for the discrimination of the three styles are β-ionone, ethyl hexanoate, isoamyl acetate, diethyl succinate and 2-phenylethanol (Numbering for the volatiles are given in Table 3.7).

The results from this study differed to some degree to results obtained in a related study on Chenin blanc styles by van Antwerpen (2011) where distinction between the three styles was not possible, based on very similar volatile compounds (Figure 3.8). The main differences between the present study and the previous work were the analytical methodologies employed.

During the previous study, four different analytical procedures were employed to analyse the content of the different compound groups in the wine samples. LLE using diethyl ether as extraction solvent in combination with GC-FID analysis was used to determine the major volatile content of the samples. The analysis of wood derived compounds was achieved by means of LLE using diethyl ether combined with GC-MS, while headspace solid phase micro-extraction (HS-SPME) coupled with GC-MS was employed for the analysis of carbonyl compounds. Lastly, solid phase extraction (SPE) was used together with another GC-FID method, for the analysis of terpenes.

As is clearly evident by the PCA scatter plot (Figure 3.8), although 72 % of the variation in the data was explained, no obvious style classification was possible using 8 volatiles deemed significant for style classification. While style classification was not possible using the previous data it was clear that higher content of diethyl succinate was correlated with the rich and ripe styles of Chenin blanc wines, while hexyl acetate, 2-phenylethyl acetate and isoamyl acetate were correlated with the fresh and fruity style Chenin blanc wines.

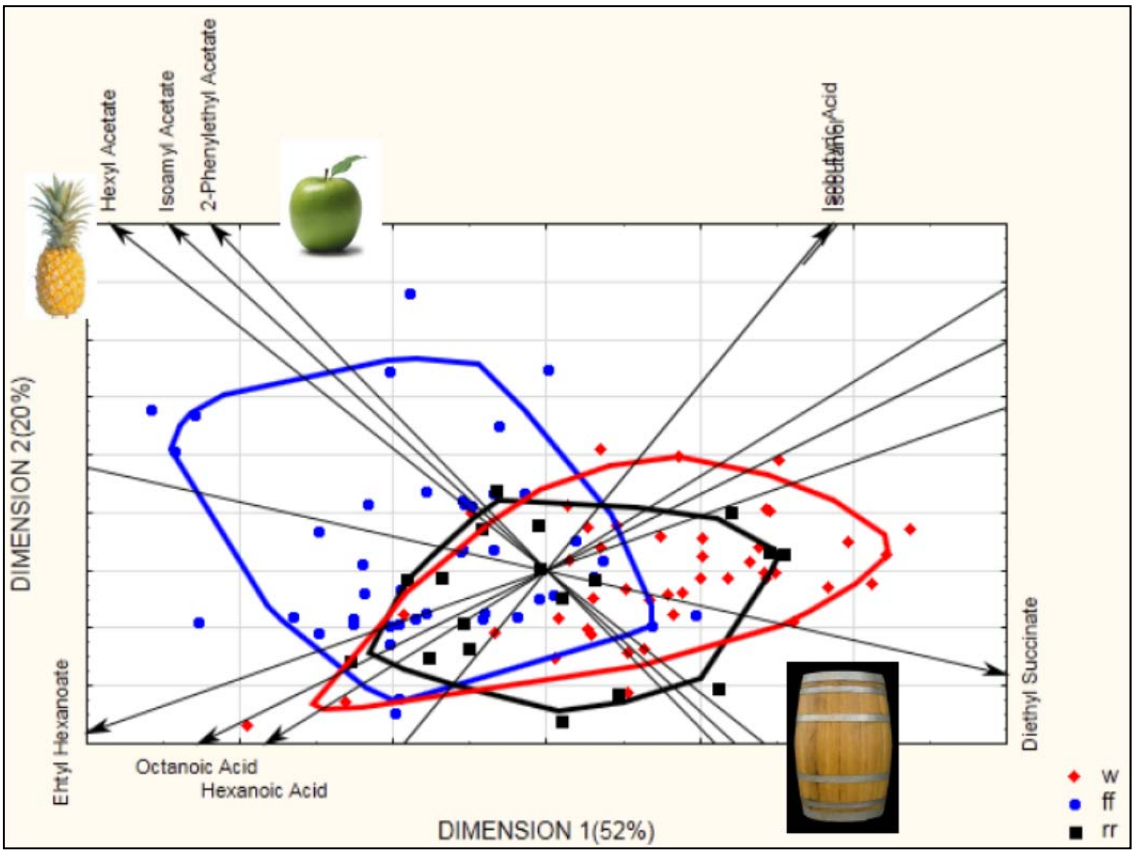

Figure 3.8 PCA scatter plot of 105 South African Chenin blanc dry and off-dry wines according to the volatile compounds which were determined as most descriptive of the styles. Where w (red, diamond) depict the wooded Chenin blanc wines, ff (blue, circle) are fresh and fruity and rr (black, squares) are rich and ripe unwooded (van Antwerpen, 2011).

3.3.2.4. Discriminant analysis

The technique DA Best Subset was applied to the data in order to classify the wines into their respective styles. The best subset model considers all possible combinations of variables to find the best set for predicting a variable. Generally good classifications were achieved between the styles, with classification rates between 93 % and 100 % using five variables per model. In the twenty most predictive DA models, three variables occurred in all of the models. These most significant volatiles for describing the three Chenin blanc styles were acetaldehyde, furfural and β-ionone. Ethyl-2-methylbutyrate occurred in seventeen of the models and was therefore also significant to the description of the three styles, as shown in the histogram in Figure 3.9.

The first two PCs of the biplot obtained using the DA determined significant volatiles, explained 70 % of the variance in the data, and is given in Figure 3.10. PC1 explained 40 % of the total variance while PC2 explained 30 %, which was the best representation of the explained variance of all the chemometric tools. Distinction between the styles was observed, although full

discrimination of all three styles was not obtained; as was the case with both the PCA style classification and the PLS style prediction models in sections 3.3.2.2 and 3.3.2.3, respectively.

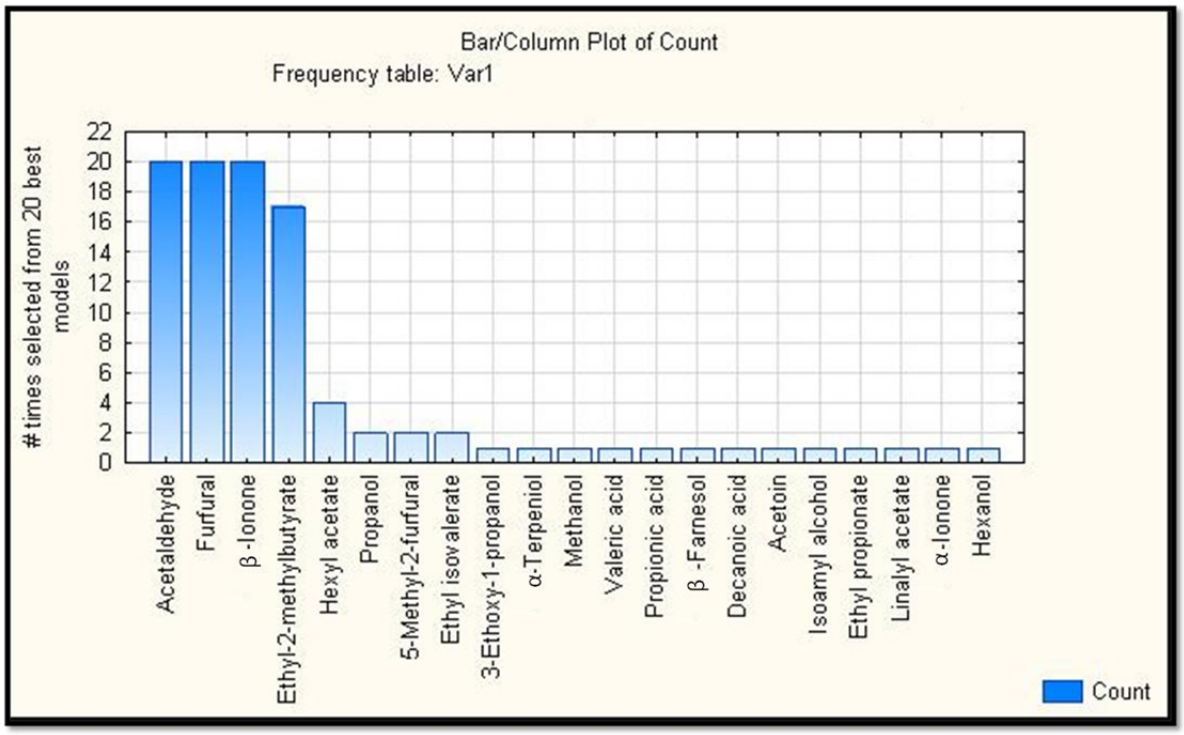

Figure 3.9 The significant volatiles for describing the styles were those which occurred in most of the DA models.

Important to note was that the rich and ripe wooded Chenin blanc style wines were associated with higher content of furfural and acetaldehyde. The rich and ripe unwooded wines were associated with higher β-ionone concentrations; while the fresh and fruity wines were correlated with lower content of each of the four volatiles determined by DA, which was in agreement with the other chemometric techniques.

The observations from the various chemometric techniques corresponded very well and this served as confirmation that style classification was indeed possible using the analysed volatiles. Important to note from the DA results was that the wood derived and maturation derived compounds were the most significant compounds in the discrimination between the wooded and unwooded styles, and some esters and terpenes were instrumental in the discrimination between the two unwooded Chenin blanc styles.

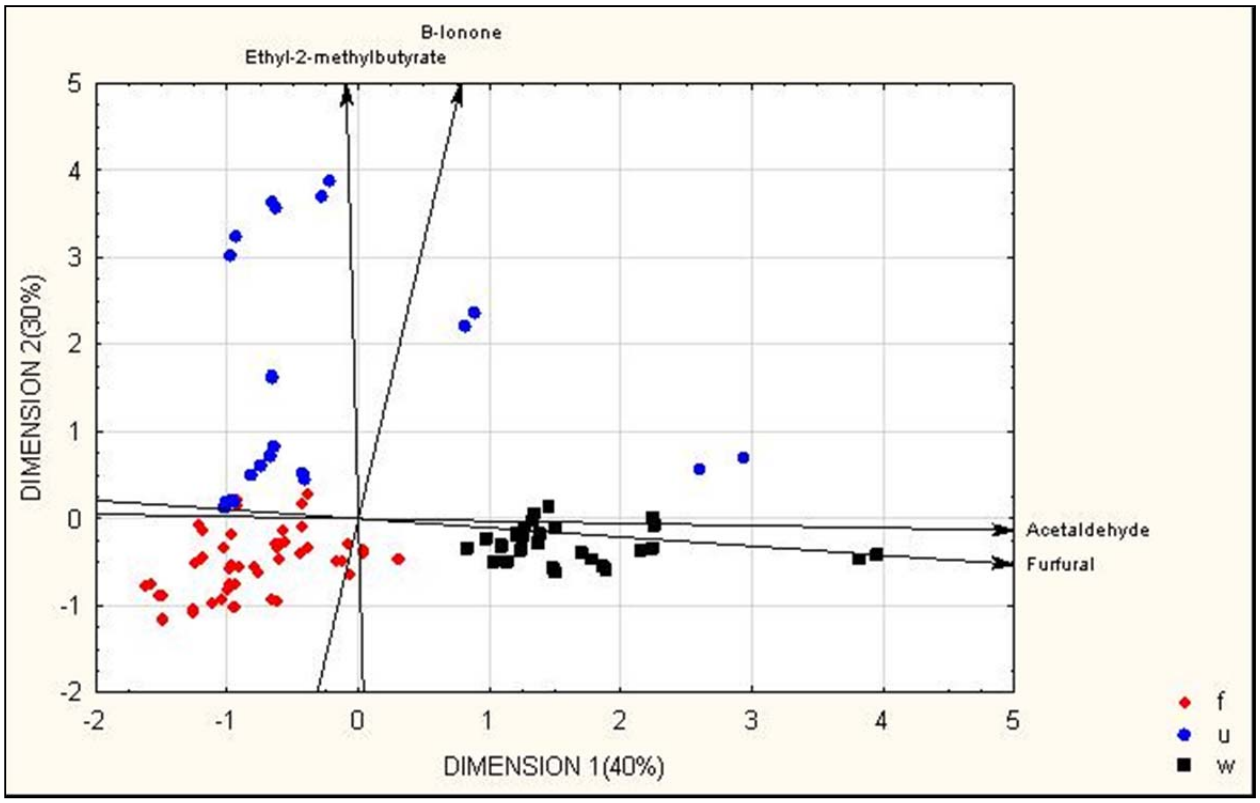

Figure 3.10 PCA scatterplot of the Chenin styles according to the four most significant volatiles. Where f (red, diamond is fresh and fruity, u (blue, circles) is unwooded rich and ripe and w (black, squares) is wooded rich and ripe.

3.4. CONCLUSIONS

During this study a rapid and simple method using LLE and GC-FID for the analysis of several different classes of volatiles in wine was developed and validated. This method allows for the identification and quantification of 57 volatile wine constituents, over a wide boiling range and polarity range, in less than 30 minutes. Validation of the method illustrated selectivity, sensitivity, repeatability and recovery of the analytes, suitable for the analysis of a wide range of wine volatiles.

The analytical method was successfully applied for the analysis of 48 commercial South African Chenin blanc wines, of three different styles, and volatile profiles of the three styles were achieved. The volatiles determined in the samples by application of the analytical method were, by means of chemometric tools, used to accurately classify the three Chenin blanc styles. Differences between the two unwooded styles were slightly less distinct than the differences between wooded and unwooded styles. The most significant volatiles involved in discriminating between the three styles were the wood and MLF derived compounds as well as the acetate esters.

This research contributes towards better understanding the differences between the volatile metabolic profiles of the South African Chenin blanc dry and off-dry wine styles. The volatile data have been added to the current wine aroma database for the benefit of the wine industry. In future, the developed analytical method, in combination with chemometrics, could possibly be applied to other wine styles, for classification and prediction as well as monitoring changes in volatile profiles over time.

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Chapter 4

Research results

Untargeted GC-MS analysis of South African Chenin blanc table wines and application of PARAFAC for resolution of complex data

This manuscript is under preparation to be submitted for publication in **Analytica Chimica Acta**

4. RESEARCH RESULTS

Untargeted GC-MS analysis of South African Chenin blanc table wines and application of PARAFAC for resolution of complex data

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ABSTRACT

Extensive research is focussed on metabolic profiling of wine and the use of untargeted gas chromatography mass spectrometry (GC-MS) to enable high throughput for the analysis of hundreds of analytes. However, data handling, processing and analysis of especially large volumes of data are often a great challenge. Multi-way analysis, specifically PARAllel FACtor Analysis (PARAFAC), is a powerful tool as it provides a means of building robust models from raw chromatographic data, which allows for the extraction of the most relevant information from the data. Furthermore, reliable and robust classification models can be built from the information obtained by PARAFAC, for example by using principal component analysis (PCA). Within this context, the aim of this work was to use PARAFAC to model raw gas chromatography mass spectrometry (GC-MS) wine aroma data, to correct for various complex phenomena, such as coelution and peak shifting. Additionally, PCA was used to discriminate between three South African Chenin blanc styles. The results demonstrated that the use of the proposed untargeted analytical and chemometric tools provided a good combination to extract relevant chemical information and aid in distinguishing the three styles by their unique volatile metabolic profiles.

Keywords: Chenin blanc, non-targeted analysis, mathematical chromatography, PARAFAC, wine aroma

4.1 INTRODUCTION

Chenin blanc, a grape variety that has its origins in the Loire valley in France, is thought to have been brought to South Africa by Jan van Riebeek in 1655 (Robinson, 2006). Today South Africa is the main producer of this varietal world-wide (Floris, 2011). It is an extremely versatile grape, adaptable to a diverse range of terroirs and as a result can be used in the production of many different styles of wines ranging from dry wooded and unwooded styles to sweet wines, sherries and brandies (Marais, 2003). The aroma and flavour of Chenin blanc can vary greatly between the different styles as well as within the styles, from floral, fresh and fruity flavours, to rich and ripe flavours associated with nuts, honey and spice (Smith, 2004). Chenin blanc was traditionally to a large extent used for the production of brandy. However, in the past decade, South African Chenin blanc has been receiving significantly more attention from both the wine industry and consumers (Brower, 2009), as this grape is extremely versatile it enables winemakers to produce a wide variety of different styles of wines, to satisfy the taste preferences of a variety of consumers.

Metabolic studies aim at the comprehensive analysis of a wide range of targeted or non-targeted small (< 1000 Da) molecules present in a sample (Olivier *et al.,* 1998). As these analytes differ significantly in abundance and physico-chemical properties, these types of studies require sophisticated analytical technologies. The ability to fingerprint samples in an untargeted manner has a significant potential for application within many fields. However, this approach has not been exploited to its full potential within wine analysis.

The analysis of volatiles and semi volatiles in the food and beverage industries is an on-going challenge, as these compounds determine the overall flavour and aroma perceived by the human sense of taste and smell. These attributes directly influence the perceived quality of the product (Monje *et al.,* 2002), and link to consumer preference and liking. A number of techniques are available for the identification and quantification of volatile compounds in wine (Ebeler, 2001; Reid *et al*., 2006), but very often gas chromatography (GC) in combination with mass spectrometry (MS) is used after suitable sample preparation. GC-MS offers high resolution and sensitivity as well as the possibility of tentatively identifying a large number of analytes by their retention times and mass spectra. But as there are such a large number of compounds in complex mixtures, it would be arduous to compare fingerprints manually. Furthermore, accuracy would be compromised due to coelution of peaks and retention time shifts.

The chemistry of wine has been thoroughly analysed, with compounds identified that are responsible for certain aromas and flavours of wine. Considering Chenin blanc, large gaps in our knowledge exist due to very limited number of research reports. As early as 1981 it was suggested, based on olfactory studies, that the guava-like aroma associated with Chenin blanc was due to the volatile thiol 4-methyl-4-mercaptopentan-2-one (4MMP), formed during fermentation (du Plessis and Augustyn, 1981). However, Ribereau-Gayon *et al., (*2000) suggested that the guava aroma in Chenin blanc could possibly be due to 3-mercaptohexyl acetate (3MHA). From this, the critical lack of chemical information regarding Chenin blanc is highlighted, since it cannot be concluded from these reports which compound, or combination of them, is responsible for this aroma attribute.

The honey aroma in white wine has been attributed to several C_{13}-Norisoprinoids (Marais, 1994), while other tropical and fruity aromas in wine are believed to be due to the presence of monoterpenes, norisoprenoids, thiols, higher alcohols and esters present above their respective odour activity values (OAV's) (Marais, 1998). Acetate esters are thought to be responsible for adding to the fresh and fruity characters of specifically young Chenin blanc wines (Marais and Pool, 1980).

Several publications spanning the past four decades involve the analysis of the volatile profile of Chenin blanc. However, very little attention has been paid to the analysis of Chenin blanc recently, and as a consequence, there is a significant gap in the knowledge of the unique volatile metabolic profiles of the different styles of Chenin blanc, which are produced lately.

With the escalating need for higher throughput analysis of samples, which requires lower chromatographic separation times, while still maintaining high resolution capabilities, there is a growing need to develop and optimise data handling methods to resolve complex chromatograms. This is especially significant with respect to slight shifts in retention times, baseline drift, peaks of low signal-to-noise, coeluting peaks and interference of background noise. These phenomena are graphically illustrated in Figure 4.2. Traditionally one or more internal standards have been used to aid in indicating problematic areas in the chromatogram. However, in cases of severe baseline drift or coelution of analytes, the capabilities of internal standards are limited (Amigo *et al*., 2008).

As a number of mass spectra are recorded across the width of each peak during the GC-MS analysis, the data from hyphenated techniques, such as GC-MS can be considered to be three-way data. Traditional software typically used for data analysis of chromatographic data

is not equipped to extract all the relevant information obtained from such complex three-way data. If coelution is extremely severe, traditional software would fail to identify the underlying analytes, despite the mass spectral data. Coeluting analytes with very similar or the same dominating mass fragments would also pose a problem for traditional data handling software (Skov and Bro, 2008). Rearranging multi-way arrays into two-way datasets and analysing them using traditional software thus inevitably result in loss of information and misinterpretation, especially with noisy, complex data (Acar and Yener, 2007).

One of the most well-known and commonly applied multi-way models for multidimensional chemical data is PARAllel FACtor Analysis (PARAFAC) (Harshman, 1970; Carroll and Chang, 1970). This method is an extension of principal component analysis (PCA) and has been shown to be able to model complex chromatographic data, yielding both underlying qualitative and quantitative information (Bro, 2006; Skov and Bro, 2008) and is often referred to as “mathematical chromatography” (Skov and Bro, 2008). Unlike in PCA, where the underlying features are identified using orthogonality constraints, in PARAFAC unique estimates for a single analyte in chromatograms and spectra can be obtained from coeluting analyte peaks, as long as the data do not deviate from the trilinear structure of the model (Skov and Bro, 2008). The three-way dataset in PARAFAC is decomposed into three loadings vectors, which represent the samples (concentration), elution time profile and mass spectral profile, respectively.

However, if analyte peaks shift between analytical runs, the trilinear structure of the data is no longer adhered to. In this case PARAFAC would no longer be a suitable technique to model the data. This led to the development of PARAFAC2, which is a less restrictive advancement of PARAFAC able to model the elution time profiles from different chromatographic runs individually, and has been illustrated to be able to handle such phenomena (de Juan and Tauler, 2001; Skov and Bro, 2008; Amigo *et al.,* 2008). Much like PARAFAC, PARAFAC2 also decomposes the three-way data array into three loading matrices, however, PARAFAC2 does not assume that each elution time profile in individual analytical runs are identical (de Juan and Tauler, 2001). Applying PARAFAC and PARAFAC2 on hyphenated chromatographic data has been reported increasingly in recent years (de Juan and Tauler, 2001; Skov and Bro, 2008).

PARAFAC is not intended to be used for detailed studies of the evolution of each metabolite over time; however, it can be used to determine which compounds change between different stages of for example various treatments, and to extract vital information on the correlation of compounds in complex datasets.

This study aims to demonstrate the suitability of using PARAFAC and PARAFAC2 to model untargeted volatile GC-MS data. Here mathematical chromatography is applied to South African Chenin blanc samples, with the resulting data used to derive preliminary classification models that could be used for future chemical identification of the three South African Chenin blanc dry and off-dry styles fresh and fruity, rich and ripe unwooded and rich and ripe wooded.

4.2. MATERIALS AND METHODS

4.2.1. Wines and samples

Twenty-one commercial South African Chenin blanc wine samples from various wine estates in the Western Cape region were selected to be representative of the fresh and fruity, rich and ripe unwooded and rich and ripe wooded styles, and were also sourced from different producing cellars, to include diverse geographic origins. The style designations are provided on the bottle labels and were verified by personal communication with the respective winemakers. All wines had above average to exceptional industry quality ratings. Table 4.1 gives a detailed description of the sample set.

For chromatographic analysis, 21 Chenin blanc samples, 3 blank samples (containing only internal standard in wine stimulant), and 3 additional samples; containing a mixture of an equal part of each of the 21 wines; were used.

4.2.2. Chemicals

HPLC grade absolute ethanol, methanol and dichloromethane as well as sodium sulphate (anhydrous), ammonium sulphate and tartaric acid were purchased from Merck (Darmstadt, Germany). Water was purified by a Milli-Q purification system (Millipore, Bedford, MA, USA). The internal standards 4-methyl-2-pentanol and 2,6-dimethyl-6-hepten-2-ol were dissolved in synthetic base wine simulant (0.5 mg L^{-1} and 0.1 mg L^{-1} respectively).

4.2.3. Synthetic base wine simulant

The synthetic base wine simulant was prepared as described by Louw *et al*., (2009) and consisted of 2.5 g L^{-1} tartaric acid from Merck (Darmstadt, Germany) and 12 % (v/v) ethanol dissolved in purified water (Millipore, Billeric, MA, USA). The pH was adjusted to 3.5 using 0.1 M sodium hydroxide (Merck, Darmstadt, Germany).

Table 4.1 List of the 21 Chenin blanc table wines analysed by GC-MS and modelled by PARAFAC.

Style	Winery name	Vintage	Geographic region
Fresh and fruity	De Krans	2010	Calitzdorp
	Eagle's Cliff	2010	Breede River
	Groot Parys Die Tweede Droom Ongehout	2010	Paarl
	Slanghoek	2009	Rawsonville
	Stellar (Organic)	2010	Klawer
	Raats	2010	Stellenbosch
	Kleine Zalze (Bush Vine)	2010	Stellenbosch
Rich and ripe unwooded	Conradie	2009	Worcester
	Hawksmoor	2008	Paarl
	Landskroon	2010	Paarl
	Dornier	2010	Stellenbosch
	Le Pommier	2010	Stellenbosch
	Douglas Green-Vinyard Creation	2010	Wellington
	Bochendal	2010	Franschoek
Rich and ripe wooded	Lammershoek	2009	Malmesbury
	Kanu KCB	2007	Stellenbosch
	Katbakkies	2008	Stellenbosch
	Simonsig Avec Chene	2009	Stellenbosch
	Perdeberg Rex Equus	2008	Paarl
	Jordan Barrel Fermented	2009	Stellenbosch
	Graham Beck Bowed Head	2009	Franschoek/Stellenbosch

4.2.4. Extraction procedure

Two and a half grams of ammonium sulphate was added to 15 mL culture tubes (Pyrex, Milian, USA). Five milliliters of wine and 100 µL of the internal standard solution (0.5 mg L^{-1} 4-methyl-2-pentanol and 0.1 mg L^{-1} 2,6-dimethyl-6-hepten-2-ol in synthetic wine) were extracted using 1 mL of dichloromethane by sonicating for 1 hour, shaking every 10 minutes. The wine/extraction solvent solution was then centrifuged at 3000 rpm for 30 minutes and the dichloromethane layer (top layer) was removed and dried on anhydrous sodium sulphate. Each wine sample was extracted in triplicate and injected into the Hewlett Packard 6890 GC-MS instrument equipped with a CTC PAL multipurpose auto sampler into a split/splitless injector (Agilent, Little Falls, USA).

4.2.5. GC-MS conditions

A Teknoram TRB-FFAP capillary column (AIT France, Houilles, France) with the dimensions 60 m length by 0.25 µm internal diameter and 0.25 µm film thickness was used. Helium gas was used as carrier gas in the constant flow of flow rate 1.5 mL min^{-1}. Three µL of each sample was injected at 250 ºC in split mode (18:1 at 27 mL min^{-1}). The initial GC oven temperature was set at 40 ºC and held for 5 minutes after which the temperature was

ramped to 225 ºC with a heating rate of 8 ºC min^{-1} and held for 1.5 minutes. Thereafter the oven was heated to 240 ºC and held for a 5 minute post run, and thereafter cooled to the initial temperature. The electron impact energy was 70 eV and the MS source and transfer line were maintained at 250 ºC and 230 ºC, respectively. Acquisition was performed in scan mode, scanning between 35-350 *m/z* at a rate of 4.45 scans sec^{-1}.

4.2.6. Multi-way analysis

PARAFAC modelling of raw GC-MS data, in .cdf format, was imported into MATLAB (version 7.9.0.529, R2009b, Mathworks Inc., USA) using the iCDF function in the software (Skov and Bro, 2008). By making use of the PLS Toolbox (Wise *et al*., 2006), which runs in MATLAB, PARAFAC and PARAFAC2 models were obtained.

The large quantity of numerical data present in all the GC-MS chromatograms would surpass the limits of the computational resources required to compute one basic PARAFAC model. Moreover, as there is always noise present, and PARAFAC is a low rank model, this would not be viable in practice. To address this problem, the chromatograms were divided into smaller sequential segments of the original GC-MS data cube, each containing a set number of components, and therein the mass spectra. This can be envisaged as executing a set of PARAFAC local models, across the retention time axis.

Non-ideal experimental behavior, such as noisy data, hinders the optimisation step in PARAFAC model fitting. In these cases, constraints based on chemical information such as non-negativity of mass spectra (and elution time profiles), were used to ensure they are chemically and physically accurate.

PCA classification models were obtained up by importing the PARAFAC and PARAFAC2 scores data into LatentiX (version 2.00, www.latentix.com, Latent5, Copenhagen, Denmark), to evaluate the underlying trends in the data and to see whether the stylistic differences could be detected.

4.3. RESULTS AND DISCUSSION

The GC-MS method was set up based on the GC-FID method previously developed, validated and described in Chapter 3 of this work.

The Chenin blanc samples as well as 3 blank samples (containing only internal standard in wine stimulant), and 3 additional samples, containing a mixture of an equal part of each of the 21 wines, were prepared using the extraction method described in section 4.2.4. The blank samples were injected to detect carry over between runs and the mixed samples were prepared so that the method could be tested with respect to all possible analytes in the total wine profile.

The sequence of analysis was set up in a manner that each sample was injected three times but not consecutively. Instead, the sequence was such that the 21 Chenin blanc samples, one mixed sample and one blank sample were each injected once, with this sequence of events repeated thrice, in varying random order. This was to ensure that no systematic error, due to sample order, would bias the resulting data (Lai, 2011).

The three-way data was unfolded into a two-way array by summing the intensities of all the mass spectral fragments per elution (TIC – Total Ion Count chromatogram). The PCA performed using all the samples, including the mixed and blank samples, explained approximately 50 % of the variance in the first 2 principal components (PC's). From the PCA scores plot, Figure 4.1, it is clear from the almost complete overlay of the mixed samples and close positioning of the blank samples that the instrument had not been subject to significant systematic error over time. This served to provide a platform for extracting valid information from subsequent chemometric investigations.

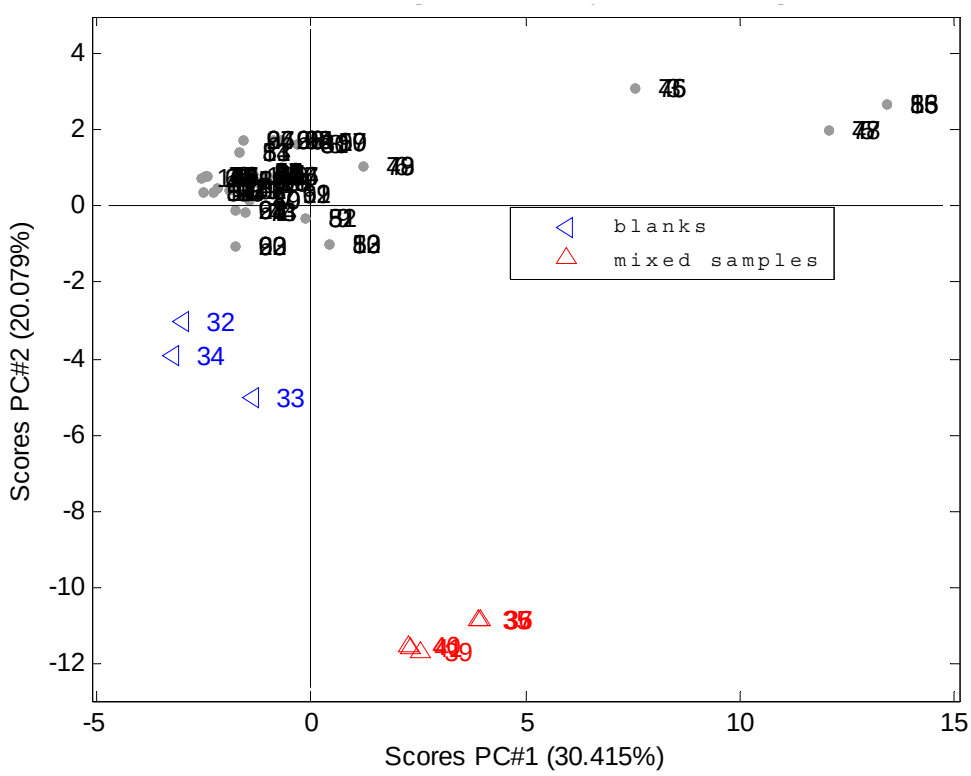

Figure 4.1. A PCA scores plot of the all samples (grey dots), including the blanks (blue, left pointing triangles) and mixed samples (red, upward pointing triangles) illustrates that the mixed samples and blanks are plotted in close proximity.

The (overlaid) TIC representative of the Chenin blanc samples are shown in Figure 4.2. As illustrated, there were a large number of peaks (55 peaks in total) and their respective sizes (area or height) varied significantly in the profiles. The various phenomena mentioned before were observed throughout the chromatogram, including (Figure 4.2 (a)), coelution (b) peak shifting, (c) low signal-to-noise peaks as well as (d) some baseline drift at the end of the chromatographic run, due to the temperature ramp.

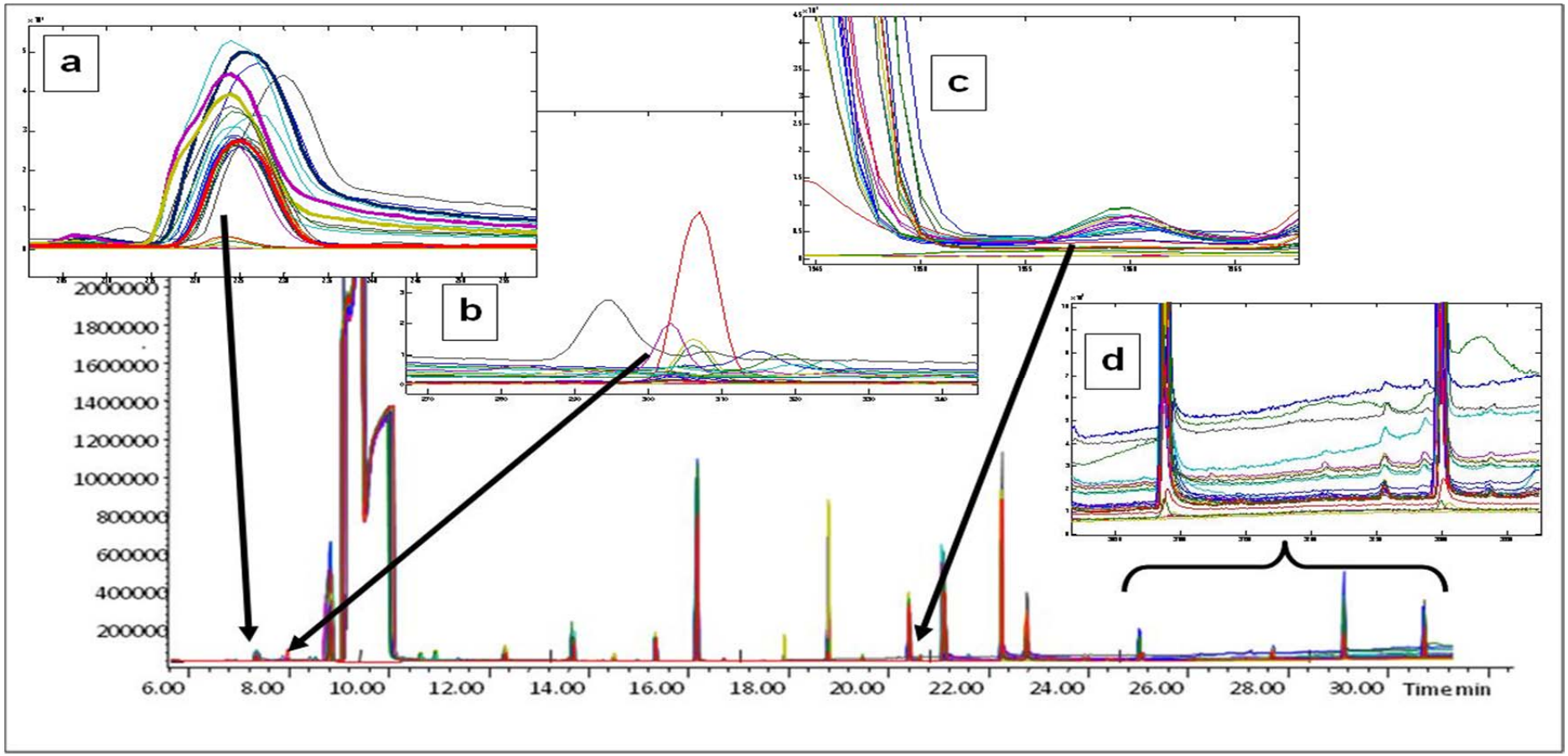

Figure 4.2. The GC-MS Total Ion Count chromatogram (TIC) of the raw data exhibited various phenomena, including (a) co-eluting peaks, (b) shifting peaks, (c) low signal-to-noise peaks as well as (d) base-line shifting due to the ramping up of the temperature at the end of the chromatographic run.

The chromatograms of the samples and mixed samples were divided into smaller data cubes, to make local models, using either PARAFAC or PARAFAC2. As previously mentioned, PARAFAC is not able to handle shifting in any direction, so peak shifting and baseline drift were handled using PARAFAC2. However, both PARAFAC and PARAFAC2 models were investigated throughout, for best possible models, consisting of the correct number of components. There are a number of ways to determine the best possible number of components; some of these include examining the residuals and the core consistency which should be above 90 % (Bro, 2010). The optimal number of components would incorporate the baseline as well as the analyte(s) within that particular local model.

When peaks slightly or completely overlapped, the loadings mode for the mass spectra was investigated to determine whether there was a case of coelution, retention time shift, or possibly both. This was done by looking at the three component PARAFAC model; for instance if there were two peaks (plus a baseline) present in a three-component PARAFAC model in the retention loadings, then the mass spectral loadings were checked as well. If the mass spectral components overlaid exactly, then two of the components were modelling the same analyte, which would indicate a peak shifting scenario. However, validation of the assumption of peak shifting was imperative, and therefore PARAFAC2 was used to model the same data cube. This combination of PARAFAC and PARAFAC2 served to cross-check the number of analytes in a region.

Some unconstrained models resulted in mass spectral profiles with small negative values. These negative values did not affect the analyte peak shape or position, but using constraints like non-negativity and/or unimodality aided in ensuring the final results were meaningful, chemically speaking. Therefore, some of the models had some constraints.

Once each sequential section of the chromatogram had been modeled accurately using PARAFAC or PARAFAC2, the scores values were captured, normalised by dividing the scores values by the scores values of the internal standard and transferred to LatentiX for further visualization and analysis.

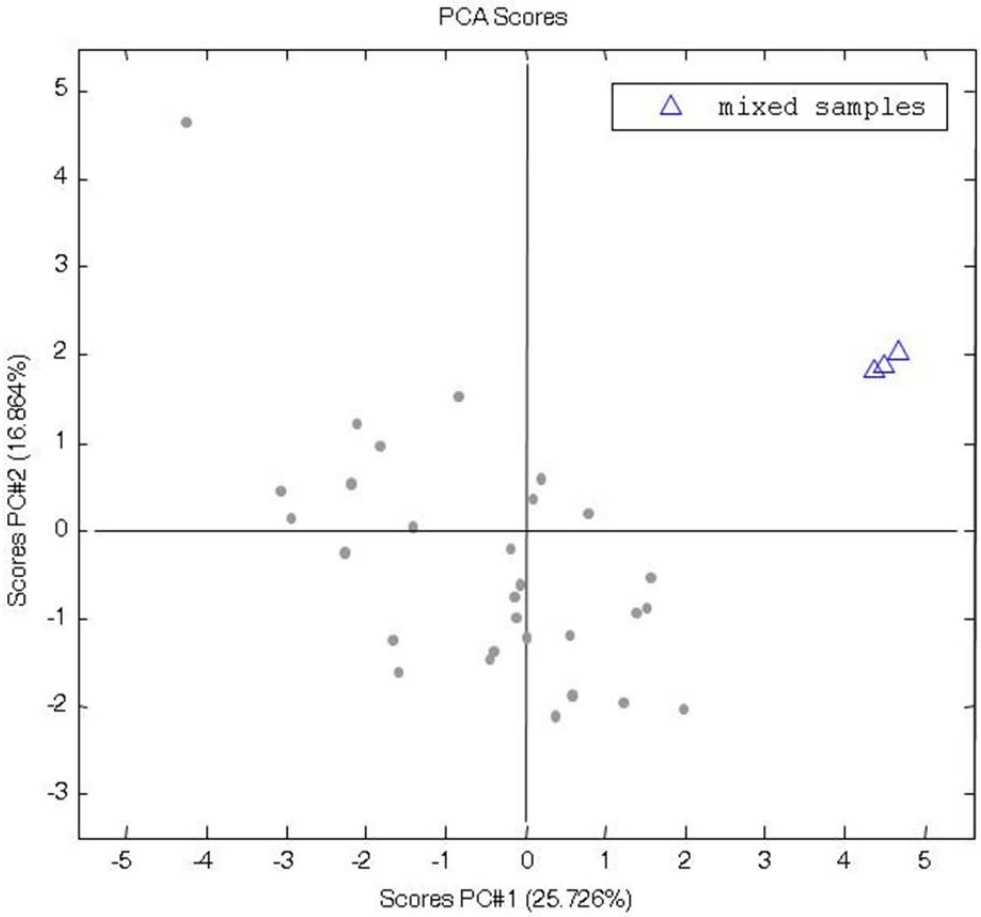

Figure 4.3 A PCA scores plot of the wine sample scores (grey dots) and mixed sample scores (blue, triangles) shows that the mixed samples were in very close proximity to one another.

Subsequently, a PCA of the normalised scores data of the wine samples and mixed samples was performed, shown in Figure 4.3. The mixed samples were situated closely together on the scores plot (blue triangles), which validated that the PARAFAC model was performing optimally in this case. Thereafter the scores for the mixed samples were removed so that the classification of styles could be performed.

The principal variables were calculated using LatentiX, and the 15 principal variables (volatiles) that explained 90 % of the total variation were selected for the style classification. This selection of principal variables was done to eliminate any over fitting of the data (Myatt and Johnson, 2009).

A PCA plot was obtained of the normalised scores data of the wine samples only, shown in Figure 4.4. PC1 and PC2 of the PCA analysis explained approximately 37 % of the variance in the data, and while the styles were somewhat discriminated between, there was some

overlap of the two unwooded styles, as seen on the scores plot, Figure 4.4 (a). Although normalisation using the internal standard should solve any inconsistencies relating to the amount of sample being injected onto the column, this approach depends entirely on the score of the internal standard peak which could also be subject to various errors, such as for example pipetting error. Therefore an additional normalisation step known as standard normal variate (SNV) was also tried, to eliminate any undesirable noise in the data. SNV has previously been used successfully for noise correction of chromatographic data (Li *et al.,* 2004; Sajewicz *et al.,* 2012).

The resulting PCA from the normalised and SNV transformed wine data scores, which described approximately 45 % of the variance in the data on PC1 and PC2, clearly distinguished between the three different styles, as seen on the scores plot in Figure 4.5 (a). As the analysis was untargeted, the variables were at this stage unknown and therefore labelled P (Peak) 1-P55, as seen in Figure 4.5 (b).

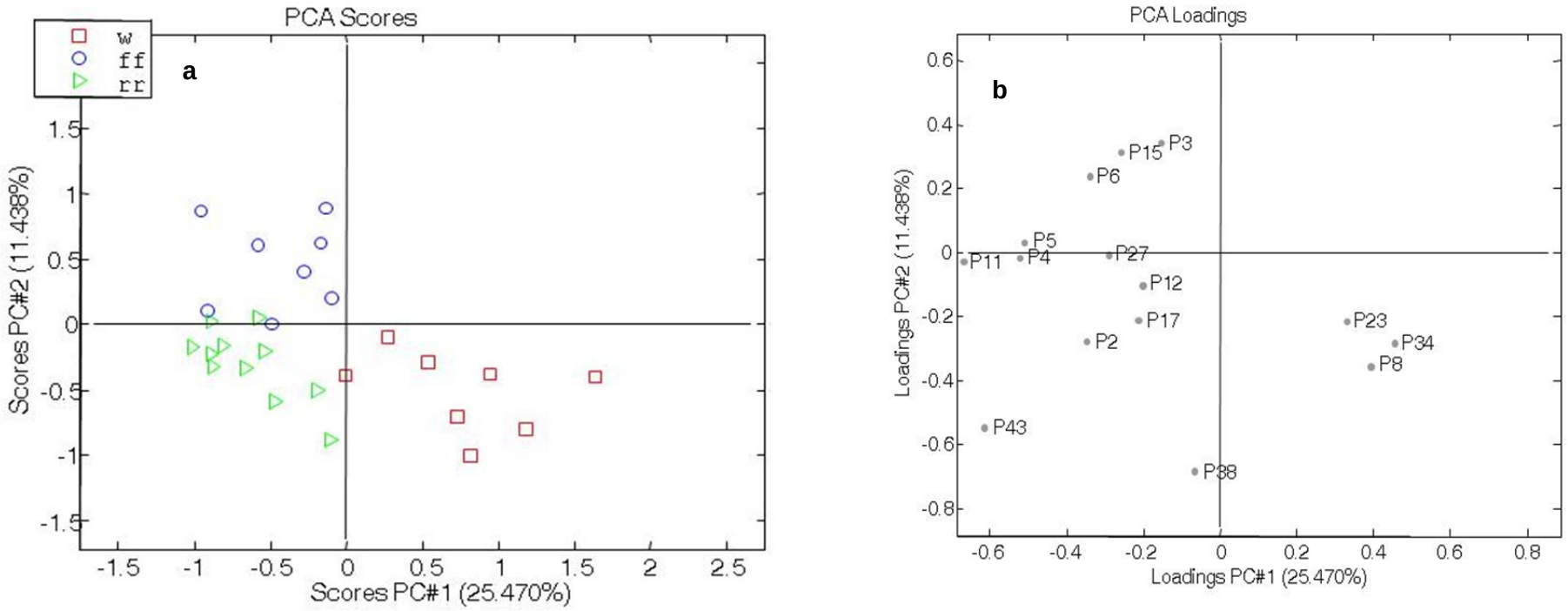

Figure 4.4 PCA of the scores values for the wine samples achieved by PARAFAC and PARAFAC2, normalised only by the internal standard. The scores plot (a) illustrated that there was some overlap of the two unwooded styles. The classification was based on the 15 principal volatiles in the loadings plot (b) that explained 90% of the total variation in the data. Note that this variation is not the variation explained by the 2 PC's in the PCA plot. Rich and ripe wooded (red, squares), fresh and fruity (blue, circles), rich and ripe unwooded (green triangles). The P on the loadings plot denotes the *peaks* in the chromatograms.

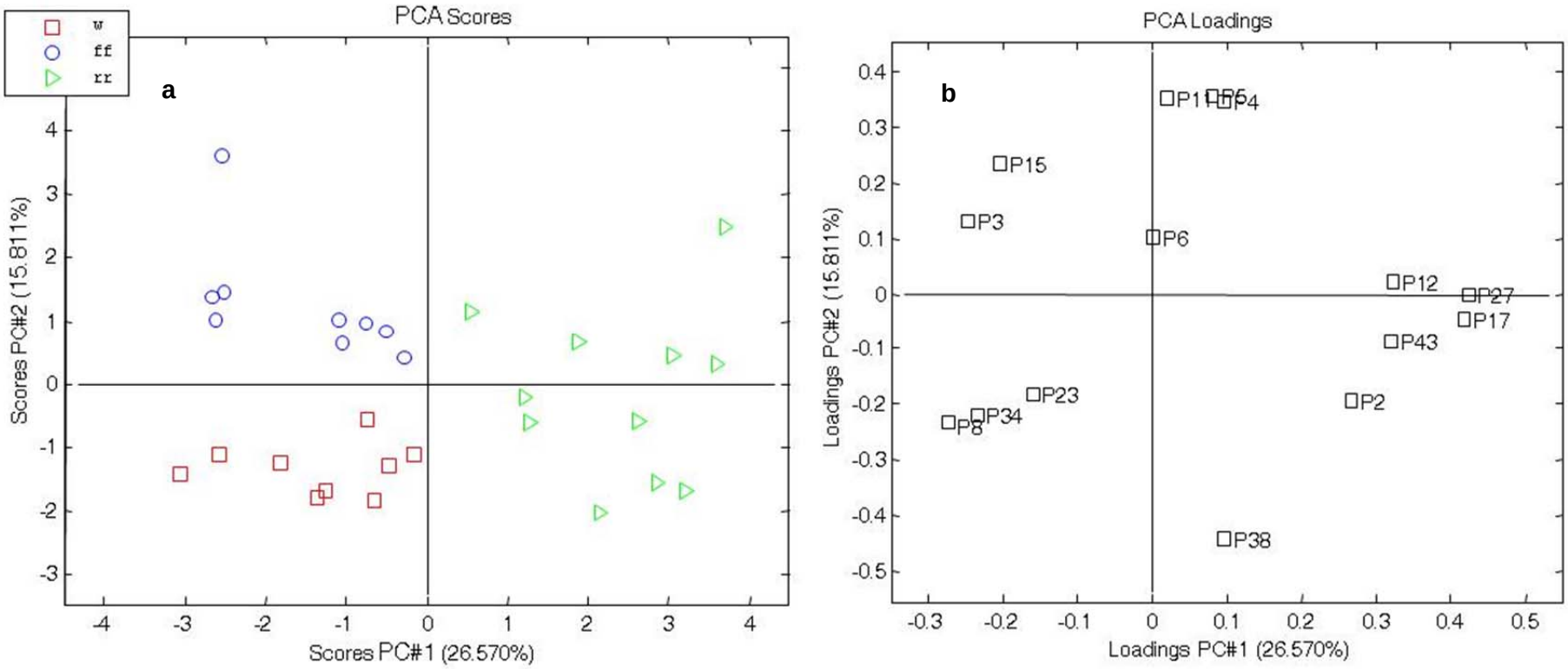

Figure 4.5 PCA of the normalised and SNV transformed scores values for the wine samples, achieved by PARAFAC and PARAFAC2. The PCA scores plot (a) illustrates that SNV transformation was necessary for PARAFAC to fully distinguish between the three styles based on the 15 volatiles in the loadings plot (b) that explained 90% of the total variation in the data. Note that this variation is not the variation explained by the 2 PC's in the PCA plot. Rich and ripe wooded (red, squares), fresh and fruity (blue, circles), rich and ripe unwooded (green triangles).

The top and bottom right hand side of the loadings plot, Figure 4.5 (b) contain the variables that were most correlated with the rich and ripe unwooded styles of Chenin blanc. These volatiles were tentatively identified by their mass spectral loadings, (by comparisons to the NIST database (Linstrom and Mallard, 2000), spectra shown in Figure 4.6). Significant volatiles were (Figure 4.6 (a) (P12)) ethyl hexanoate, which lends wine a fruity aroma of apples, strawberries and anise (Swiegers *et al.,* 2005) above 14 µg L^{-1} (Cullere *et al.,* 2004) and (Figure 4.6 (c) (P17)) 2-methyl-1-butanol (active amyl alcohol), which exhibits banana and fine fruit aromas as well as rich marzipan notes when present above 65 mg L^{-1} (Rocha *et al.,* 2006; Meilgaard, 1975; Wondra and Berovic, 2001).

These results correlate well with results obtained earlier in Chapter 3 of this work, where the unwooded styles were associated with higher content of ethyl hexanoate. The findings also agree well with results obtained recently by sensorial analysis of the rich and ripe unwooded Chenin blanc styles, which exhibited tropical and ripe fruit aromas (Bester, 2011). However, during the latter study it was not possible to discriminate between the two unwooded styles (fresh and fruity and rich and ripe unwooded) on a sensory basis. Nonetheless, separation of the wooded and unwooded styles was achieved.

The variables most correlated with the wooded style were found in the lower left quadrant of the loadings plot; Figure 4.5 (b). Upon comparing the mass spectral loadings from the PARAFAC model, to mass spectra from the NIST database (Linstrom and Mallard, 2000) they were tentatively identified as (P-34, Figure 4.7 (a)) diacetyl (2,3-butanedione), (P-23, Figure 4.7 (c)) ethyl lactate (propanoic acid, 2-hydroxy, ethyl ester) and (P-8, Figure 4.8 (a)) acetaldehyde. The mass spectra comparisons are depicted in Figure 4.7 and Figure 4.8. Diacetyl and ethyl lactate are known to be present at higher concentrations in wines that have undergone some malolactic fermentation activity (Bartowsky and Henschke, 1995). In South Africa, the wooded Chenin blanc styles are normally preferred to undergo some malolactic fermentation (O'Kennedy, 2009), but not the unwooded styles. When present at low concentrations diacetyl is known to contribute positive aromas of nutty, toasty and contributes to the complexity of the wine; however at higher concentrations the buttery, butterscotch aroma can become overpowering (Martineau and Henick-Kling, 1995). Although ethyl lactate is generally not considered to contribute to the aroma of wine, it has been found to before to lend raspberry and lactic nuances to some white wines (Tominaga *et al.,* 1998). Acetaldehyde is produced by yeast during fermentation (Cheynier, 2005) although fairly low amounts remain (3-30 mg L^{-1}) at the end of alcoholic fermentation (Schneider, 2009). Usually the presence of acetaldehyde in white wines is indicative of oxidation

(Bartowsky and Pretorius, 2009) but it is typically observed in wines matured in wooden barrels (Schneider, 2009).

The above findings correlate well with findings by Bester (2011) with regards to the sensorial descriptors of the styles. In this previous and related study it was illustrated that the wooded styles were associated with aromas of toasty, butterscotch and nutty. Using various classification techniques, discrimination of the wooded from unwooded styles only was found to be possible, based on sensorial attributes only. It was therefore suggested that there were two main styles of dry and off-dry South African Chenin blanc, wooded and fresh and fruity (Bester, 2011).

The variables that were highly correlated with the fresh and fruity styles are found in the top left quadrant of the loadings plot, Figure 4.5 (b). Upon comparing the mass spectral loadings from the PARAFAC model, to spectra in the NIST database (Linstrom and Mallard, 2000), they were identified as (P6, Figure 4.9 (a)) isoamyl acetate (1-butanol, 3-methyl-, acetate) and (P15) isobutyl acetate, illustrated in Figure 4.9 (c). Isoamyl acetate is ubiquitous to young, fresh white wines and confers aromas of apple and pear, as well as tropical aromas of banana and melon. Isobutyl acetate has been reported to lend complexity to high quality white wines as well as a fruity and tropical aroma of banana and figs (Killian and Ough, 1979, Bakker and Clarke, 2004; Simpson and Miller, 1983) and has been noted as one of the most important acetate esters in wine (Lambrechts and Pretorius, 2000).

These results were in agreement with the results obtained by sensorial analysis for the fresh and fruity styles, where the style was associated with aromas of tropical fruit and apples (Bester, 2011). However unlike in that sensory study, where separation of the two unwooded styles was not possible, this current work shows that classification of all three Chenin blanc styles is possible by means of mathematical chromatography. Additionally this work has indicated that in future, for the classification of specifically these three Chenin blanc styles, it may not be necessary to use more than the most significant variables for the analyses.

When performing untargeted chromatographic profiling, extremely complex multidimensional datasets are obtained. Multivariate data analysis is required to make correlations and identifications of components in the dataset. This approach can aid researchers in correlating larger ranges of metabolites, without need for prior assignment of the chemical structures. This is time saving as it removes the necessity for using hundreds of reference standards during initial stages of analysis. However, confirmation of any identifications made

of interesting compounds, would be necessary to be absolutely sure of biological and chemical meaning of correlations found.

Applying untargeted analysis of wine volatiles would aid in high throughput analysis of samples and aid in rapidly analysing trends or interesting changes during the various stages of winemaking as well as potentially identifying new wine volatiles.

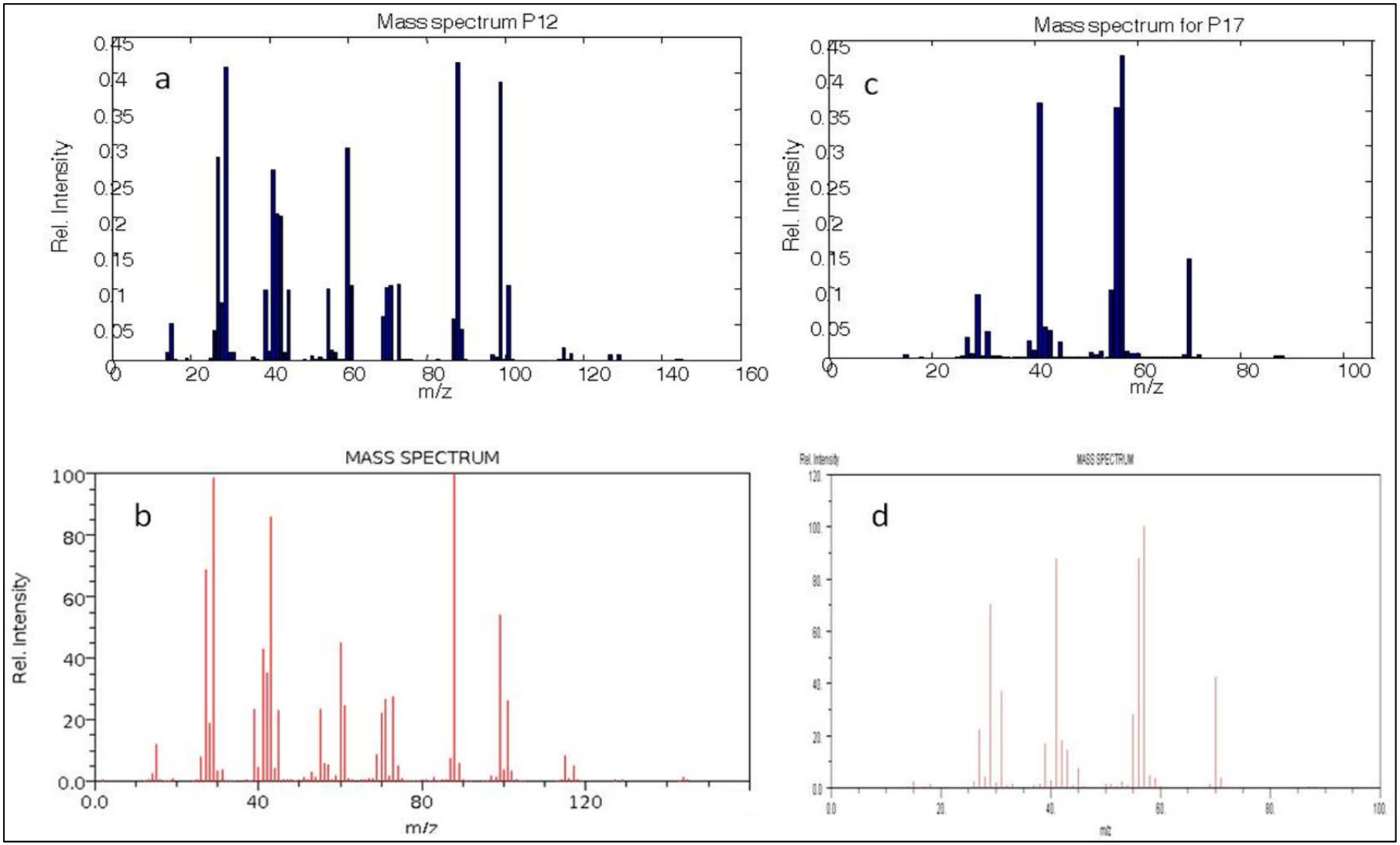

Figure 4.6 (a) The mass spectral loadings from the P12 PARAFAC model corresponded to spectra in the NIST database for (b) ethyl hexanoate, while the mass spectral loadings from (c) the P17 PARAFAC model corresponded with (d) 2-methyl-1-butanol in NIST (Linstrom and Mallard, 2000)

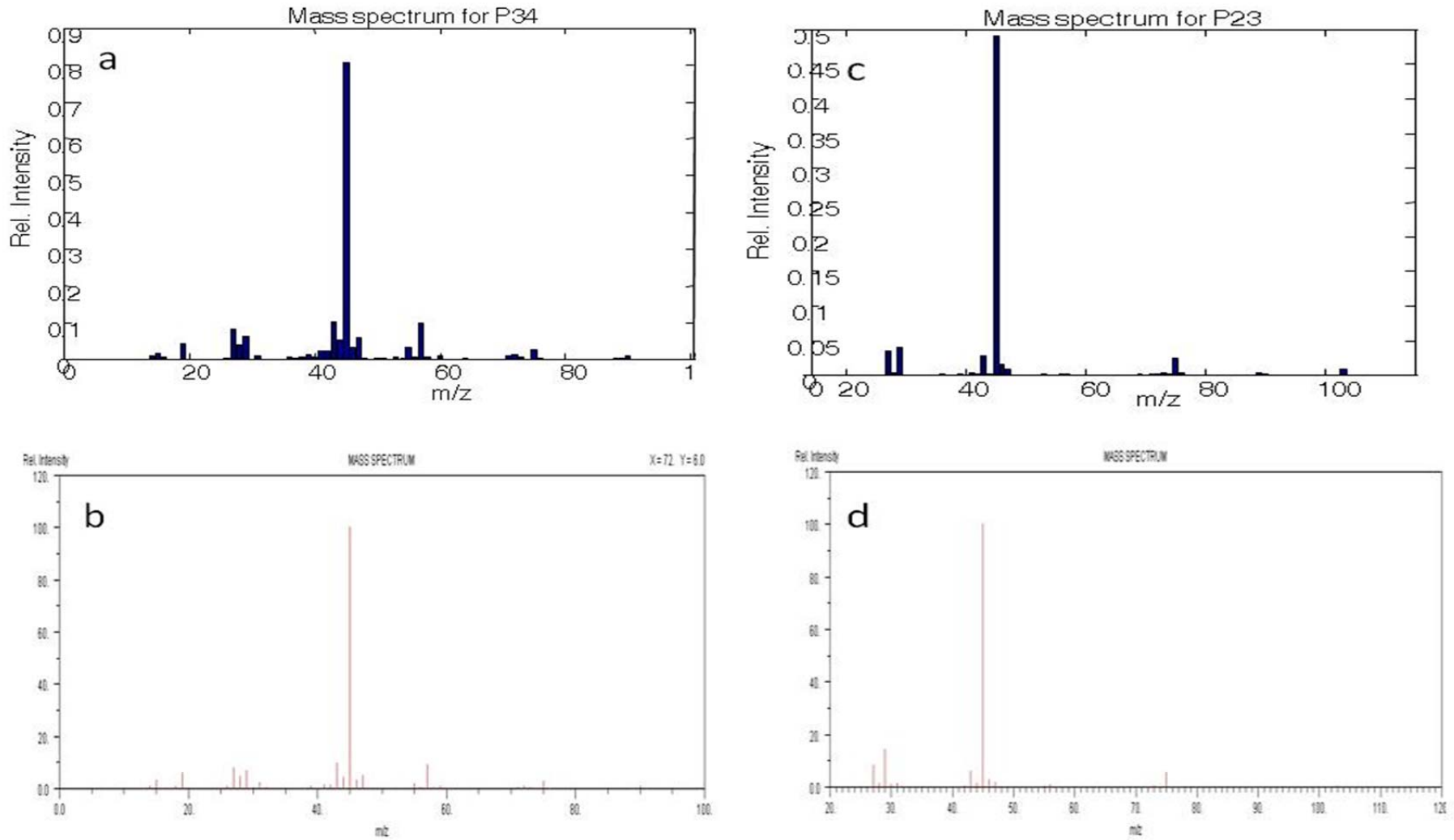

Figure 4.7 (a) The mass spectral loadings of variable P34 from the PARAFAC model were compared to the spectra of (b) 2,3-butanediol, from NIST. (c) The mass spectral loadings for variable P23 from the PARAFAC model compared to the mass spectra of (d) ethyl lactate in the NIST database (Linstrom and Mallard, 2000)

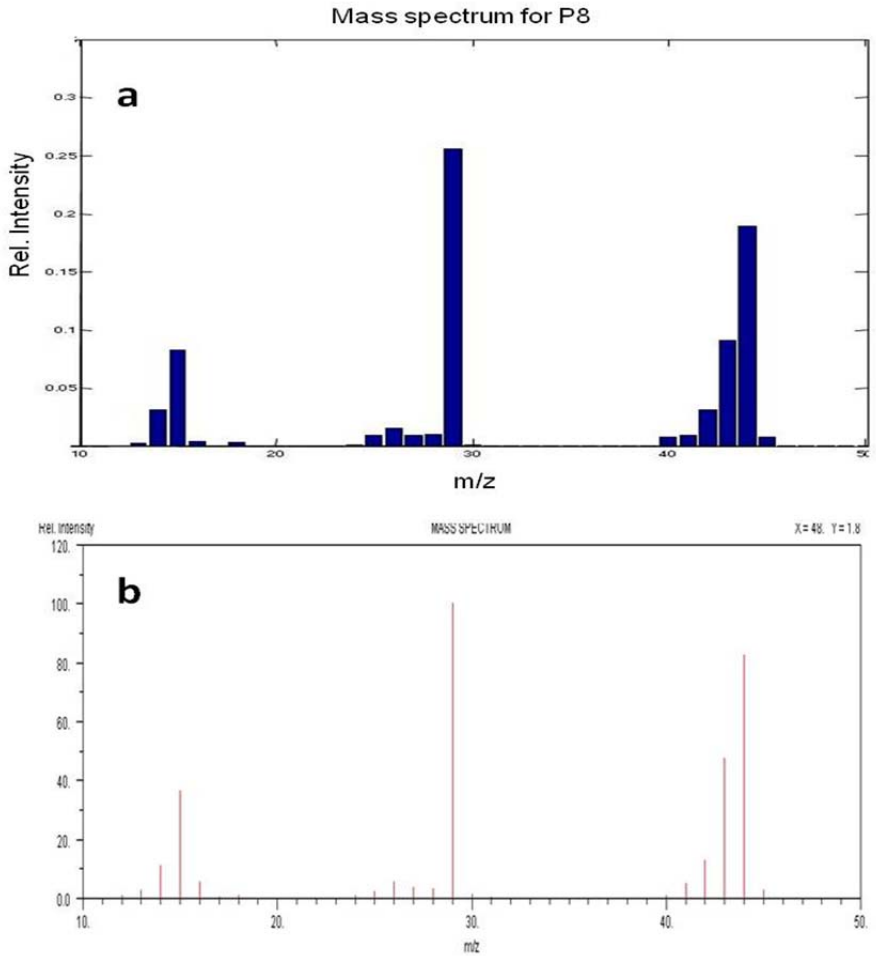

Figure 4.8 (a) The mass spectral loadings of variable P8 from the PARAFAC model were compared to the spectra of (b) acetaldehyde in the NIST database (Linstrom and Mallard, 2000).

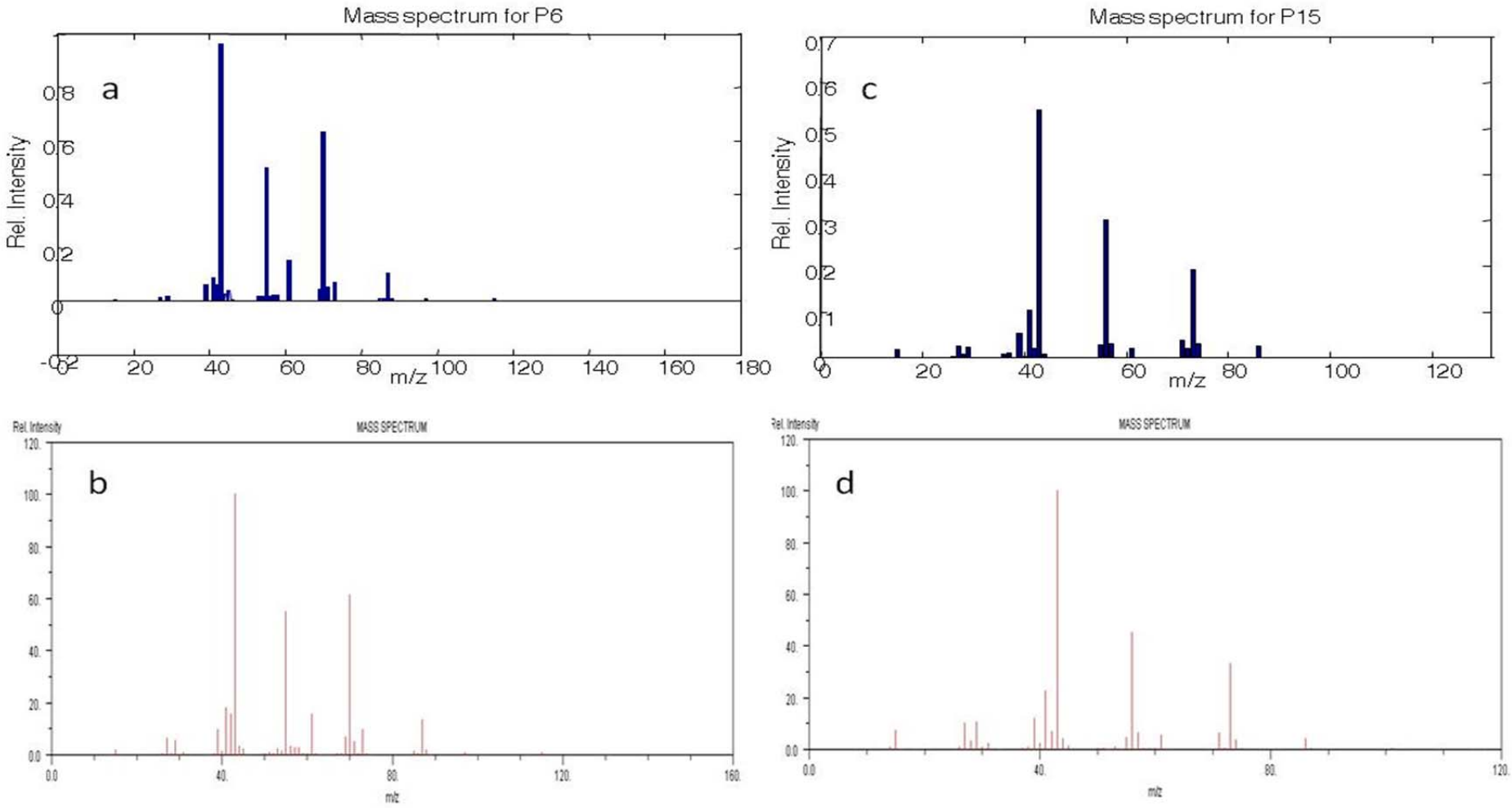

Figure 4.9. (a) The mass spectral loadings of variable P6 from the PARAFAC model indicate that the compound was (b) isoamyl acetate from the NIST database, while the mass spectral loadings of (c) variable P15 compared well to that of (d) isobutyl acetate from the NIST database (Linstrom and Mallard, 2000)

4.4 CONCLUSIONS

During this study, untargeted GC-MS analysis of volatiles in Chenin blanc was performed on 27 South African dry Chenin blanc wines of three dry and off-dry styles. The sample preparation and GC-MS method were based on the method developed in Chapter 3 and found to be suitable for this purpose. Thereafter PARAFAC was used to model the obtained GC-MS data for accurate characterisation of different styles of wine within a single cultivar.

The PCA scores plot of the PARAFAC data showed very good discrimination between the three styles. Upon investigating the various volatiles associated with the groups, acetate esters were found to be highly correlated with the fresh and fruity style, while volatiles associated with malolactic activity were associated with the rich and ripe wooded Chenin blanc wines. The unwooded rich and ripe style were correlated with ethyl hexanoate and amyl alcohol. The results found with the untargeted style classification confirm the results found with the targeted analysis (chapter 3). However, the advantage of the untargeted analysis is that the selection of variables is completely unbiased; this provides a much firmer basis of extracting the variables that contribute to the discrimination between the three styles. In addition the arduous task of setting up calibration curves for many volatile compounds, described in chapter 3, can be avoided using the untargeted approach combined with mathematical chromatographic data analysis tools. Furthermore these results indicate that untargeted GC-MS analysis of Chenin blanc together with PARAFAC modeling could be applied for future volatile metabolic profiling and style classification within varieties.

Based on the aroma contribution reported in literature for the style discriminating volatile compounds identified in this study, an interpretation in terms of the aroma contribution of these to the wine styles can be made. A sensory evaluation of the wines used in this study was undertaken in a concurrent project (Bester, 2011). In that study, sensory attributes that were discriminative between the styles were found that correlate well with the findings of the untargeted analysis.

In conclusion it was demonstrated that the results obtained by the untargeted GC-MS analysis and PARAFAC modelling could be used to accurately characterise the three styles, with indications of the aroma and flavour profile for each individual style. This was performed without the need for analysing numerous standards and setting up vast numbers of calibration curves.

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Chapter 5

General conclusions

5. GENERAL CONCLUSIONS

5.1 Conclusions

The tasks of this study were to focus on the chemical characterisation of Chenin blanc dry and off-dry wine styles. Based on the literature review, the shortcomings in our current analytical protocols for analysis of wine aroma compounds, as well as the handling and processing of large volumes of data were identified.

As stated in the introduction to this thesis, the specific aims of this study were: (i) to do chemical profiling of aroma compounds in Chenin blanc wines, by development of a rapid, high throughput gas chromatographic flame ionization detection (GC-FID) method for quantification of these compounds; (ii) to develop a protocol for handling the large volume of analytical data created by GC mass spectrometry (GC-MS) analysis, and investigate methods to overcome inherent chromatographic problems such as coelution of analytes, retention time shifts and baseline drifts; and, (iii) to develop preliminary models, based on targeted analysis (using GC-FID) as well as untargeted analysis (using GC-MS) to discriminate between the fresh and fruity, rich and ripe unwooded and rich and ripe wooded styles.

In terms of the first aim, targeted volatile metabolic profiling Chenin blanc wine was done by a new GC-FID method that was developed, using a dichloromethane liquid-liquid extraction for sample preparation. This method proved to be well suited to accurately quantify 57 volatile aroma compounds in wine, in less than 30 minutes, and the new method considerably extended the previous number of compounds (27 fermentation derived compounds) that were routinely analysed in our laboratories. Upon validation, it was proved that the method is robust in terms of linearity, repeatability, accuracy and sensitivity. The newly developed method was then applied to 48 Chenin blanc wines to gain a preliminary overview of the distribution of aroma compounds in this cultivar. The quantified data will be captured by the existing South African wine industry aroma compounds database, that to date contains no data on Chenin blanc wines. More wines will also be analysed in future by this method to expand the Chenin blanc component of the database.

The outputs of the first objective were thus both of a fundamental, as well as applied nature. A significant contribution was made to the analytical capacity for high throughput analysis of wine volatile compounds, and the quantified information captured can be used in future for the purposes of benchmarking and comparisons within the wine industry.

In terms of the second aim, wine analysis using untargeted GC-MS analysis provided another dimension to this study. Vast amounts of information concerning the chemical make-up of samples analysed, are typically collected in these analyses, but often left unexplored. This is mainly due to the manual data processing being extremely laborious and time-consuming. The untargeted approach, using GC-MS data processed with PARAFAC, resulted in the development of a workflow with which the vast amount of data could be handled, processed and useful information extracted from the analysis. Indeed, the application of the workflow is to be encouraged in wine science research, where applicable, in order to do in-depth data mining and extract maximum useful information from the chromatograms. Following PARAFAC analyses, the data were subsequently subjected to pattern recognition techniques to derive style classification models. This approach seemed to be as effective as attempts to classify the wines based on the data obtained with the targeted approach, discussed in the next paragraph. The main advantage of the untargeted approach is that it is unbiased in selection of compounds, therefore giving a more comprehensive insight into the metabolites involved in processes such as fermentation, and, if applicable, the effects of lees contact, malolactic fermentation and wood ageing.

For the third objective, style classification was done using the volatile data obtained using the newly developed GC-FID method, by selecting the most influential variables with partial least squares regression-discriminant analysis (PLS-DA) as well as linear-DA. It was found that very good discrimination between the three styles was possible based on these statistical and chemometric approaches. Results from both GC-FID and GC-MS analyses illustrate that the compounds responsible for this discrimination could possibly be interpreted in terms of literature reports regarding winemaking regimes (i.e. lees contact, fermentation conditions, malolactic fermentation and ageing) for other cultivars. It is interesting to note that in a related project on style classification of the same set of wines, using sensory data generated by a trained panel, reasonably good discrimination between the wooded and unwooded Chenin blanc styles (fresh and fruity and rich and ripe unwooded) was obtained, while discrimination between the two unwooded styles was not possible. This once again highlights the value of instrumental measurements, as opposed to human sensory measurements, that are less accurate and repeatable. Since the style classifications indicated on the labels are intended for consumers, it remains to be decided whether Chenin blanc dry and off-dry wine style classification should not rather be simplified to include two classes only, namely wooded and unwooded. Nevertheless, the contribution of the chemical data to style profiling lies much more in verification of the winemaking techniques than label information, and the chemical compound-based classification models show potential for future style verification and authentication purposes.

Additional outputs of this study are listed in Addendum A. In summary the outcomes of this project were the establishment of a high throughput GC-FID method that can be used for volatile metabolic profiling of other wine cultivars, as well as an established workflow for handling large volumes and complexity in three-way analytical data. These outputs are important from both a fundamental research perspective as well as an applied perspective, and make a significant contribution towards unravelling Chenin blanc wine chemistry.

ADDENDUM A

METHOD VALIDATION DATA

ADDENDUM A

OBJECTIVE AND SCOPE OF THE METHOD

A method suitable for the extraction and analysis of major and minor wine volatiles with gas chromatography flame ionization detection.

TYPE OF COMPOUNDS AND MATRIX

Esters, aldehydes, fatty acids, alcohols, terpenes, carbonyl compounds and wood derived compounds in white wine using 4-methyl-2-pentanol (0.5 mgL^{-1}) and 2,6-dimethyl-6-hepten-2-ol (0.1 mgL^{-1}) as the internal standards

MATERIALS AND METHODS

Chemicals:

Dichloromethane, absolute ethanol as well as sodium sulphate (anhydrous), ammonium sulphate and sodium hydroxide were purchased from Merck (Darmstadt, Germany). Tartaric acid was purchased from Sigma-Aldrich (St. Louis, MO, USA). The water was purified through a Milli-Q purification system (Millipore, Bedford, MA, USA). The chemicals, all of which were of analytical grade, were purchased from Riedel de Haën (Seelze, Germany), Sigma-Aldrich (St. Louis, MO, USA), Fluka (Buchs, Switzerland), and Aldrich (Steinheim, Germany), are shown in Table 1.

Synthetic base wine simulant

A synthetic base wine simulant was prepared as described by Louw et al. (2009) and consisted of 2.5 gL^{-1} tartaric acid from Merck and 12 % (v/v) ethanol dissolved in purified water. The pH was adjusted to 3.5 using 0.1 M sodium hydroxide.

Gas

UPH hydrogen gas as carrier gas, UPH nitrogen as make up gas for the detector flow, and UPH air for the flame ionization detector (AFROX, South Africa).

Table 1 List of chemical standards used in this work, and their purity.

	Analyte	Supplier	Purity (%)
Alcohols	1-Propanol	Fluka	>99.8%
	Methanol	Sigma-Aldrich	>99.9%
	n-Butanol	Fluka	>99.5%
	Isobutanol	Fluka	>99.5%
	4-Methyl-2-pentanol	Fluka	>99%
	Isoamyl alcohol	Aldrich	>99%
	1-Pentanol	Fluka	>99%
	2-Phenylethanol	Merck	>99%
	Hexanol	Merck	>98%
	1-Octen-3-ol	Fluka	>98%
	2,6-Dimethyl-6-hepten-2-ol	Fluka	>99.5%
	3-Methyl-1-pentanol	Sigma-Aldrich	>97%
	3-Ethoxy-1-propanol	Sigma-Aldrich	>97%
	4-Methyl-1-pentanol	Sigma-Aldrich	>95%
Acetate esters	Hexyl acetate	Fluka	>99%
	Ethyl phenylacetate	Aldrich	≥98%
	Ethyl acetate	Sigma-Aldrich	>99.7%
	Isoamyl acetate	Riedel de Haën	>98%
	2-Phenylethyl acetate	Fluka	>99%
	Ethylphenyl acetate	Fluka	>99%
Acids	Propionic acid	Fluka	>99.5%
	Isovaleric acid	Fluka	>99%
	Isobutyric acid	Fluka	>99.5%
	Valeric acid	Fluka	>99%
	Hexanoic acid	Aldrich	>99.5%
	Octanoic acid	Aldrich	>99.5%
	Acetic acid	Merck	>98%
	Decanoic acid	Sigma	>98%
	Butyric acid	Fluka	>99.5%
Esters	Ethyl butyrate	Fluka	>98%
	Ethyl-2-methylbutyrate	Aldrich	≥98%
	Ethyl isovalerate	Aldrich	≥98%
	Ethyl propionate	Aldrich	≥97%
	Ethyl lactate	Fluka	>99%
	Diethyl succinate	Fluka	>98%
	Ethyl ocatanoate	Fluka	>98%
	Ethyl hexanoate	Fluka	>99%
	Ethyl decanoate	Aldrich	>99%
	Ethyl-3-hydroxybutanoate	Sigma-Aldrich	≥97%
Carbonyls	Acetoin	Fluka	>97%
	Diacetyl (2,3-Butanedione)	Fluka	>99.5%
	Acetaldehyde	Fluka	>99.5%
Terpenes	α-Terpeneol	Sigma-Aldrich	>99%
	Citronellol	Fluka	>99%
	Fenchone	Aldrich	>98%
	β-Ionone	Aldrich	>97%
	α-Ionone	Aldrich	>90%
	β-Farnesol	Fluka	>99%
	Limonene	Fluka	>99%
	Linalool	Sigma-Aldrich	>99%
	Linalool oxide	Aldrich	>97%
	Linalyl acetate	Aldrich	≥97%
	Geraniol	Fluka	>99%
	Nerol	Fluka	>99%
Wood derived	Furfuryl alcohol	Aldrich	>98%
	Furfural	Sigma-Aldrich	99%
	5-Methyl-2-furfural	Sigma-Aldrich	99%
	Guaiacol	Aldrich	>98%
	Whiskey lactone	Aldrich	>98%

Column and instrument

A J & W DB-FFAP capillary GC column (Agilent, Little Falls, Wilmington, USA), with the dimensions 60 m length by 0.32 mm internal diameter and 0.5 µm film thickness. The instrument was a Hewlett Packard 6890, gas chromatograph equipped with a flame ionization detector (GC-FID) (Agilent, Little Falls, USA)

Gas chromatographic conditions

The initial oven temperature was set at 40 ºC and held for 5 minutes, after which the temperature was increased by 8 ºCmin^{-1} to 225 ºC, and held for 1.2 minutes. 3 µL of the sample was injected at 200 ºC in split mode (18:1, with a split flow rate of 98.9 mL min^{-1}). The column flow rate was 5.5 mL min^{-1} and a post run of 5 minutes, with oven temperature at 240 ºC and a flow rate 6 mL min^{-1} cleaned off the column from any contaminants of high boiling point, making the total run time 29.3 minutes. The detector temperature was set at 250 ºC.

Sample preparation

Before the addition of wine, 2.5 grams of ammonium sulphate was added to 15 mL culture tubes (Pyrex, Milian, USA). Five milliliters of wine and 100 µL of the internal standard solution (0.5 mgL^{-1} 4-methyl-2-pentanol and 0.1 mgL^{-1} 2,6-dimethyl-6-hepten-2-ol in synthetic base wine) were extracted using 1 mL of dichloromethane by sonicating for one hour with shaking every 10 minutes. The extract was then centrifuged at 3000 rpm for 30 minutes and the dichloromethane layer (top layer) removed and dried on anhydrous sodium sulphate. Each wine sample was extracted in triplicate and injected into the Hewlett Packard 6890 GC-FID (Agilent, Little Falls, USA) in duplicate.

RESULTS

Calibration was performed for all the volatile compounds at the ranges stated in Table 2, with the calibration curves given in order of elution of the compounds, (Figures 1-57).

Recoveries in wine for all compounds, lowest levels of quantification and detection as well as the slope and correlation coefficient (R^2) are given in Table 2.

The repeatability and intermediate repeatability, expressed in % relative standard deviation (% RSD) are given in Table 3.

Table 2. Recoveries, limits of detection and quantification, slopes, correlation coefficient and calibration ranges for aroma compounds in wine

	Compound	Recovery in wine (%) ± RSD (%)	LOD[a] mg L^{-1}	LOQ[b] mg L^{-1}	R^2	Slope	Range mg L^{-1}
Alcohols	Methanol	89.10 ± 3.68	0.24	0.81	0.997	0.070	9.10 - 901.70
	Propanol	97.44 ± 2.12	0.02	0.08	0.998	0.779	2.00 - 201.00
	Isobutanol	83.78 ± 1.77	0.02	0.07	0.999	0.884	1.25 - 103.80
	Butanol	89.64 ± 1.47	0.02	0.07	0.997	0.822	0.25 - 20.30
	Isoamyl alcohol	83.12 ± 1.35	0.02	0.06	0.998	0.989	5.54 - 477.30
	Pentanol	82.87 ± 2.69	0.02	0.05	0.999	0.926	1.00 - 100.00
	4-Methyl-1-pentanol	86.26 ± 1.67	0.02	0.06	0.999	0.974	1.00 - 100.00
	Hexanol	77.44 ± 2.59	0.01	0.05	0.999	1.174	0.34 - 30.90
	3-Ethoxy-1-propanol	83.07 ± 2.24	0.03	0.09	0.999	0.623	1.00 - 100.00
	3-Methyl-1-pentanol	85.80 ± 1.58	0.02	0.06	0.999	0.968	1.00 - 100.00
	1-Octen-3-ol	88.69 ± 2.25	0.02	0.07	0.999	0.851	0.10 - 100.00
	2-Phenyl Ethanol	87.83 ± 1.43	0.02	0.05	0.999	1.070	0.64 - 51.00
Carbonyls	Acetaldehyde	92.68 ± 7.31	0.03	0.05	0.999	0.630	0.10 - 100.00
	Diacetyl	97.40 ± 2.31	0.03	0.04	0.999	0.373	0.50 - 100.00
	Acetoin	81.75 ± 3.08	0.04	0.12	0.999	0.465	1.00 - 100.00
Acetate esters	Ethyl Acetate	88.90 ± 0.99	0.03	0.09	0.998	0.623	3.61 - 360.80
	2-Phenylethyl Acetate	83.78 ± 6.84	0.02	0.06	0.999	0.937	0.25 - 20.60
	Isoamyl Acetate	85.25 ± 2.43	0.03	0.10	0.999	0.715	0.24 - 19.20
	Hexyl Acetate	80.71 ± 4.38	0.02	0.08	0.997	0.738	0.252 - 21.90
	Ethyl phenylacetate	85.23 ± 2.09	0.02	0.06	1.000	0.999	1.00 - 100.00
	Ethyl propionate	84.65 ± 3.60	0.03	0.11	0.998	0.556	1.00 - 100.00
	Ethyl-2-methylpropanoate	81.06 ± 3.84	0.03	0.10	0.998	0.610	1.00 - 100.00
	Ethyl Butyrate	101.74 ± 7.45	0.03	0.09	0.998	0.654	0.25 - 22.00
Esters	Ethyl-2-methylbutyrate	84.50 ± 2.02	0.02	0.08	0.999	0.685	1.00 - 100.00
	Ethyl isovalerate	86.17 ± 2.09	0.02	0.08	0.999	0.712	1.00 - 100.00
	Ethyl Hexanoate	82.09 ± 2.81	0.03	0.08	0.999	0.705	0.35 - 30.6
	Ethyl Lactate	77.66 ± 3.21	0.04	0.12	0.999	0.461	6.25 - 500.20
	Ethyl octanoate	102.99 ± 2.58	0.02	0.06	0.999	0.945	0.07 - 4.00
	Ethyl-3-hydroxybutanoate	84.49 ± 2.89	0.03	0.10	0.999	0.544	0.35 - 100.00
	Ethyl decanoate	89.64 ± 5.04	0.14	0.47	0.999	1.215	0.50 - 3.50
	Diethyl Succinate	74.32 ± 2.35	0.03	0.09	1.000	0.618	0.35 - 31.40

Table 2 continued

	Compound	Recovery in wine (%) ± RSD (%)	LOD[a] mg L^{-1}	LOQ[b] mg L^{-1}	R^2	Slope	Range mg L^{-1}
Terpenes	Limonene	69.73 ± 1.34	0.04	0.11	0.999	0.125	0.1 - 1.00
	Fenchone	73.65 ± 1.26	0.02	0.04	0.999	0.258	0.04 - 1.00
	Linalool Oxide	73.83 ± 3.15	0.04	0.15	0.999	0.079	0.05 - 1.00
	Linalool	65.02 ± 8.16	0.02	0.08	0.999	0.188	0.08 - 1.00
	Linalyl acetate	85.96 ± 3.26	0.02	0.06	0.999	0.138	0.06 - 1.00
	Citronellol	71.78 ± 1.29	0.02	0.06	0.999	0.193	0.01 - 1.00
	α-terpeneol	81.15 ± 5.75	0.02	0.03	0.999	0.383	0.01 - 1.00
	Nerol	99.52 ± 6.59	0.02	0.03	0.999	0.455	0.03 - 1.00
	Geraniol	79.20 ± 1.06	0.02	0.04	1.000	0.229	0.04 - 2.00
	β-Ionone	102.11 ± 1.57	0.03	0.09	1.000	0.137	0.09 - 1.00
	α-Ionone	88.90 ± 1.07	0.03	0.11	0.999	0.086	0.10 - 1.00
	β-Farnesol	95.22 ± 9.67	0.04	0.1	0.998	0.109	0.1 - 5.00
Acids	Acetic Acid	77.68 ± 1.26	0.12	0.39	0.999	0.155	22.50 - 1000.00
	Propionic Acid	115.49 ± 4.83	0.04	0.15	0.999	0.402	0.35 - 100.00
	Isobutyric acid	76.36 ± 7.21	0.03	0.11	0.999	0.560	0.25 - 20.90
	Butyric Acid	85.57 ± 8.47	0.03	0.1	0.999	0.566	0.25 - 21.20
	Iso-Valeric Acid	100.73 ± 1.10	0.03	0.09	0.999	0.640	0.45 - 39.30
	Hexanoic Acid	74.12 ± 2.21	0.02	0.08	0.999	0.731	0.38 - 29.70
	Valeric Acid	97.87 ± 5.53	0.05	0.16	0.999	0.640	0.25 - 20.70
	Octanoic Acid	81.19 ± 0.97	0.03	0.08	0.999	0.822	0.5 - 40.4
	Decanoic Acid	101.62 ± 1.38	0.03	0.1	0.998	0.650	0.63 - 56.00
Wood derived	Furfuryl alcohol	95.84 ± 7.11	0.05	0.16	0.999	1.011	0.16 - 25.00
	Furfural	86.66 ± 5.07	0.01	0.03	0.999	0.943	1.00 - 100.00
	5-Methyl-2-furfural	79.67 ± 9.44	0.02	0.05	0.999	0.557	0.04 - 35.00
	Guaiacol	90.17 ± 4.96	0.02	0.08	0.999	0.710	0.1 - 20.00
	Whiskey lactone	97.26 ± 2.50	0.03	0.09	0.999	1.120	0.10 - 20.00

Table 3 Repeatability and intermediate repeatability (expressed in% RSD) for the developed GC-FID method in white wine and synthetic wine.

Compound class	Volatile compound	Repeatability		Intermediate repeatability	
		White wine	Synthetic	White wine	Synthetic
Alcohols	Methanol	3.97	5.70	4.17	5.98
	Propanol	3.71	2.27	3.90	2.39
	Isobutanol	1.46	0.76	1.53	0.79
	Butanol	0.11	0.38	0.12	0.40
	Isoamyl Alcohol	1.45	6.31	1.53	6.62
	Pentanol	0.57	0.76	0.60	0.80
	4-Methyl-1-pentanol	0.37	0.42	0.39	0.44
	3-Methyl-1-pentanol	0.35	0.40	0.37	0.42
	Hexanol	0.26	0.16	0.27	0.17
	3-Ethoxy-1-propanol	0.62	0.34	0.65	0.36
	1-Octen-3-ol	0.52	0.23	0.55	0.24
	2-Phenyl Ethanol	0.94	0.53	0.99	0.55
Carbonyl compounds	Acetaldehyde	2.32	2.73	2.43	2.86
	Diacetyl	0.59	0.67	0.62	0.70
	Acetoin	0.97	0.81	1.02	0.85
Acetate esters	Ethyl Acetate	1.47	1.65	1.54	1.73
	Ethyl Phenylacetate	0.48	0.46	0.50	0.48
	2-Phenylethyl Acetate	0.36	0.19	0.37	0.21
	Isoamyl Acetate	0.13	0.42	0.14	0.44
	Hexyl Acetate	0.20	0.18	0.21	0.18
Esters	Ethyl Ocatanoate	0.25	0.13	0.26	0.14
	Ethyl Decanoate	0.10	0.50	0.11	0.52
	Diethyl Succinate	0.74	0.38	0.78	0.40
	Ethyl Butyrate	0.50	0.41	0.52	0.43
	Ethyl Propionate	0.82	0.39	0.86	0.41
	Ethyl-3-hydroxybutanoate	0.45	0.69	0.47	0.72
	Ethyl Isovalerate	0.49	1.00	0.52	1.05
	Ethyl-2-methylbutyrate	0.50	1.37	0.52	1.43
	Ethyl Lactate	0.89	2.37	0.94	2.48
	Ethyl-2-methylpropanoate	0.85	0.63	0.89	0.66
	Ethyl Hexanoate	0.25	0.17	0.26	0.18
Terpenes	Limonene	3.25	9.44	3.41	10.01
	Fenchone	2.38	4.80	2.50	5.04
	Linalool Oxide	7.47	6.20	7.85	6.51
	Linalool	9.88	7.80	10.37	8.68
	α-Terpeniol	5.95	2.98	6.24	3.11
	Linalyl Acetate	9.71	9.92	10.18	10.41
	Citronellol	4.15	9.42	4.35	9.88
	Geraniol	2.50	6.85	2.63	7.19
	Nerol	8.81	6.97	9.25	7.31
	α-Ionone	2.43	8.20	2.55	8.61
	β-Ionone	4.12	4.37	4.32	4.59
	β -Farnesol	4.94	2.61	5.19	2.74
Acids	Acetic Acid	6.61	7.85	6.94	8.23
	Propionic Acid	5.95	0.60	6.25	0.63
	Isobutyric Acid	0.46	0.36	0.48	0.38
	Butyric Acid	0.62	0.25	0.65	0.26
	Iso-Valeric Acid	0.11	0.43	0.12	0.45
	Valeric Acid	0.54	0.11	0.57	0.13
	Hexanoic Acid	0.28	0.31	0.29	0.40
	Octanoic Acid	0.13	0.72	0.14	0.75
	Decanoic Acid	0.20	0.53	0.21	0.56
Wood derived	Furfural	0.51	0.53	0.54	0.56
	Furfuryl Alcohol	0.62	0.35	0.65	0.37
	5-Methyl-2-furfural	0.44	0.18	0.52	0.19
	Guaiacol	0.23	0.39	0.24	0.41
	Whiskey Lactone	0.14	0.34	0.15	0.36

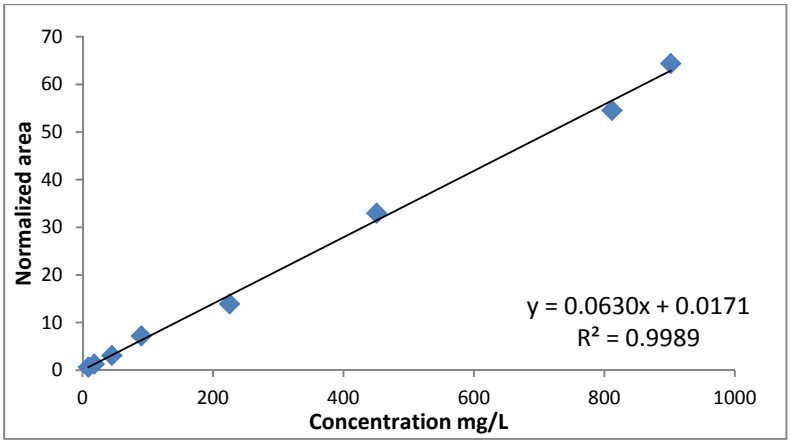

Figure 1. Calibration curve for acetaldehyde

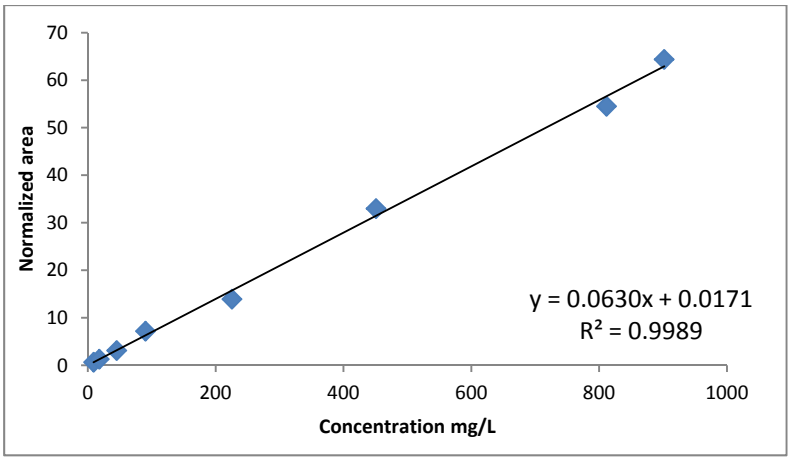

Figure 2. Calibration curve for Methanol

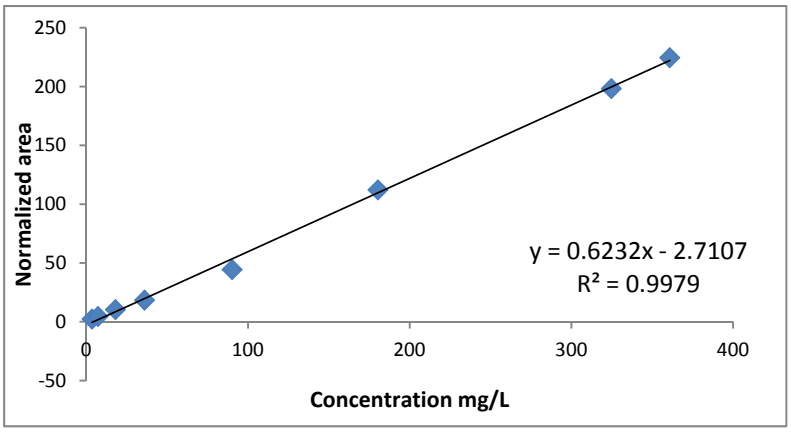

Figure 3. Calibration curve for ethyl acetate

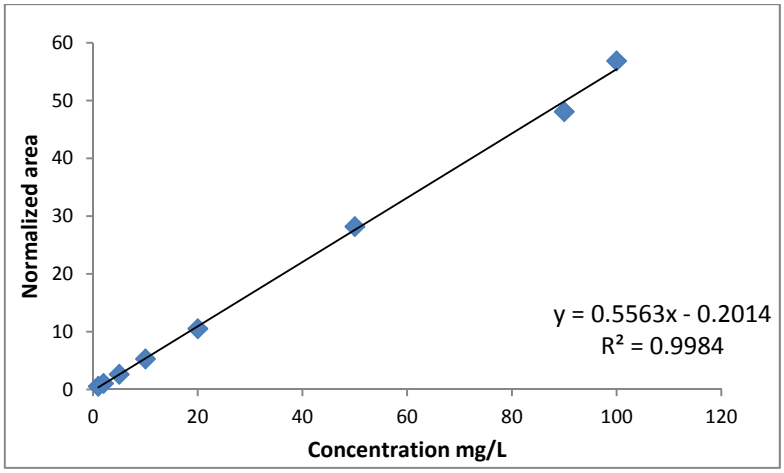

Figure 4. Calibration curve for ethyl propionate

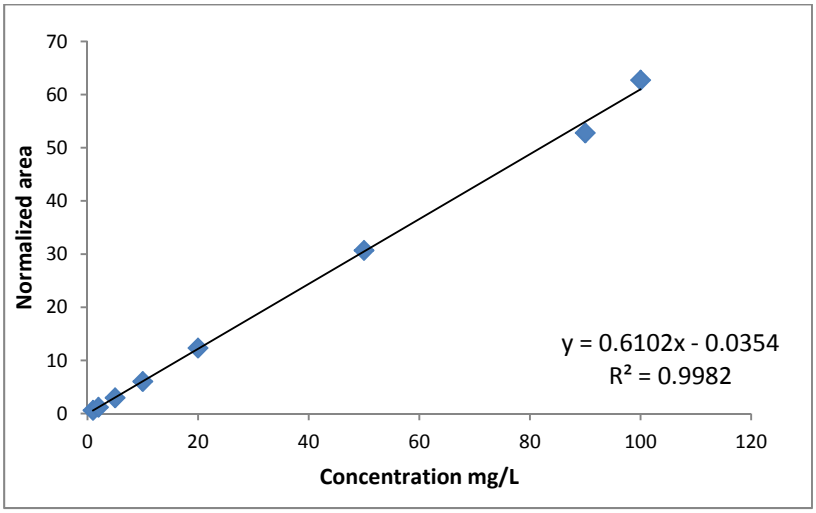

Figure 5. Calibration curve for ethyl-2-methylpropanoate

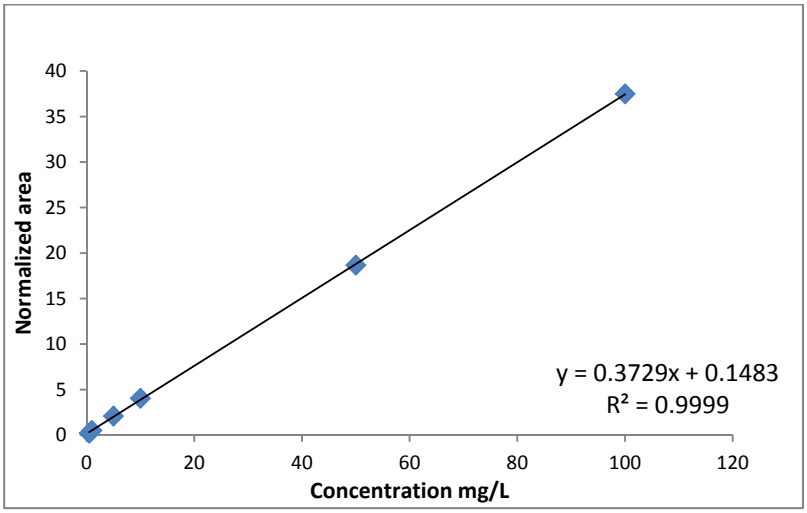

Figure 6. Calibration curve for diacetyl

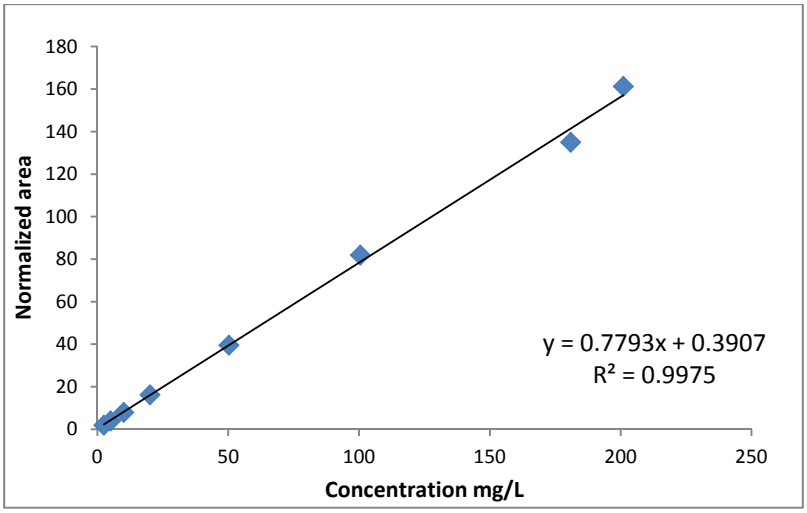

Figure 7. Calibration curve for propanol

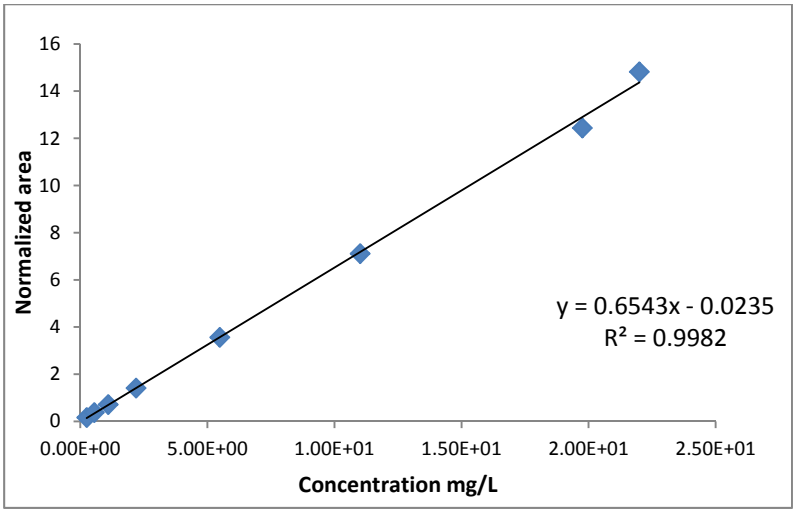

Figure 8. Calibration curve for ethyl butyrate

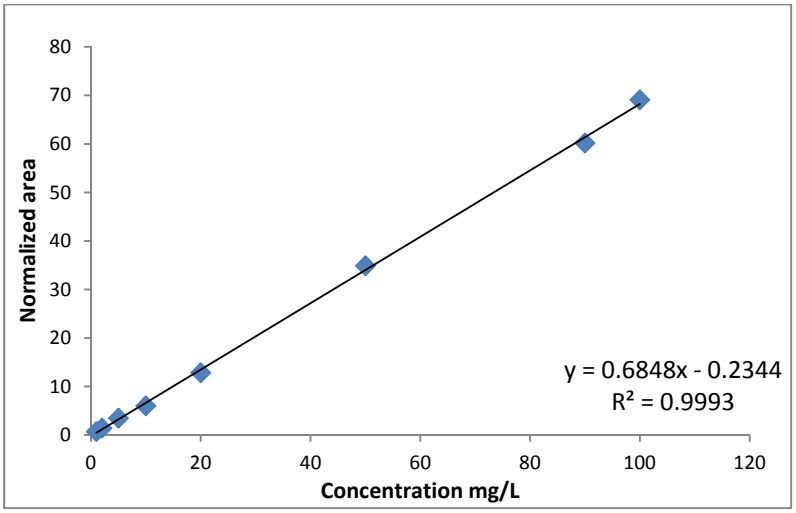

Figure 9. Calibration curve for ethyl-2-methylbutyrate

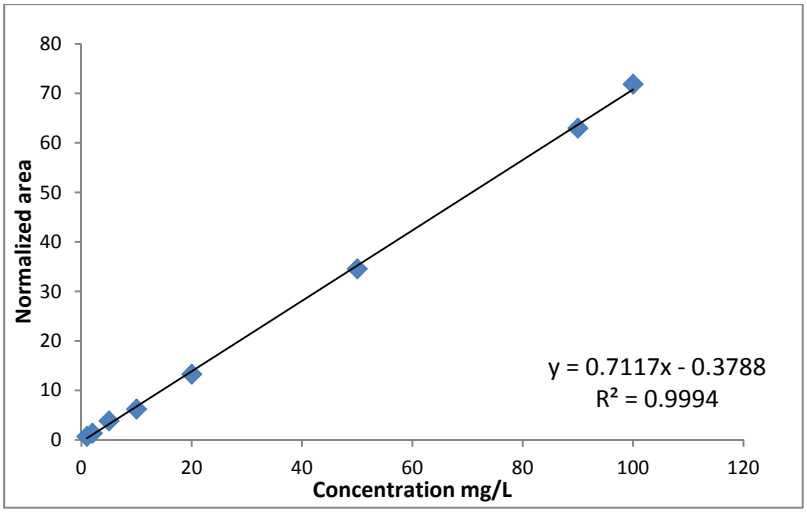

Figure 10. Calibration curve for ethyl isovalerate

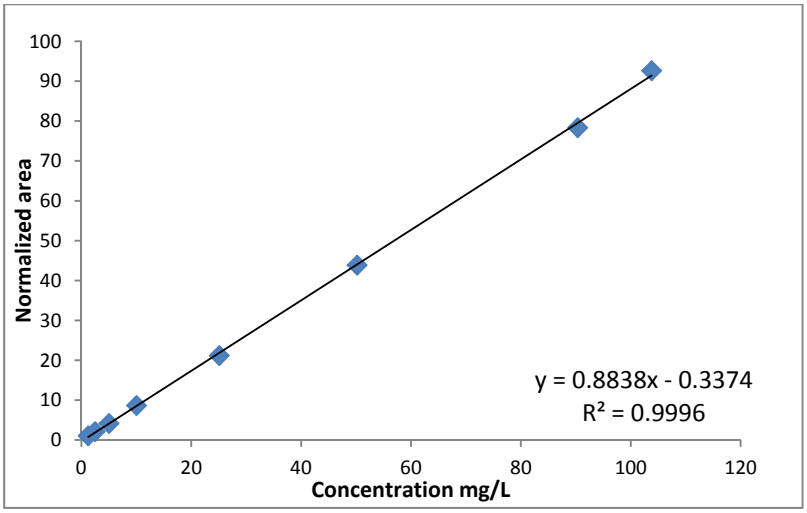

Figure 11. Calibration curve for ethyl isobutanol

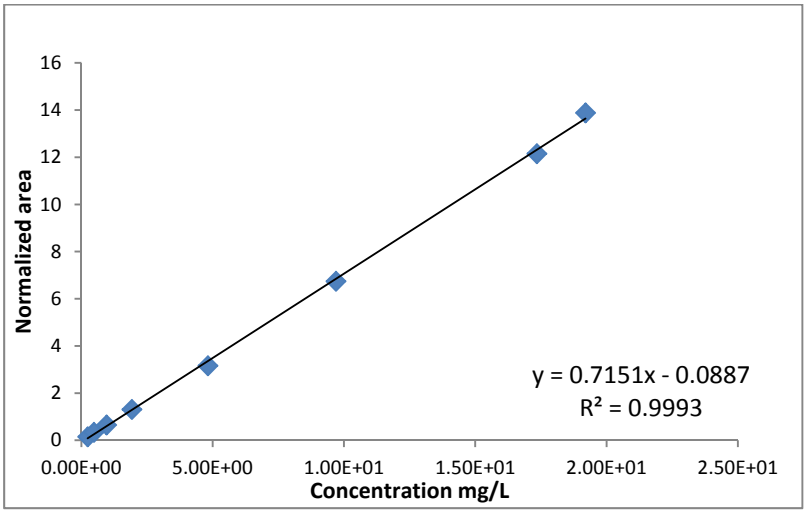

Figure 12. Calibration curve for isoamyl acetate

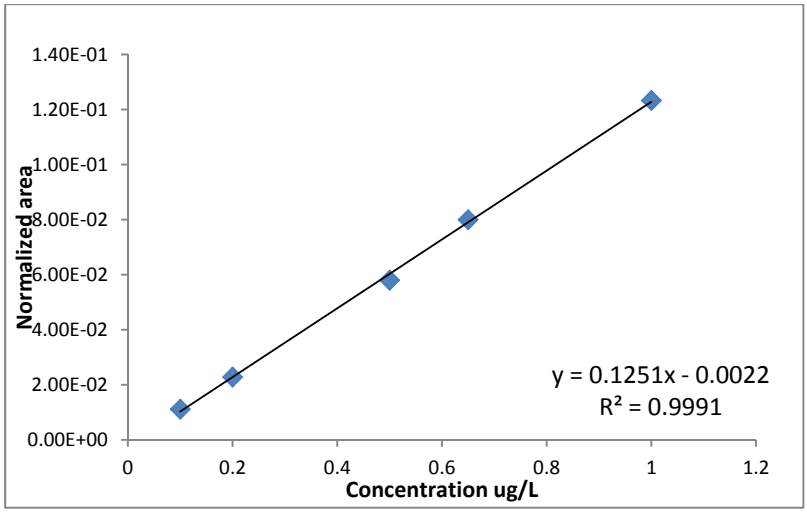

Figure 13 Calibration curve for limonene

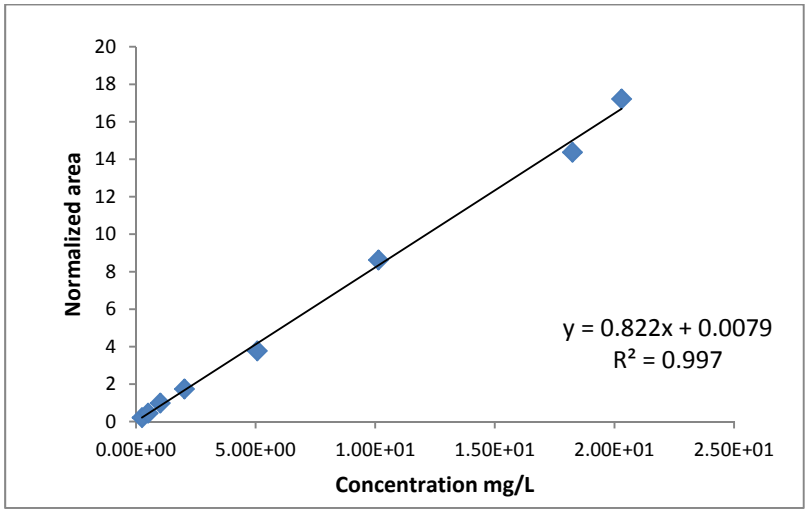

Figure 14. Calibration curve for butanol

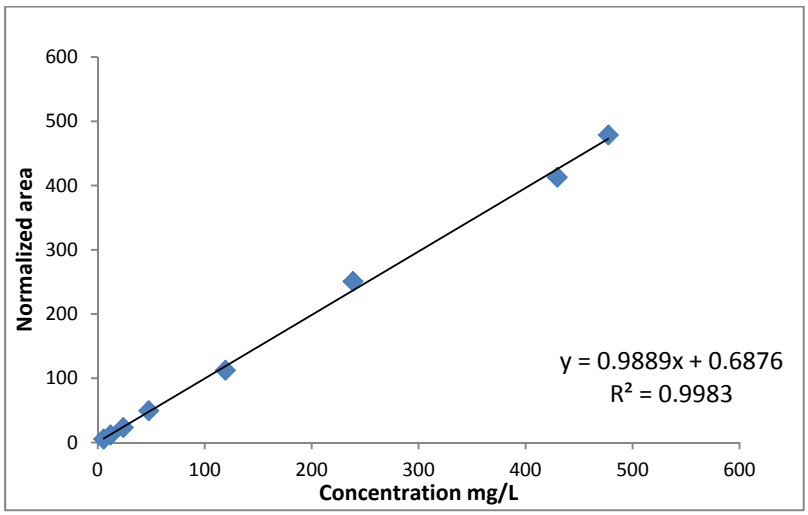

Figure 15. Calibration curve for isoamyl alcohol

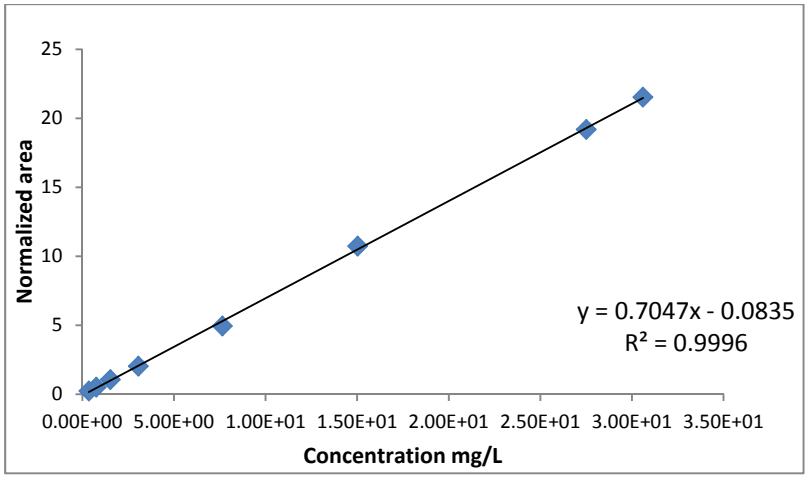

Figure 16. Calibration curve for ethyl hexanoate

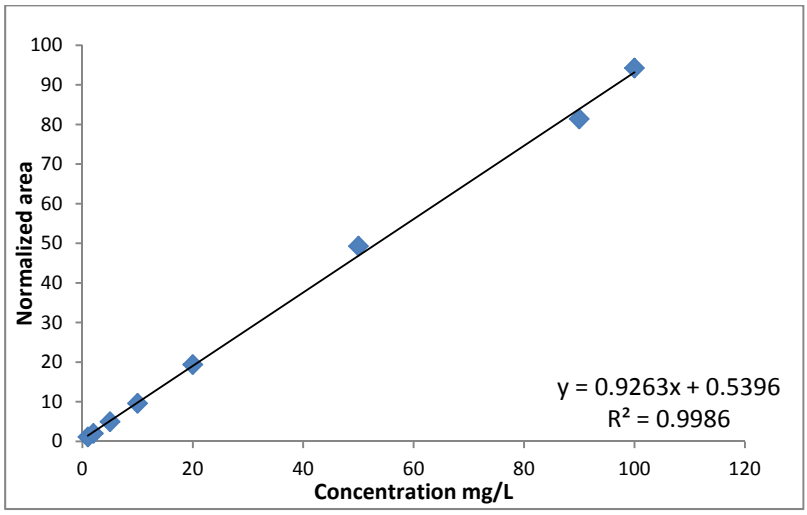

Figure 17. Calibration curve for pentanol

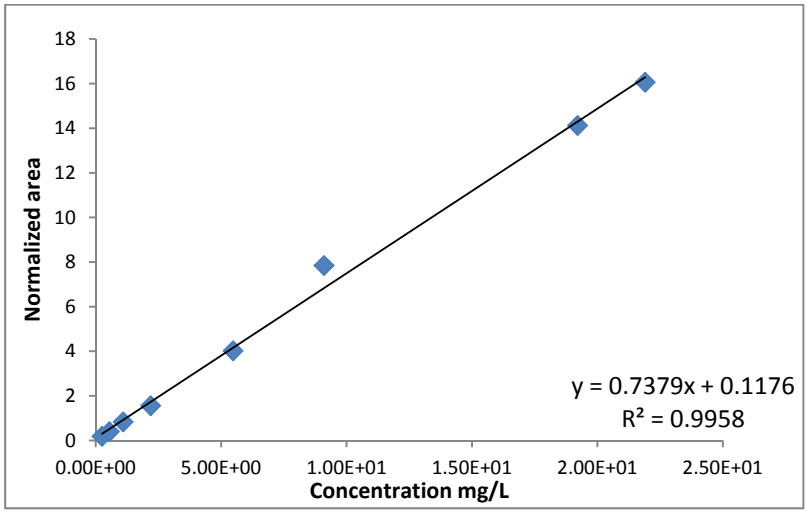

Figure 18. Calibration curve for hexyl acetate

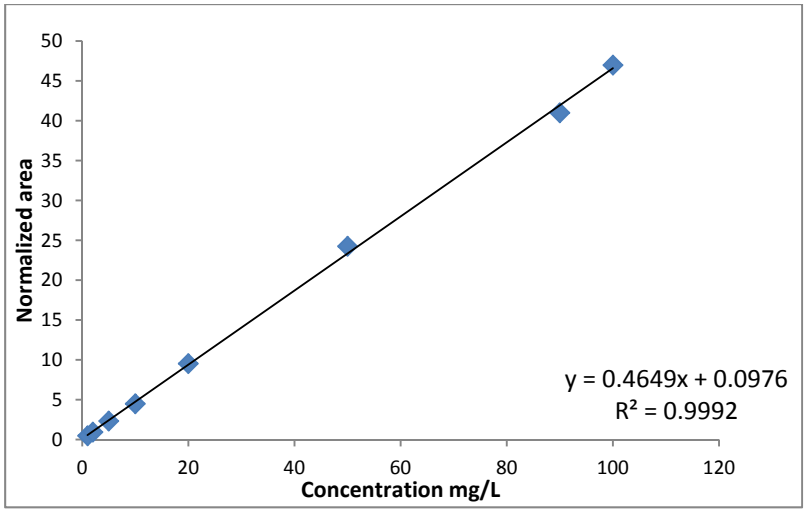

Figure 19. Calibration curve for acetoin

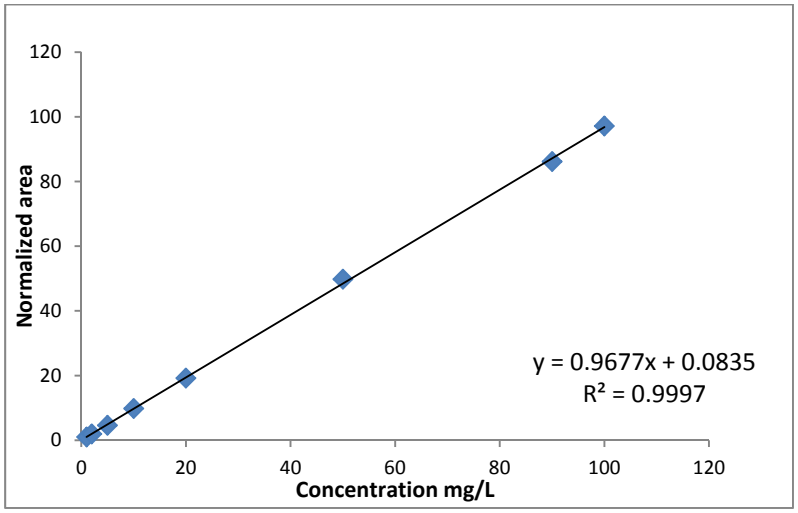

Figure 20. Calibration curve for 3-methyl-1-pentanol

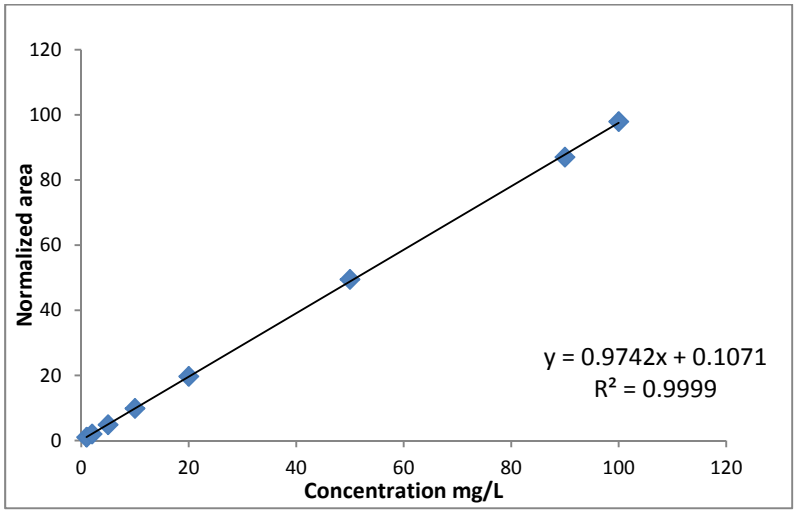

Figure 21. Calibration curve for 4-methyl-1-pentanol

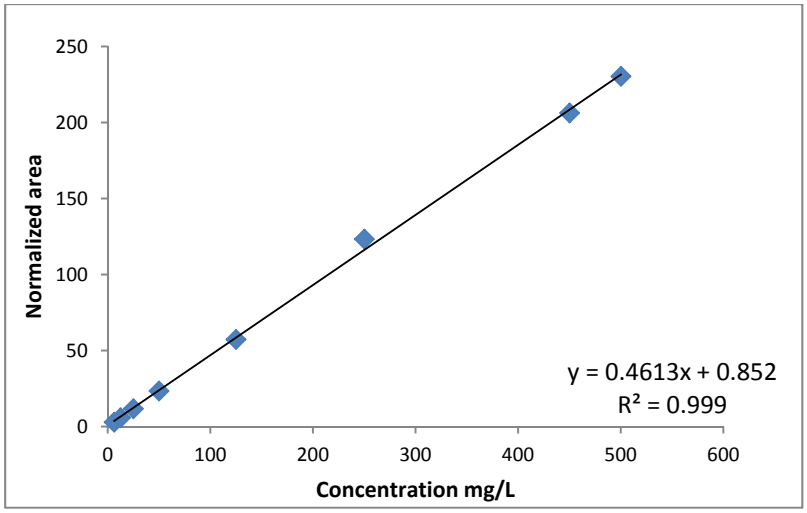

Figure 22. Calibration curve for ethyl lactate

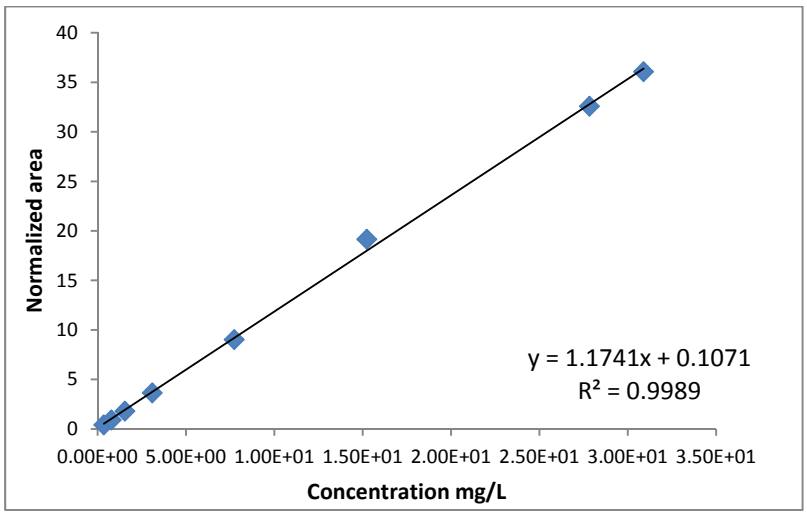

Figure 23. Calibration curve for hexanol

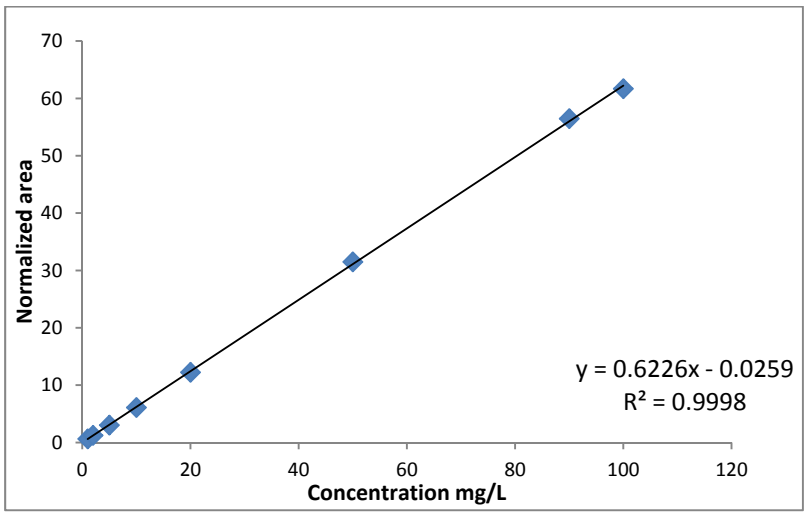

Figure 24. Calibration curve for 3-ethoxy-1-propanol

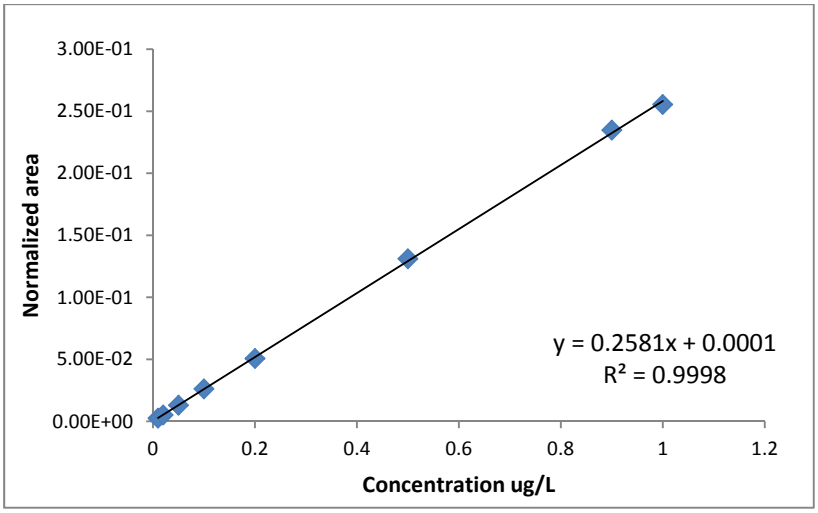

Figure 25. Calibration curve for fenchone

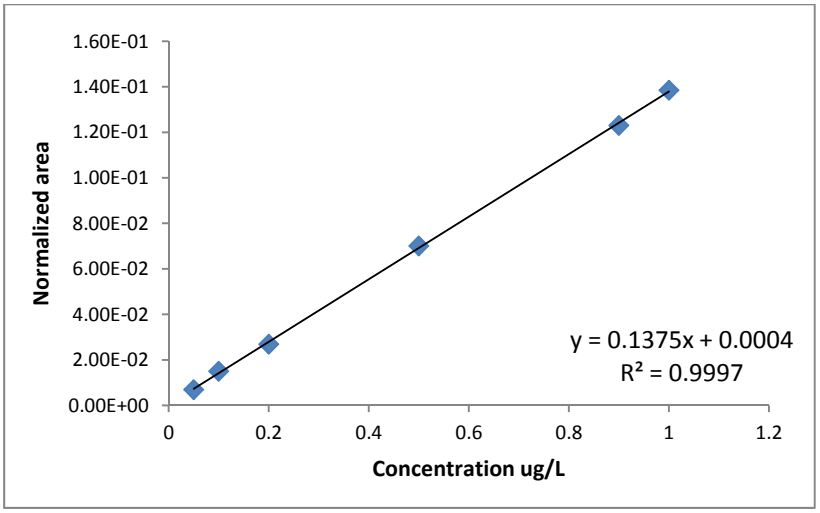

Figure 26. Calibration curve for linalyl acetate

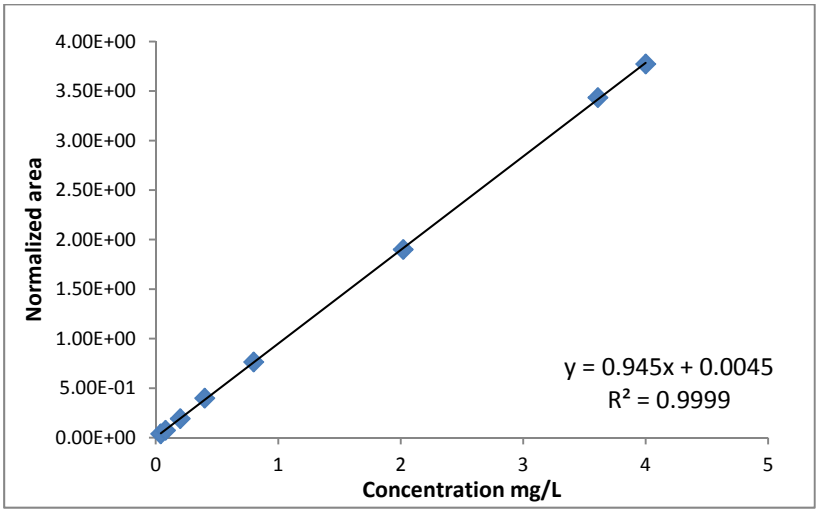

Figure 27. Calibration curve for ethyl octanoate

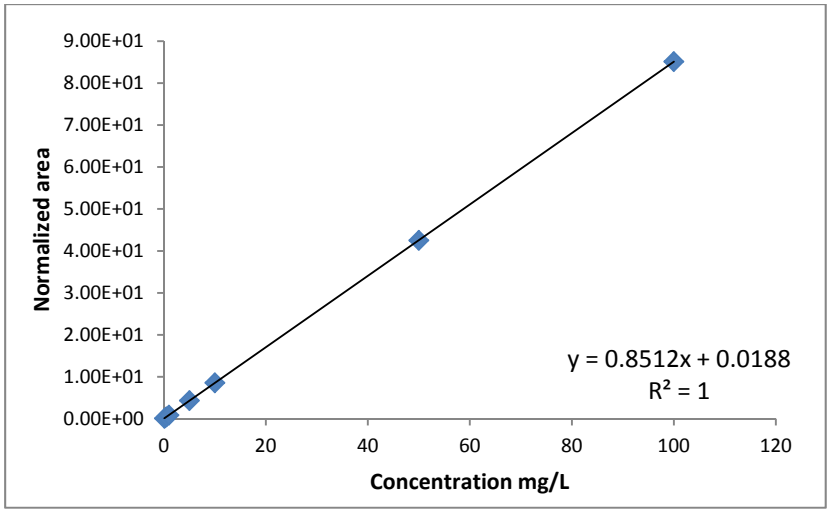

Figure 28. Calibration curve for 1-octen-3-ol

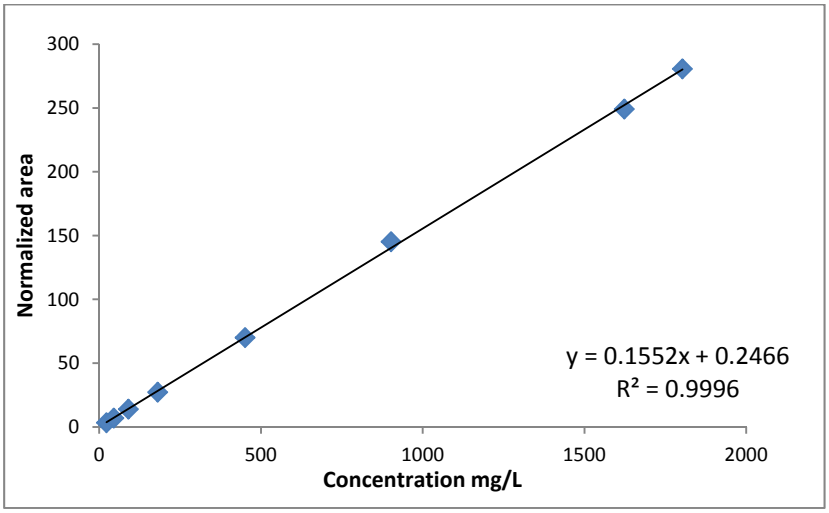

Figure 29. Calibration curve for acetic acid

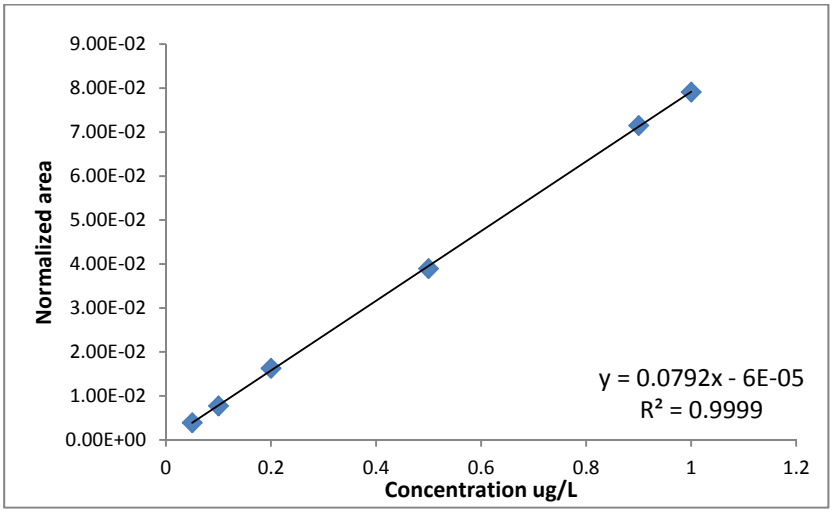

Figure 30. Calibration curve for linalool oxide

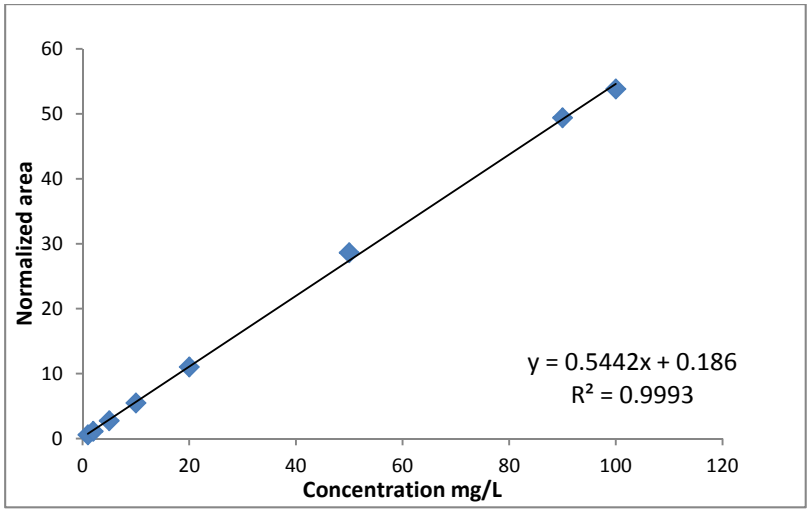

Figure 31. Calibration curve for ethyl-3-hydroxybutanoate

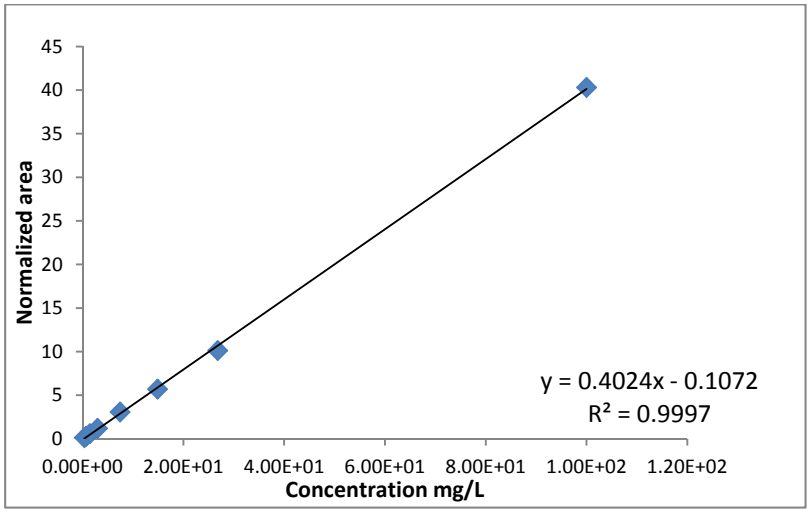

Figure 32. Calibration curve for propionic acid

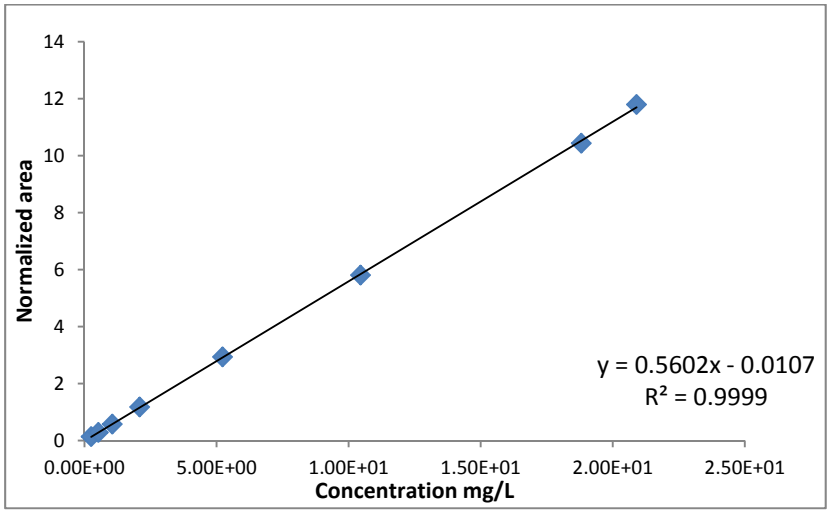

Figure 33. Calibration curve for isobutyric acid

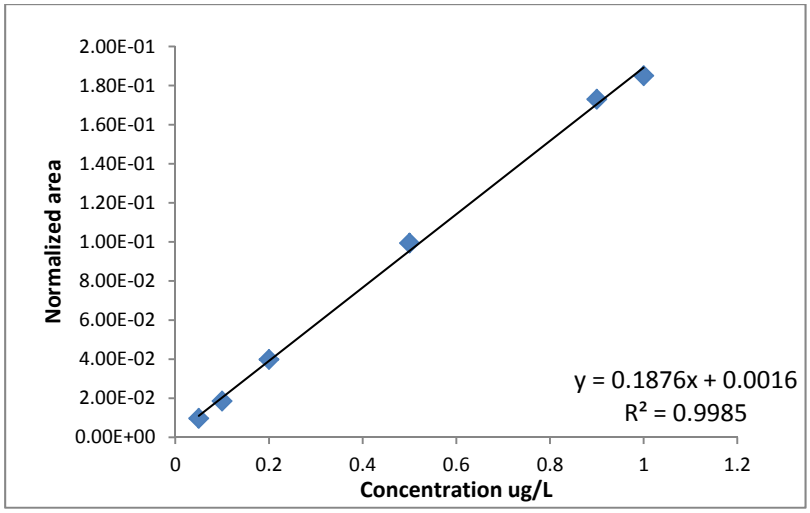

Figure 34. Calibration curve for linalool

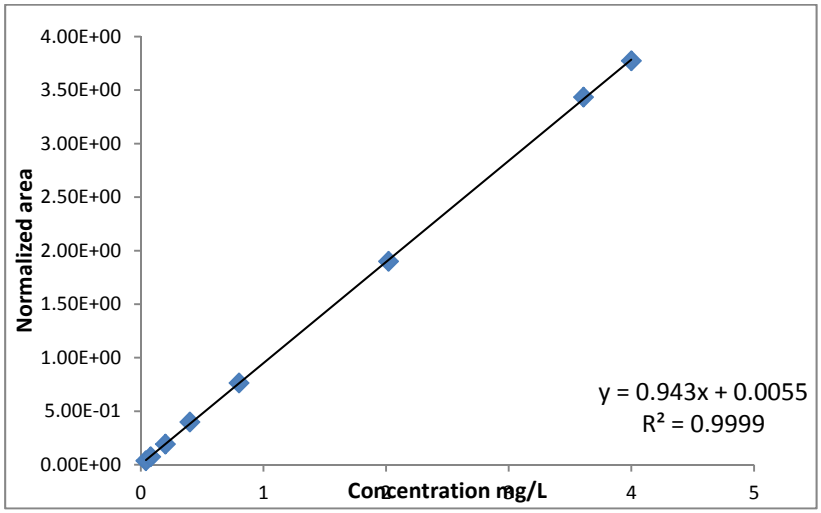

Figure 35. Calibration curve for furfural

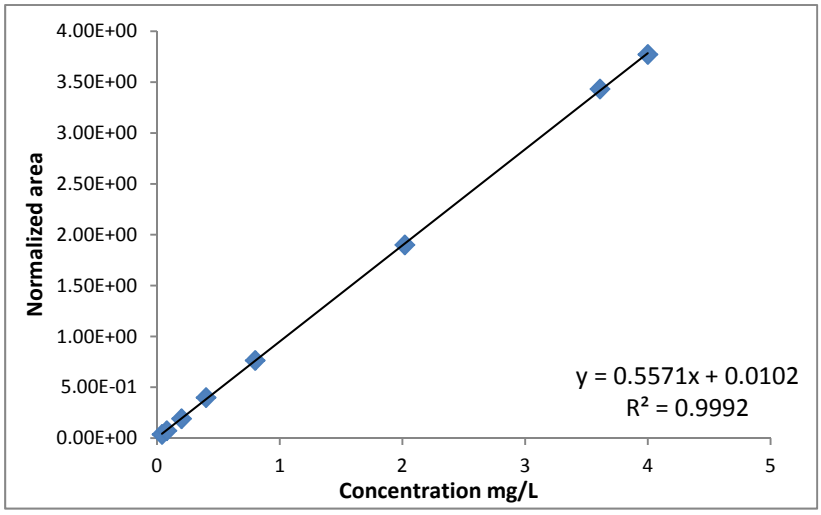

Figure 36. Calibration curve for 5-methyl-2-furfural

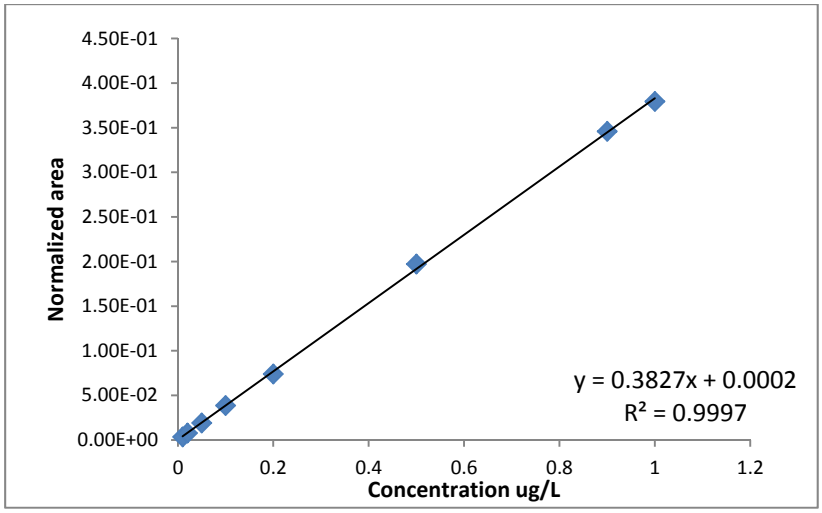

Figure 37. Calibration curve for α-terpeniol

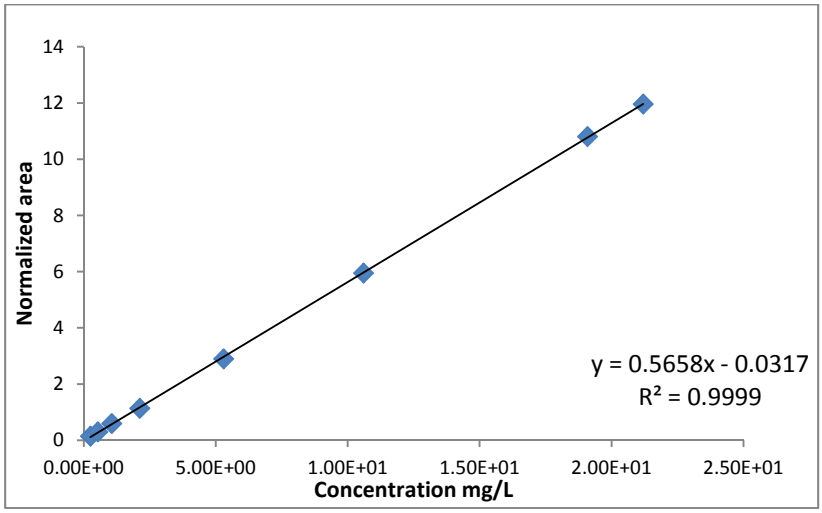

Figure 38. Calibration curve for butyric acid

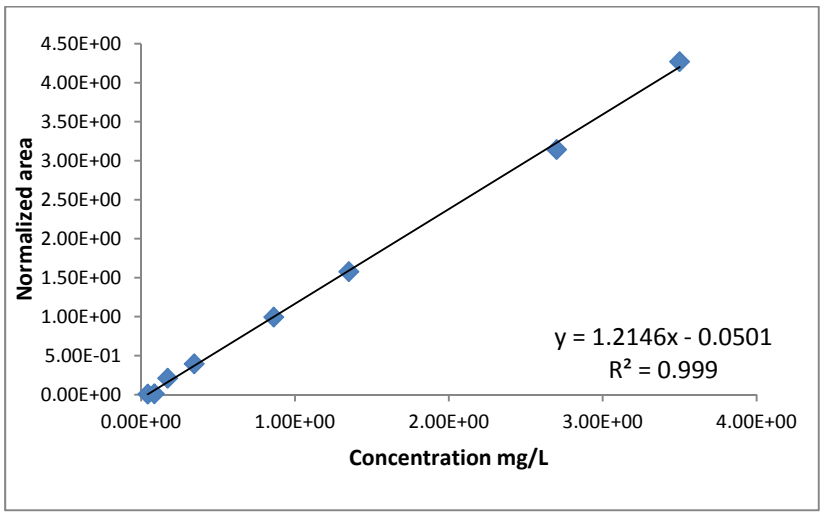

Figure 39. Calibration curve for ethyl decanoate

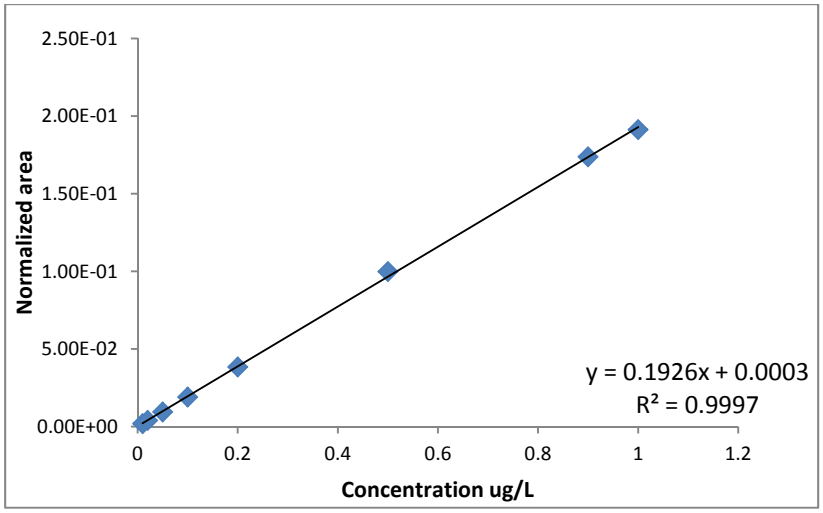

Figure 40. Calibration curve for citronellol

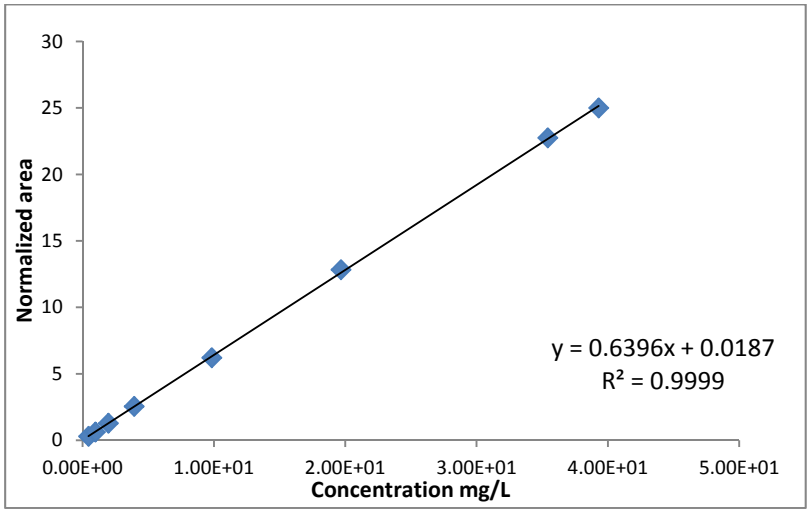

Figure 41. Calibration curve for isovaleric acid

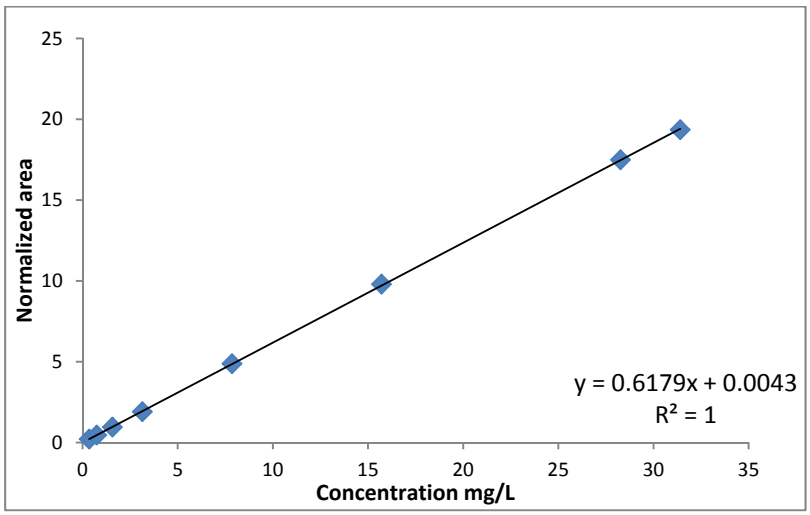

Figure 42. Calibration curve for diethyl succinate

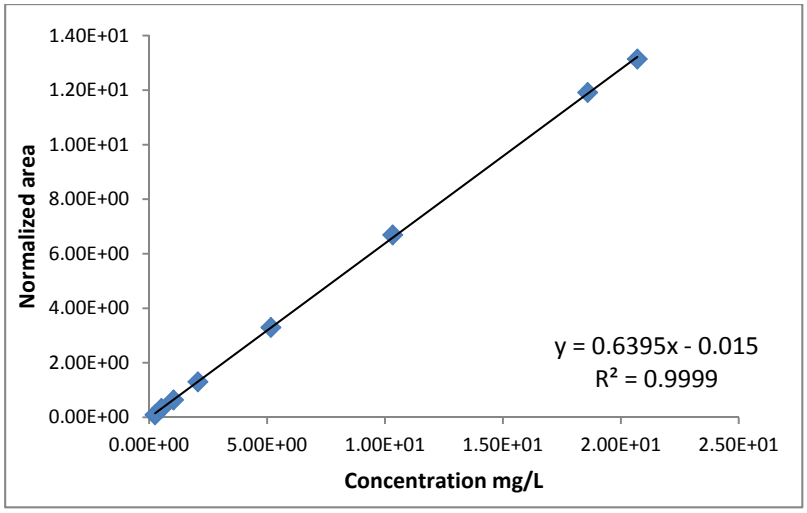

Figure 43. Calibration curve for valeric acid

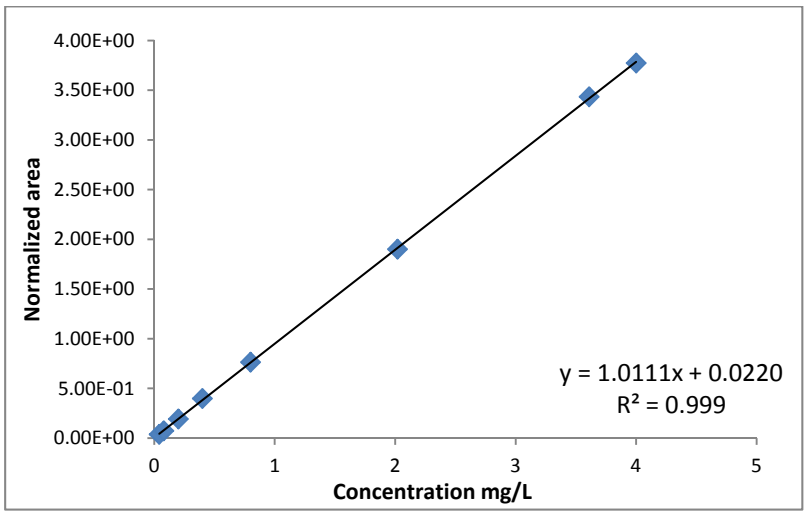

Figure 44. Calibration curve for furfuryl alcohol

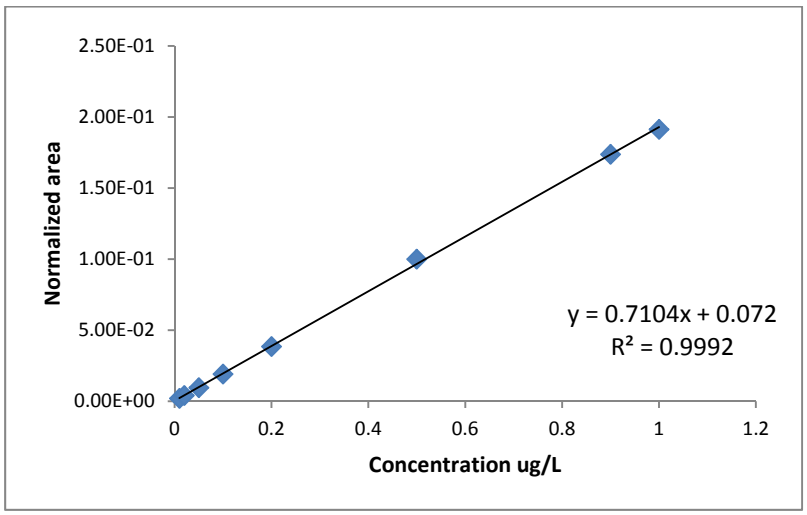

Figure 45. Calibration curve for guaiacol

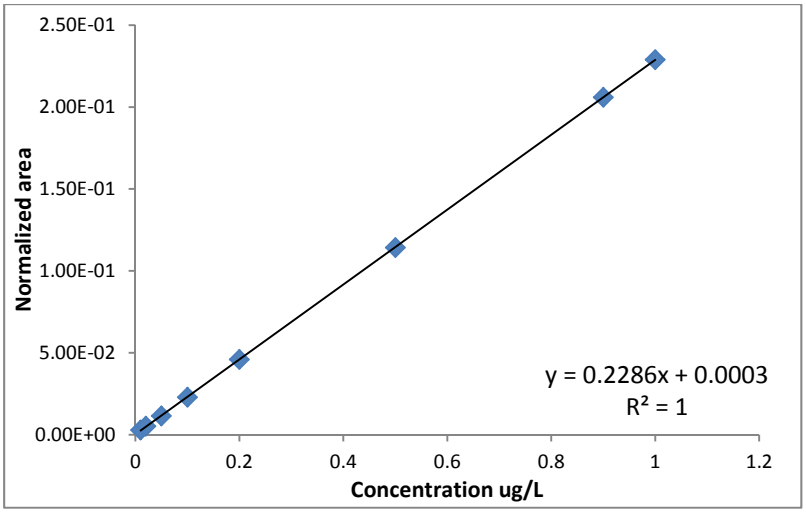

Figure 46. Calibration curve for geraniol

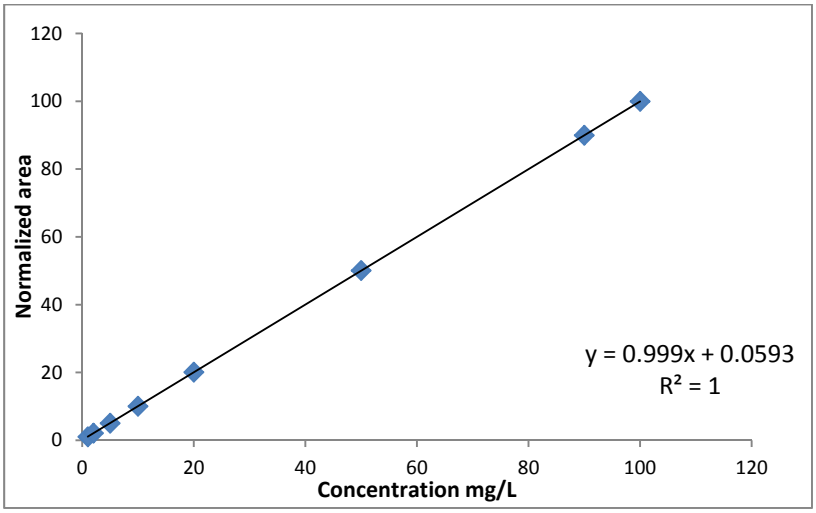

Figure 47. Calibration curve for ethyl phenyl acetate

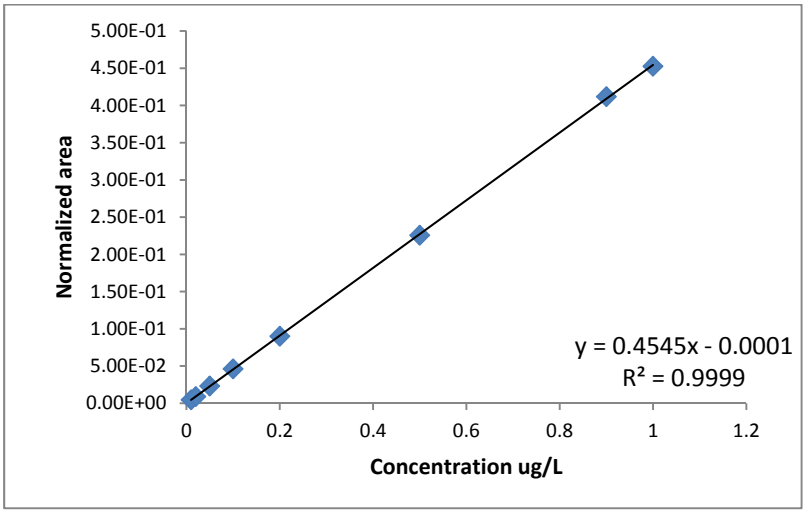

Figure 48. Calibration curve for nerol

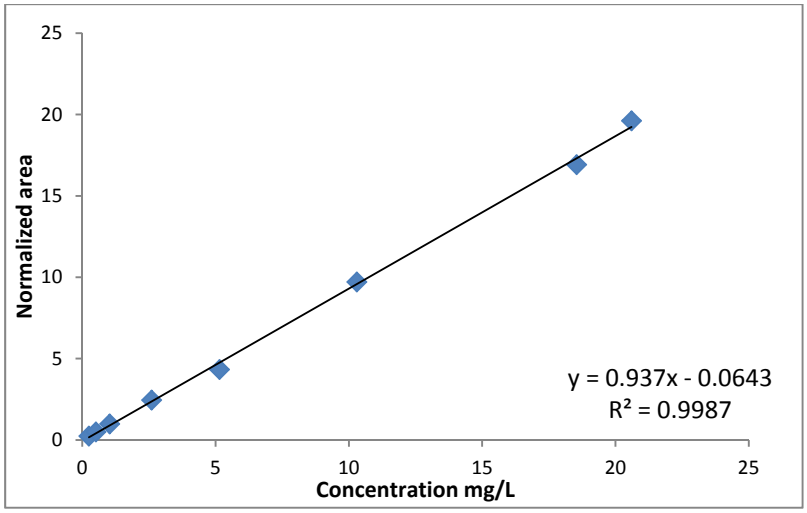

Figure 49. Calibration curve for 2-phenylethyl acetate

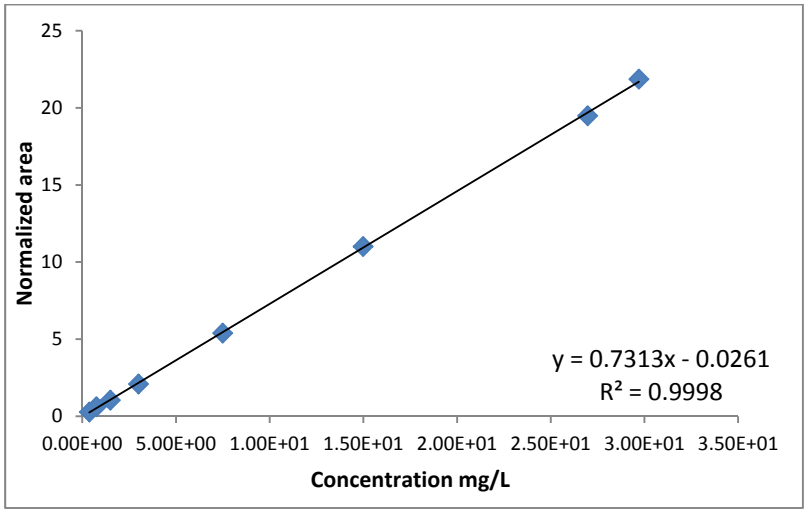

Figure 50. Calibration curve for hexanoic acid

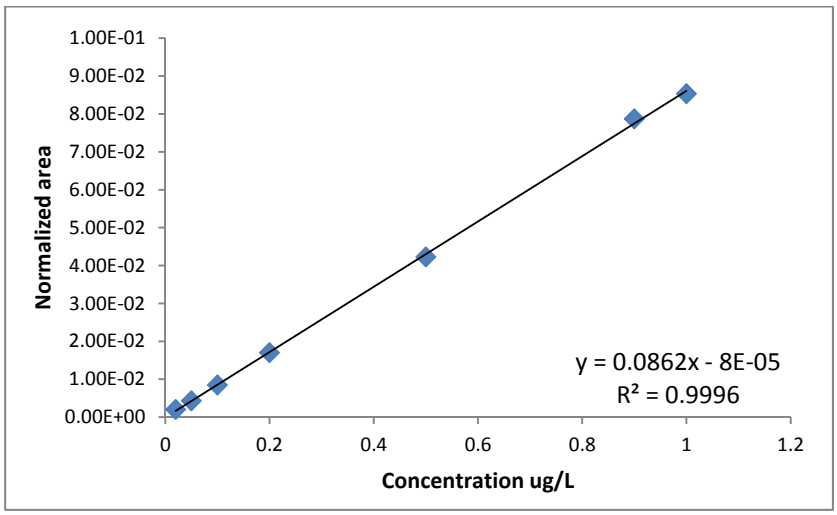

Figure 51. Calibration curve for α-ionone

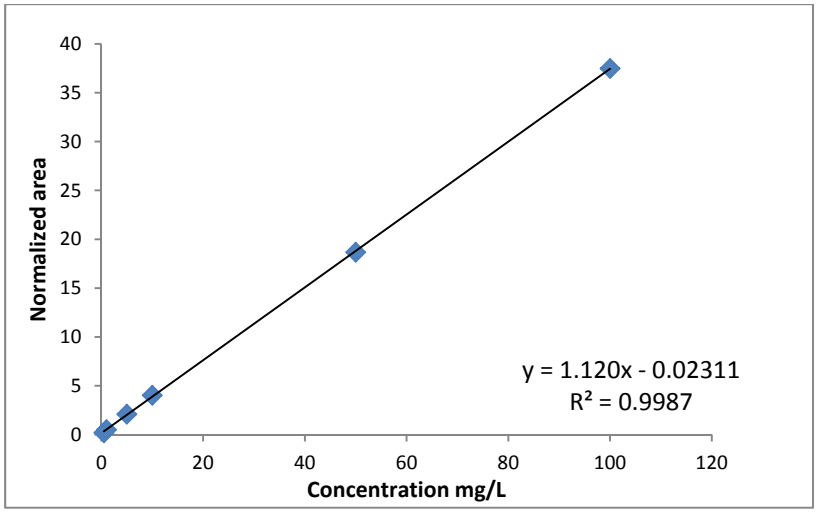

Figure 52. Calibration curve for whiskey lactone

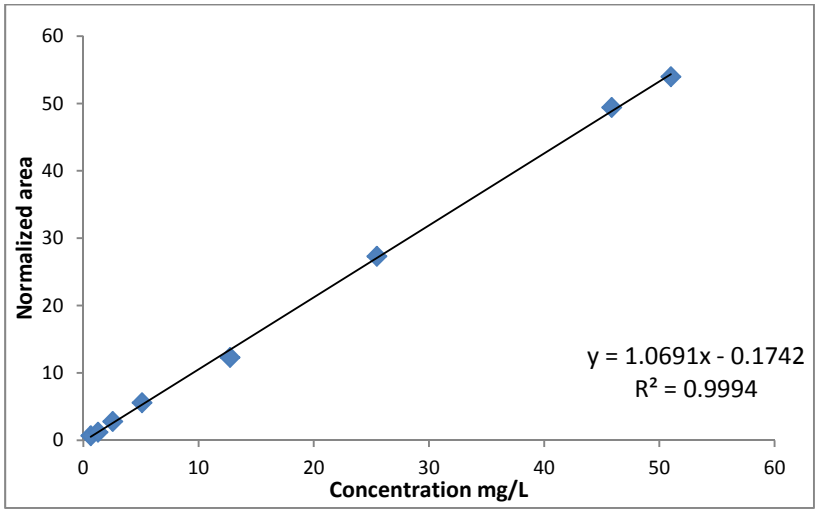

Figure 53. Calibration curve for 2-phenyl ethanol

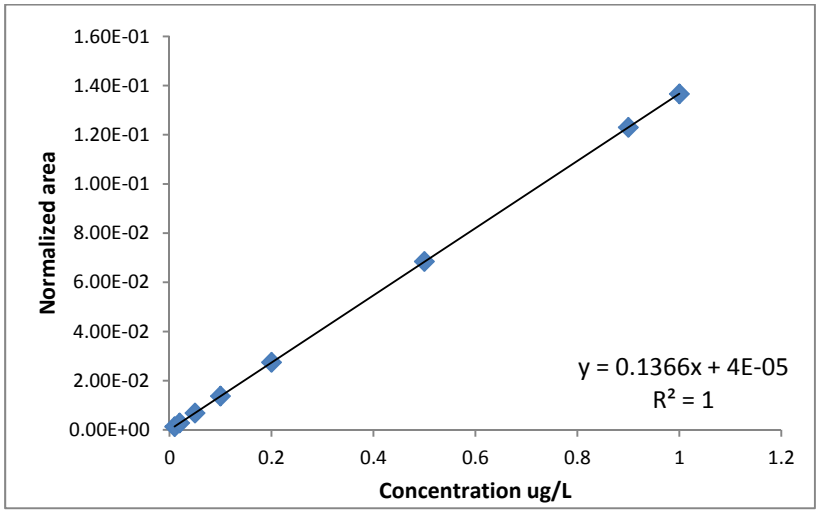

Figure 54. Calibration curve for β-ionone

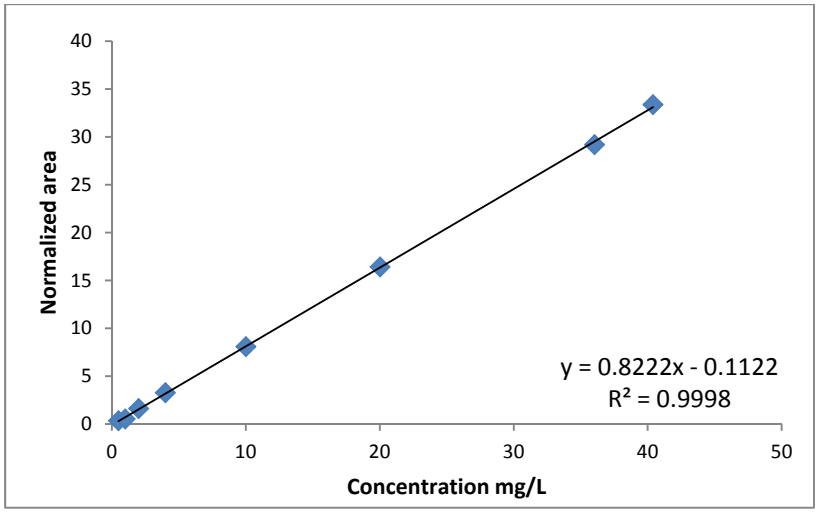

Figure 55. Calibration curve for octanoic acid

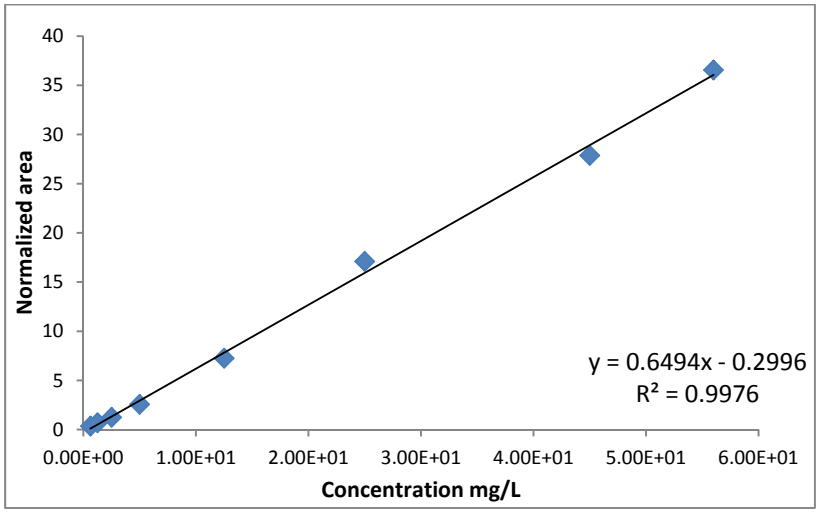

Figure 56. Calibration curve for decanoic acid

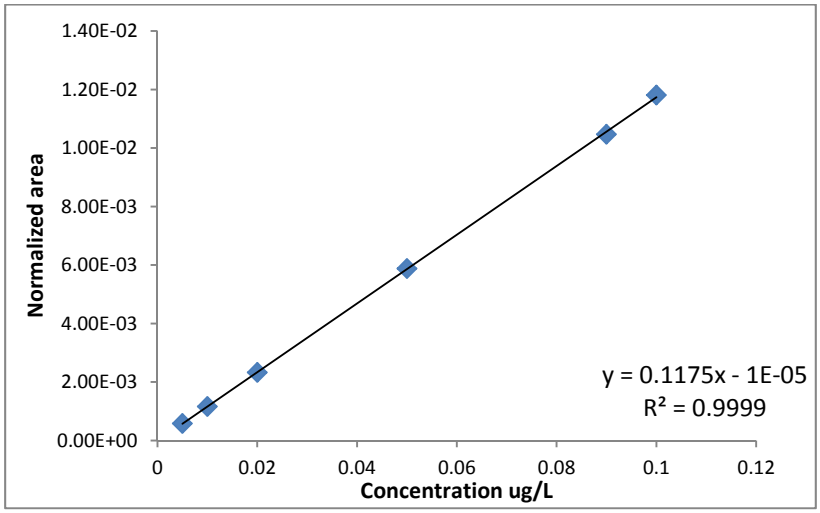

Figure 57. Calibration curve for β-farnesol

CONCLUSION:

The parameters evaluated are within the acceptable limits for food analysis, as per the United States Food and Drug Administration (2001).

Please find below a list of the parameters used for method validation and a short description of each

SELECTIVITY/SPECIFICITY

The selectivity of a method is defined as the ability of the method to accurately measure an analyte in the presence of interferences that may be expected to be present in the sample matrix. The method should be tested with standards and with real samples. Selectivity and specificity are used interchangeably.

LINEARITY

The linearity of an analytical method is its ability (within a certain range) to extract test results that are directly proportional to the concentration of analytes in samples (or proportional by means of a well defined mathematical calculation). Linearity is typically determined by a series of standards whose concentrations span 80-120 % of the expected concentration range of the analyte.

RANGE

Concentration interval from upper to lower concentration, for which the calibration curve is linear (detector response is linear vs concentration).

RECOVERY/ACCURACY

The accuracy of an analytical procedure is the level of agreement between a reference value and a measured value. The recovery of an analyte is the amount that is quantified relative to the amount present in the reference standard.

PRECISION

The precision of a method is the extent to which individual test results of multiple injections the same standard/sample agree. Precision can be subdivided into three categories: repeatability, intermediate precision and reproducibility (given below).

REPEATABILITY

Repeatability is the within-day repeatability, and it is obtained when the analysis is carried out in one laboratory by a single operator using one piece of equipment over a relatively short time-span.

INTERMEDIATE PRECISION

The intermediate precision is a longer-term variability (inter-day repeatability) of the measurement process. It is determined by comparing the results of a method run within a single laboratory, with multiple operators using different equipment, over a few days.

REPRODUCIBILITY

The reproducibility is the precision between laboratories and is often used for standardisation of methods.

LIMIT OF QUANTITATION/ DETECTION

The limit of detection (LOD) is the minimum amount of analyte in a sample that can be detected but not necessarily quantified. It is the lowest possible injected amount that results in a peak of height three times as high as the baseline noise.

The limit of quantitation (LOQ) is the minimum amount of analyte that can be quantitatively determined with accuracy and precision. It is the minimum injected amount that gives a peak height ten times higher than baseline noise.

REFERENCES

US Department of Health and Human Services, Food and Drug Administration Centre for Drug evaluation and Research (CDER) Centre for Veterinary Medicine (CVM) – Guidance for Industry, Bioanalytical Method Validation. Accessed June 2010 from: http://www.fda.gov/ (2001).

ADDENDUM B

PUBLICATIONS AND PRESENTATIONS

ADDENDUM B

The following posters and publications were derived from this work:

Publications:

D. Fracassetti, N. Lawrence, A.G.J. Tredoux, A. Tirelli, H.H. Nieuwoudt & W.J. du Toit. 2011. Quantification of glutathione, catechin and caffeic acid in grape juice and wine by a novel ultra-performance liquid chromatography method. *Food Chemistry* 128: 1136-1142.

Posters and presentations at conferences:

D. Fracassetti, N. Lawrence, A.G.J. Tredoux, A. Tirelli, H.H. Nieuwoudt, W.J. du Toit. Quantification of glutathione in must and wine by ultra-performance liquid chromatography. Thirty Second Conference of the South African Society for Enology and Viticulture, Lord Charles Hotel, Somerset West. 18-19th November 2010, poster presented

N. Lawrence, D. Fracassetti, A. Tirelli, H. H, Nieuwoudt, W. J. du Toit, A. G. J. Tredoux. Development of Ultra-performance liquid chromatography (UPLC) methods for the analysis of antioxidants in wine. Thirty Second Conference of the South African Society for Enology and Viticulture, Lord Charles Hotel, Somerset West. 18-19th November 2010, oral presentation

N. Lawrence, A.G.J. Tredoux, H.H. Nieuwoudt, T. Skov, Development of an advanced multidimensional technique for Ultra-high performance liquid chromatography with photodiode array detector (UPLC-DAD) for more adequate handling of complex data in wine analysis. International symposium Analitika, Stellenbosch, South Africa, 5-9th December 2010, Poster presented

N. Lawrence, D. Fracassetti, H.H. Nieuwoudt, A. Tirelli, W. J. du Toit, A.G.J. Tredoux. Development and validation of an analytical method for the quantification of glutathione in white wine and juice by ultra-performance liquid chromatography (UPLC). International symposium Analitika, Stellenbosch, South Africa, 5-9th December 2010, poster presented

N. Lawrence, A. G. J. Tredoux, H. H. Nieuwoudt, T. Skov. Improved handling of chromatographic wine aroma data. 12th Scandinavian Symposium on Chemometrics, Billund, Denmark, 7-10th June 2011, poster presented.