Libraries and Learning Services

University of Auckland Research Repository, ResearchSpace

Copyright Statement

The digital copy of this thesis is protected by the Copyright Act 1994 (New Zealand).

This thesis may be consulted by you, provided you comply with the provisions of the Act and the following conditions of use:

- Any use you make of these documents or images must be for research or private study purposes only, and you may not make them available to any other person.
- Authors control the copyright of their thesis. You will recognize the author's right to be identified as the author of this thesis, and due acknowledgement will be made to the author where appropriate.
- You will obtain the author's permission before publishing any material from their thesis.

General copyright and disclaimer

In addition to the above conditions, authors give their consent for the digital copy of their work to be used subject to the conditions specified on the Library Thesis Consent Form and Deposit Licence.

The Characterisation of Central Otago Pinot Noir Wines

Tanya Rutan

A thesis submitted in partial fulfilment of the requirements for the degree of Doctor of Philosophy in Chemistry

The University of Auckland 2016

Abstract

The importance of Pinot noir for the New Zealand wine industry has grown and stands second only to Sauvignon blanc in production volume. This thesis investigates the chemical composition of the aroma of Central Otago Pinot noir and its sensory description with the aim of characterising it in a way that an association is made with the typicality of the region. The first stage included a general exploration of the composition of Pinot noir where the chemical aroma, phenolic, tannin composition and colour properties of 105 Pinot noir wines from New Zealand, Australia, France and USA were determined using five different analytical methods across two vintages. The main finding was that the chemical constituents of Pinot Noir wines can vary both between and within different growing regions. There were several compound families where perception thresholds were exceeded for most of the wine samples, including C_{13} norisoprenoids, higher alcohols, esters of isoacids and fatty acids and cinnamic esters; these can be considered of importance to the overall varietal aromas of Pinot Noir wines. The second aim was to focus on Pinot noir wines from Central Otago where both chemical composition and sensory attributes were explored using reconstitution studies. Aroma Extract Dilution Analysis (AEDA) was applied to two distinct Central Otago Pinot noir wine styles, a blended estate and a single vineyard premium, over two consecutive vintages. The aroma compounds and/or compound families of importance varied across the wines, while a number of compounds were in common. A total of 42 odorants were identified in the AEDA study with flavour dilution (FD) factors ranging from 3 to 19683, with over 20 having FD > 81. The compounds with the highest FDs for the Estate wines were fruity esters and phenylethyl alcohol, while for the Premium wines the norisoprenoids and volatile phenols originating from oak had the highest FDs. The aroma reconstitution experiments, which considered both chemical and sensorial properties, revealed no overwhelming differences when compound families were omitted. Overall, these results suggest that Central Otago Pinot noir wines do not depend on a few key odorants for their aromatic complexity, but instead on the interactions of many aromatic compounds. An additional aim of the study was to measure the

effects on wine quality and volatile composition of two cluster thinning regimes on *Vitis vinifera* cv. Pinot noir in vineyards located in Central Otago across three seasons. The main finding here was that cluster thinning may have an effect on ripening times and the non-volatile and volatile chemical composition of the subsequent wines. These effects may also lead to detectable sensory differences in the final product. It would seem that crop thinning, while a costly practice due to increased labour and yield reduction, is effective in influencing wine quality. However, a particularly intense level of thinning was not necessary to achieve differences in the wines, as a moderate level of thinning also provided a marked enhancement to several attributes.

Acknowledgements

I am very grateful to so many people that have assisted me along the way on this long journey to accomplish this dream.

I would like to thank The University of Auckland, Ministry of Business Innovation and Education and New Zealand Wine Growers for the funding and support.

An enormous thank you to my supervisor, Prof. Paul Kilmartin, for his support, advice and encouragement over the years. His patience and kindness meant more than he will ever know.

Another massive thank you to Mandy Herbst-Johnstone for her support, assistance in the lab and assistance with just about everything. Our days in the lab will never be forgotten as they were some of the best times in my life. Thanks for being such a great friend.

A big thanks to the Wine Science team over the years. Jan Robertson always kept the lab stocked and gave great advice. Randy Weaver, for sharing all your winemaking knowledge. Frank Benkwitz for sharing all your knowledge on GC-O and reconstitution studies and for being a great friend. Dang Dung Nyguyen, thanks for all your help with the phenolics work and for being a wonderful friend.

I would like to thank Benedicte Pineau and Michelle Beresford and the rest of the team at Plant and Food research for assisting with the sensory studies.

I am ever so grateful for the opportunity to work with Matt Dicey at Mt. Difficulty. Thanks for all your winemaking knowledge and allowing me to invade your winery. James Dicey and the Grape Vision team for assisting me in my vineyard trials. Robin Dicey for all your great wisdom on just about everything.

My greatest gratitude goes to my partner, Len Ibbotson, who has tolerated me through the many years on this long journey. Thanks for your patience, kindness, knowledge, understanding and most of all for your love. Big thanks to my little angels, Sophia Rose and Edgar Allan who make every day brighter and make all the stress leave my body with one big cuddle.

Table of Contents

Chapter 1 1

Introduction 1

1.1 Project aims 3

1.2 Thesis structure 5

Chapter 2 6

Review of the Literature 6

2.1 Varietal aromas 6

2.1.1 Terpenes 7

2.1.2 C_{13} Norisoprenoid derivatives 11

2.1.3 Sulfur compounds with a thiol function 14

2.7.4 C_6 aldehydes and C_6 alcohols 17

2.1.5 Aminobenzoate and phenylpropanoid esters 20

2.2 Aromas formed by biological transformation 23

2.2.1 Higher fermentation alcohols 24

2.2.2 Fatty acids 27

2.2.3 Esters 30

2.2.4 Volatile phenols 34

2.2.5 Oak derived volatiles 37

2.3 Sensory analysis 39

2.3.1 QDA® 39

2.3.2 Panellist selection 40

2.3.3 Development of descriptors 40

2.3.4 Training the panel 41

2.4 Controlling bias 42

2.4.1 Panellist physiological bias 43

2.5 Disadvantages of QDA® 44

2.6 Descriptive analysis of Pinot noir wines 45

2.7 Identification of key aroma compounds of Pinot noir wines by means of linking chemistry with sensory 47

2.7.1 GC-O 48

2.7.1.1 Detection frequency 48

2.7.1.2 Dilution to threshold 49

2.7.1.3 Direct intensity 50
2.7.2 Isolation and identification 50
2.7.3 Quantitative determination of the odorants 52
2.7.4 Reconstitution studies 53
2.7.5 Roles odorants can play in wine 54
2.8 Influences on aroma compound formation and their roles in wine 55
2.9 Summary 57
Chapter 3 59
Survey of major aroma compounds and phenolics in Pinot noir wines from New Zealand, France, Australia and USA 59
3.1 Introduction 61
3.2 Materials and methods 62
3.2.1 Wine samples 62
3.2.2 Aroma extraction and analysis 63
3.2.3 Phenolic analyses 65
3.2.3.1 Tannin analyses 66
3.2.3.2 Colour analyses 66
3.2.4 Reagents 66
3.2.5 Statistical analysis 67
3.3 Results and discussion 67
3.3.1 Aroma compounds 67
3.4 Conclusions 71
Chapter 4 83
Characterisation of the aroma of Central Otago Pinot noir wines using sensory reconstitution studies 83
4.1 Introduction 85
4.2 Materials and methods 86
4.2.1 Wine samples 86
4.2.2 Reagents 87
4.2.3 Isolation of volatile compounds for gas chromatography-mass spectroscopy (GC-MS) analyses 88
4.2.4 Isolation of volatile compounds for gas chromatography-olfactometry analysis (GC-O) 90
4.2.5 Aroma extract dilution analysis (AEDA) 90
4.2.6 Deodorisation of wines 91
4.2.8 Sensory analysis 91

4.2.9 Statistical analysis 92
4.3 Results and discussion 93
4.3.1 GC-O and AEDA 93
4.3.2 Chemical composition and OAVs 96
4.3.3 Aroma reconstitution experiments 96
4.4 Conclusions 99
Chapter 5 112
Effect of Cluster Thinning *Vitis vinifera* cv Pinot noir on Wine Volatile and Phenolic Composition and Quality 112
5.1 Introduction 114
5.2 Materials and methods 116
5.2.1 Vineyard site and experimental design 116
5.2.2 Yield components 117
5.2.3 Canopy characterisation 117
5.2.4 Juice composition 117
5.2.5 Winemaking 118
5.2.6 Reagents 118
5.2.7 Isolation of volatile compounds for GC-MS analyses 119
5.2.8 Polyphenol analysis 120
5.2.8.1 Tannin analysis 121
5.2.9 Sensory analysis 122
5.2.10 Statistical analysis 123
5.3 Results and discussion 123
5.3.1 Canopy and fruit characterisation 123
5.3.2 Basic juice composition 123
5.3.3 Quantification of aroma compounds 124
5.3.4 Phenolics composition 127
5.3.5 Sensory difference tests 128
5.4 Conclusions 129
Chapter 6 142
Conclusions and Future Work 142
6.1 Chemical Survey of Pinot noir Wines from Different Countries 143
6.2 Characterising the Key Aroma Compounds in Central Otago Pinot noir wines 144
6.3 Effects of Cluster Thinning on the Aromatic and Phenolic Compounds of Pinot noir Wines 146
6.4 Summary 147

List of Tables

Chapter 2

Table 2.1 Monoterpenes - olfactory description and perception thresholds 9

Table 2.2 C_{13} Norisoprenoids - olfactory descriptions and perception thresholds 12

Table 2.3. Sulfur compounds with a thiol function - olfactory descriptions and perception thresholds 16

Table 2.4 C_6 Alcohols - olfactory descriptions and perception thresholds 19

Table 2.5 Amino benzoates and phenyl propanoids - olfactory descriptions and perception thresholds 20

Table 2.6 Higher alcohols - olfactory descriptions and perception thresholds 25

Table 2.7 Fatty acids - olfactory descriptions and perception thresholds 29

Table 2.8 Esters - olfactory descriptions and perception thresholds 33

Table 2.9 Volatile phenols including oak derivatives- olfactory descriptions and perception thresholds 36

Chapter 3

Table 3.1 Supplier, concentration range, instrument employed, ions, retention times, and R^2 values for the phenolic (mg/L) used in this study 72

Table 3.2 Supplier, concentration range, instrument employed, ions, retention times, and R^2 values for the aroma compounds (µg/L) used in this study 74

Table 3.3 Mean concentrations of aroma compounds (µg/L), phenolics (mg/L), tannins (mg/L) and colour properties (absorbances) for the 2005-2006 and 2006-2007 surveys. Minimum and maximum range includes both surveys 75

Chapter 4

Table 4.1 Linearity, quantification data and ions chosen for SIM mode for the compounds analysed in this study 99

Table 4.2 Compounds selected for the composition of the recombinant models 102

Table 4.3 Sensory descriptors used in trained panel evaluations 103

Table 4.4 Compounds detected by aroma detection dilution analysis in conjunction with their corresponding retention indices (RI) on two columns, and flavour dilution (FD) factors for the 2009 and 2010 Estate and Premium wines obtained on the INNOWax column 104

Table 4.5 Concentration (µg/L) and odour activity values (OAV) of aroma compounds in the Estate and Premium wines for 2009 and 2010 vintages 106

Table 4.6 Omission tests for Estate and Premium complete models 109

Chapter 5

Table 5.1 Linearity, quantification data and perception thresholds for the volatile compounds and phenolics and phenolics analysed in this study 129

Table 5.2 °Brix, pH, TA, bunch weight and harvest date for the grapes harvested in 2009 to 2011 with corresponding r coefficients 131

Table 5.3 Volatile composition of wines made from Pinot noir grapes at different crop yields, achieved through bunch thinning for the 2009 vintage 132

Table 5.4 Volatile composition of wines made from Pinot noir grapes at different crop yields, achieved through bunch thinning for the 2010 vintage 133

Table 5.5 Volatile composition of wines made from Pinot noir grapes at different crop yields, achieved through bunch thinning for the 2011 vintage 134

Table 5.6 Phenolic composition (mg/L) and tannins (mg/L epicatechin equivalents) of wines made from Pinot noir grapes at different crop yields, achieved through bunch thinning for the 2009 vintage. 135

Table 5.7 Phenolic composition and tannins (mg/L epicatechin equivalents) of wines made from Pinot noir grapes at different crop yields, achieved through bunch thinning for the 2010 vintage 136

Table 5.8 Phenolic composition and tannins (mg/L epicatechin equivalents) of wines made from Pinot noir grapes at different crop yields, achieved through bunch thinning for the 2011 vintage 137

Table 5.9 Results from triangle test for wines made from the 2009 to 2011 harvests 138

Table 5.10 Results of paired comparison test for wines made from the 2009 to 2011 harvests 139

List of Figures

Chapter 2

Figure 2.1 Biosynthesis of monoterpenes in grapes 8

Figure 2.2 Biosynthesis of C_{13} norisoprenoids 11

Figure 2.3 Proposed pathways for sulfur compounds with a thiol function 15

Figure 2.4 Biosynthetic pathway for C_6 aldehydes and alcohols 18

Figure 2.5 Biosynthetic pathway for amino benzoates and phenylpropanoids 22

Figure 2.6. Derivation of yeast fermentation flavour compounds 23

Figure 2.7 Biosynthesis of higher alcohols 24

Figure 2.8 Biosynthesis of fatty acids 28

Figure 2.9 Esterification 30

Figure 2.10 Biosynthesis of ethyl phenols 35

Figure 2.11. *Cis* and *trans* isomers of beta-methyl-gamma-octalactone 37

Chapter 3

Figure 3.1a Principal component analysis showing the relations between the chemical parameters for the 2005-2006 survey 77

Figure 3.2b Principal component analysis showing the position of the wines in relation to the chemical parameters for the 2005-2006 survey 78

Figure 3.3a Principal component analysis showing the relations between the chemical parameters for the 2006-2007 survey 79

Figure 3.4b Principal component analysis showing the position of the wines in relation to the chemical parameters for the 2006-2007 survey 80

Chapter 1

Introduction

The importance of Pinot noir for the New Zealand (NZ) wine industry has grown and stands second only to Sauvignon blanc in production volume. According to NZ Wine Growers, the increased growing area from 4773 hectares in 2010 to 5509 hectares in 2014 has seen a 54% increase in harvested fruit from 23,655 to 36,499 tonnes, respectively. This increase in production has been followed by a growth in the export sales of Pinot noir in the last five years, with 6.1 million litres exported in 2009 up to 10.7 million litres in 2014.

Since the 1990s, plantings have expanded throughout all regions in the South Island and also in a few selected sites in the North Island with each region claiming its own distinct style. The noticeable differences in the wines from different areas led to many questions and subsequently to a two year research project examining the chemical properties of juice and wine including aroma, phenolics, tannins and all standard measurements typically taken before, during and after fermentation. The research was conducted by MSc students Sonal Maharaj and Tanya Rutan across two growing seasons, 2008 and 2009 respectively. Three wineries from each of three major Pinot noir growing region participated in the research: Martinborough – Craggy Range, Martinborough Vineyards and Escarpment; Marlborough – Villa Maria and Pernod Ricard (2 vineyards); Central Otago – Felton Road, Mt. Difficulty and Olssens. The results indicated that there were chemical differences in the wines with Central Otago appearing to be the most distinctive. This thought provoking information caught the attention of several winegrowers in the Central Otago region and it was clear that while the sample size was small, further research

should be undertaken examining the chemical and sensory components of the wines coming from this unique region.

Historically, Central Otago was noted as being a highly suitable region for growing Pinot noir grapes by Romeo Bragato and the first vines were planted by Jean Feraud in 1864. However, stone fruit prevailed and there was little interest in planting more until the 1950s and later significant commitments while modest were made in the 1970s and then a rapid expansion in the mid-1990s. In 1996 there were 11 wineries in the Central Otago region accounting for just 4.6% of the national total; in 2014 this had risen to 132 wineries making 18.9% of the national total. Over the same period, the area planted with vines rose from 92 hectares, 1.4% of the national total, to 1,932 hectares and 5.4%, respectively, with 77% being Pinot noir.

Central Otago is the world's southernmost wine region and NZ's highest. There are six sub-regions, namely, Wanaka, Gibbston Valley, Bannockburn, Bendigo, Alexandra and Cromwell/Lowburn/Pisa. While the sub-regions lie within close proximity, the soils can vary considerably and the mountainous terrain means each occupies a unique niche of climate, aspect and altitude; nevertheless, each sub-region has characteristics common to all. In general the region has a semi-continental climate with marked daily and seasonal temperature extremes. The summers are short and hot with high sunshine (1973 annual average hours), autumn is cool and dry with low humidity (637 mm annual average rainfall), while winter is cold with snowfall and spring is accompanied by high winds and frequent frosts. Overall, the structure of the soil consists of heavy deposits of rough edged mica and other metamorphic schists in silt loams that make for a free draining base, making irrigation essential. While this severe landscape can make for harsh growing conditions, it also provides beneficial assets for adding complexity and distinctiveness to the wines.

One of the largest producers in the Bannockburn sub-region and one of NZ's iconic wineries is Mt. Difficulty Wines Ltd. Participating in the aforementioned research (Maharaj [1] and Rutan [2]) for both years, Mt. Difficulty is dedicated and eager to be at the forefront of the wine industry by

using science to craft wines that speak with a sense of place. Established in 1992, Mt Difficulty Wines is home to some of Central Otago's oldest and most revered vineyards. They believe that the individual vineyards have a specific terroir that is largely influenced by climate and offer a variety of soil textures, from open gravels to heavier clays. They are all high in pH, low in fertility, and include light sands, clays, loams and gravels. Manipulation of the soil by man, which came about as a result of hydraulic mining and sluicing in the gold mining era of the late 1800's, can also be found on some blocks.

With the variations in soils, microclimates and grape clones that each site has, the team at Mt. Difficulty found that each location contributed significantly different features to the final product. The philosophy of the company is to make wines that display the characteristics that are particular to their terroir and thus tell the unique story of its own place. This mission required the company to gain more knowledge about their vineyards and wines. The success of their Pinot noir wines is undoubtedly linked to its distinctive flavours, therefore, applying the knowledge gained from the previous research, a new project was developed.

1.1 Project aims

Commencing in 2010, a PhD research project on characterising the primary odorants of Central Otago Pinot noir was funded by the Ministry of Business Innovation and Employment (formerly Foundation for Research, Science and Technology) and NZ Wine Growers. The ultimate goals of this three year project were to identify potential impact and/or key aroma compounds in Central Otago Pinot noir and determine the effects that different levels of crop thinning, a common viticultural practice, may have on these compounds as well as other parameters of the wine. Mt. Difficulty's Pinot Noir wines, winery and vineyard sites were used in undertaking this research.

The emphasis and focus during the first stage of this project was to quantify known impact compounds identified in other varietal wines as well as compounds that commonly occur in red wines. This data would provide information on the concentration ranges and ratios of the

volatiles found across a broad selection of Pinot noir wines from distinct growing regions around the world. In addition, field trials were set up in one of Mt Difficulty's largest and oldest vineyards. The trial included different crop thinning regimes from which wines were made and chemically analysed. Traditionally this type of study has only examined extreme cases, all or none, of thinning treatments and has not replicated the typical growing conditions or made the wines on a commercial scale. In addition, the analyses of the volatile composition followed by sensory analysis of such wines had not been previously undertaken. These trials and subsequent analyses were repeated across three seasons.

The second phase of the research project involved an attempt to identify new compounds via gas chromatography studies using aroma extraction dilution analysis. This screening tool gave some insight into the potential importance of each compound through assigning flavour dilution factors. The compounds identified were quantified with methods developed specifically for each aromatic family, and odour activity values were calculated conveying more insight into each compound's potential importance and role. The ultimate step in understanding each compound's importance is through recombinant studies which were performed as the final phase of the research. Due to various limitations and costs, many researchers have been unable to undertake such studies. For this analysis two of the original Mt. Difficulty Pinot Noir wines were deodorised and the aroma reconstituted commercially available aroma compounds. This novel approach of using the original wine instead of a model wine solution provided for a more precise evaluation, as this approach takes the non-volatile interactions in the wine matrix into consideration. Further recombinant models were created where particular aroma families were excluded as a way to assess their role in the overall aroma. The original and omission models were presented to a trained sensory panel. This data, along with the previous analyses, were used to determine the roles and potential importance of the volatiles in Central Otago Pinot noir wines.

1.2 Thesis structure

Including this introduction, this thesis contains six chapters. Chapter 2 is a literature review lending insight on the thought process surrounding the sequencing of the project and information and validation of why particular methodologies were used. This chapter also includes relevant past and current work completed on Pinot noir wines. Chapters 3, 4 and 5 are presented as complete journal papers including abstract, introduction, aims, methodology, results, discussion and conclusions for each respective project goal discussed in Chapter 1. Chapter 6 provides the overall conclusions from the project and future research. A complete list of references is at the end of Chapter 6 listed numerically as they appear in the thesis.

Chapter 2

Review of the Literature

The main focus of this chapter is to give some insight into the aroma compounds typically present in Pinot noir wines and their sensorial effects. The review begins with introducing aroma compounds typically present in most wines and their proposed biological and chemical pathways. These details are followed by historical and current studies which focused on methodologies used in determining aroma composition in Pinot noir wines.

Secondly, the review introduces methods used in determining sensorial effects of aroma compounds in wine where panellists are utilised. Both pros and cons of these methods are considered. Finally, various practices by which aroma composition can be altered in wines are reviewed briefly with a more comprehensive examination of cluster thinning of which was the selected approach for consideration in this research.

2.1 Varietal aromas

Aromas that arise from grape-derived compounds are commonly known as varietal aromas. These aroma compounds are often reflections of a particular *Vitis vinifera* variety and a region's soil and climate. Many of these compounds have been found to exist in the grapes and in the grape vine's leaves as non-volatile precursors (glycosides and cysteine conjugates), and are released upon the rupturing of the cell walls during harvesting and winemaking procedures. The cell decompartmentation is accompanied by liberation of precursors and enzymes. The released glycosides undergo hydrolysis via glycoside hydrolases, which are present in the grape juice or originate from yeast metabolism. *Saccharomyces cerevisiae* also mediates the cleavage of non-volatile cysteinylated precursors in grape juice to release volatile thiols.

2.1.1 Terpenes

Terpenes are widespread in nature, mainly in plants as constituents of essential oils. Many terpenes are hydrocarbons, although oxygen containing compounds such as alcohols, aldehydes, or ketones are also found and are known as terpenoids. The building block of terpenes is the hydrocarbon isoprene, $CH_2=C(CH_3)-CH=CH_{2.,}$ and terpenes are therefore classified according to the number of isoprene units. The monoterpenes (10 carbon atoms) and sesquiterpenes (15 carbon atoms), formed from two and three isoprene units, are the most odoriferous. These compounds are biologically synthesized from isopentyl pyrophosphate (IPP) and dimethyl lallyl pyrophosphate (DMAPP). These precursors are formed either through the cystonic melvalonic-acid (MVA) pathway [3] or through the plastidial 2-C-meth lery thritol-4-phosphate (MEP) pathway [4]. Subsequently, monoterpenes are formed from geranyl pyrophosphate (GPP) (Figure 2.1) and exist in grapes mainly as odourless glycosidic precursors (eg. linalyl, geranyl, neryl β-D-glucosides). These odourless monoterpenyl glycosides can be hydrolysed by acids or by means of grape or yeast derived enzymes.

Enzymic hydrolysis of these glycosides occurs when the sugar linkage is cleaved by either α-L-arabinofuranosidase, α-apiosidase or α-L-rhamnosidase and consequently releases arabinose, rhamnose, apiose and the corresponding monoterpenyl β-D-glucosides. Subsequently, the liberation of the aglycon moiety occurs through the activity of grape or yeast derived β-D-glucosidases. Acidic hydrolysis of monoterpenyl glycosides occurs from natural β-D-glucosidases found in the grape stimulated by the acidic conditions of must and wine. This type of hydrolysis can induce a molecular rearrangement of the monterpenols, to form other monoterpene alcohols. These transformations also occur and are important during ageing of wines [5].

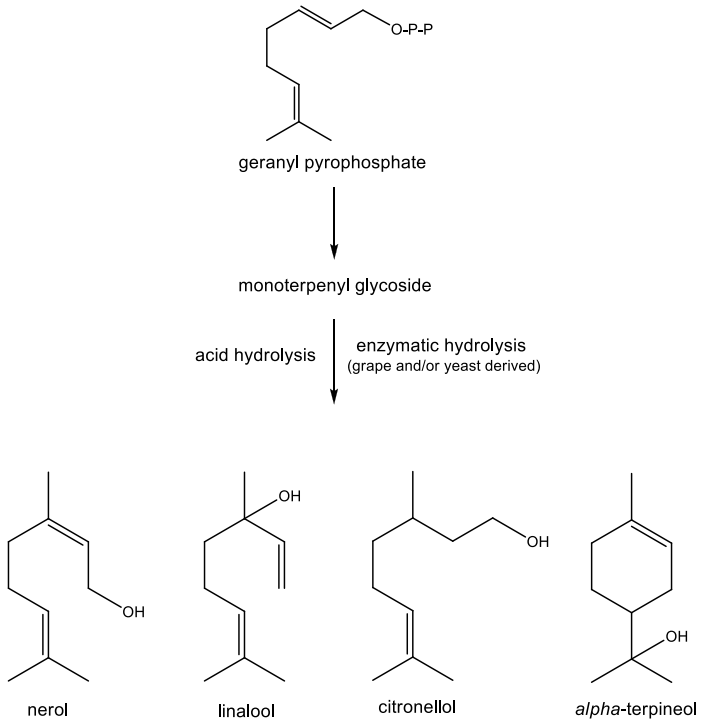

Figure 2.1 Biosynthesis of monoterpenes in grapes [5]

Approximately 60 terpene compounds have been identified in grapes, 20 as recently as 2007 [6] suggesting that as extraction methods improve, there may be more yet to be discovered in grapes. The monoterpene alcohols are the most fragrant lending floral notes to wine aromas. The terpene content varies considerably from cultivar to cultivar, with some Muscat varieties depending on them for cultivar distinction. Red varieties are not phenotypically characterised by high concentrations of terpenes, although low levels are usually present and their role in red wine aroma, particularly in Pinot noir, is still under investigation. Early studies identified only small amounts of linalool in Californian Pinot noir extracts [7] and trace amounts of linalool and α-terpineol in Burgundy extracts [8]. Linalool was identified in all six Oregon Pinot noir samples across two vintages [9] with the gas chromatography-olfactometry technique (GC-O). The panellists gave descriptors of floral, cherry, peach and heavy fruit. Additional studies quantified concentrations of linalool (5-24 μg/L), geraniol (3-18 μg/L), nerol (nd-6 μg/L), α-terpineol (9-62 μg/L), and citronellol

Table 2.1 Monoterpenes - olfactory description and perception thresholds

Compound	Olfactory description	Olfactory perception threshold (µg/L)
Geraniol	Rose, geranium	20 [10]
Nerol	Rose	400 [11]
Linalool	Floral, rose, lavender	25 [10]
α-terpineol	Floral, lily of the valley	250 [10]
Citronellol	Citronella	100 [10]

(<18 µg/L) utilising gas chromatography mass spectrometry (GC-MS) after enzymatic and chemical hydrolysis of glycosylated precursors [12]. A later study, utilising solid phase micro-extraction (SPME) post fermentation [13] also observed similar concentrations of terpenes after the inoculation of the must with varying yeasts. Both studies revealed concentrations well below perception thresholds. Girard *et al.* [14] identified geraniol (6-18 µg/L) at different concentration upon manipulating vinification treatments but again levels were below perception threshold. Research wines vinified with grapes from different regions in NZ (Martinborough, Marlborough, and Central Otago) over two seasons showed concentrations below threshold [1, 2]; however a more recent study with NZ Pinot noir reported concentrations exceeding the threshold with a range from 41-170 µg/L [15]. It has been observed that Pinot noir wines made from grapes grown in low and ultra-low vigour zones displayed higher concentrations of some terpenes [16] and with 100% leaf removal higher concentrations of some bound forms of terpenoids [17].

Other studies regarding the contribution of terpenes in Pinot noir were carried out on Oregon Pinot noir wines employing GC-O with aroma extract dilution analysis (AEDA). The study revealed linalool (floral) and geraniol (dried fruit) to be important odorants in the acidic/water fraction with moderately high FD ≥ 64, while α-terpineol (floral) showed only potential importance with FD ≥ 16. Citronellol (fruity, rosy) showed little to no importance with FD ≤ 4, although, in the neutral fraction, citronellol displayed more importance with FD ≥ 16 with green,

lemon, and fruity odours [18]. The same research group in an additional study using stir bar sorptive extraction (SBSE) and GC-MS quantified concentrations of linalool, nerol, geraniol, and citronellol from different grape maturities [19]. The four monoterpenes studied were present in the wines at low concentrations and increased with grape maturity with the exception of linalool which decreased. One explanation may be the transformation of linalool to geraniol and nerol during wine production, with geraniol and nerol being further converted to citronellol via enzymatic reactions, a compound that exhibits a lower perception threshold than the other terpenes [20]. Even though concentrations were below perception thresholds, Fang and Qian [18] suggested the monoterpenes quantified may contribute to the floral and cherry flavours of Pinot noir wines. Furthermore, it is thought that terpenes can form lactones and ketones as wine ages, however, the overall change in terpene content has little effect on the fragrance of red wines where their occurrence was minimal [21]. It is proposed that additive or synergistic effects of the various terpenes can make the prediction of sensory influences difficult. A recent Oregon study demonstrated an increase of linalool, citronellol and geraniol in wines made from postharvest dehydrated grapes where grapes were harvested at 22° and 24° Brix and exposed to a temperature controlled (22°C) dehydration air tunnel until they reached 25° and 27° Brix, respectively. However, concentrations presented again were below perception threshold [22]. A sensory comparison analysis was not performed to compare the control versus treated wines; therefore the contribution of this increase is still unknown.

One of the latest studies evaluating terpenes in Pinot noir found that pre-fermentation cold maceration, a common winemaking practice for Pinot noir, with *Saccharomyces cerevisiae* isolates resulted in over twice the concentration of β-citronellol when compared to no maceration [23]. An additional study using stir bar sorptive extraction gas chromatography mass spectrum revealed that most terpeneols accumulated at early grape development stages and their concentration did not increase much during the later stages of ripening, and some even decreased [24].

2.1.2 C_{13} Norisoprenoid derivatives

Norisoprenoids are presumed to originate from direct degradation of carotenoid molecules such as β-carotene, lutein, neoxanthin and violaxanthin. The norisoprenoids emerge from an oxidative cleavage of the carotenoidal molecule between the C_9 and C_{10} position yielding norisoprenoids with 13 carbon atoms. The majority of these C_{13} compounds occur in grapes in the form of glycosylated precursors (released during crushing), which are hydrolysed under acidic must and wine conditions or by fungal enzymes to the aroma active compound [11].

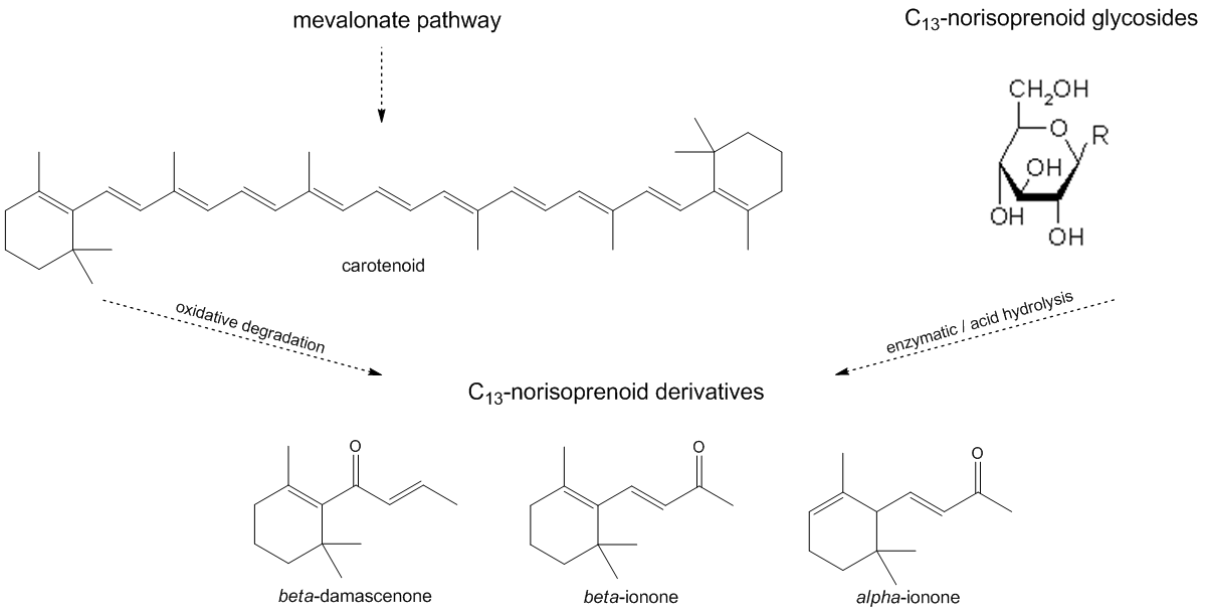

Figure 2.2 Biosynthesis of C_{13} norisoprenoids [11]

The norisoprenoids are divided into two groups, non-megastigmane and megastigmane, with the C_{13} norisoprenoids falling into the latter. Structurally, the megastigmanes are a benzene cycle substituted on carbons 1, 5 and 6 and an unsaturated aliphatic chain with four carbon atoms attached to C_6 [11]. The derivatives containing 13 carbons (C_{13}) are present in all grape varieties and have been the main focus in wine aroma research.

Table 2.2 C_{13} norisoprenoids - olfactory descriptions and perception thresholds

Compound	Olfactory description	Olfactory perception threshold (µg/L)
β-damascenone	Apple, honey, floral	0.05 [10], 7 [25]
β-ionone	Violets, raspberry	0.09 [10]
α-ionone	Woody, floral, violet	2.6 [10]

The C_{13} norisoprenoids, β-damascenone and β-ionone, both with low aroma perception thresholds, can play significant roles in the aromas of both white and red wines. β-ionone lends a unique violet aroma to red wines while β-damascenone exhibits varying odours depending on its concentration. Low concentrations were reminiscent of lemon balm aromas where as high concentrations were described apple, rose and honey [26].

Trace amounts of β-damascenone were detected in Burgundy Pinot noir wines with GC-FID and GC-MS [8] and later in an Italian Pinot noir [13] using SPME and GS-MS; however, no β-ionone was detected. A more reliable method for quantification of β-damascenone and β-ionone was developed using a stable isotope dilution assay [27]. Several vintages of red cultivars, including Pinot noir, from different regions in France were analysed. The research group observed that Pinot noir concentrations of β-damascenone varied between vintages with mean concentrations of 2.76 µg/L in 1995 and 4.49 µg/L in the 1996. The β-ionone concentrations ranged from 86 ng/L to 1475 ng/L in Pinot noir wines; encompassing the lowest and highest concentrations amongst the cultivars. Concentrations of β-damascenone in the NZ research Pinot noir wines ranged between 0.1 and 2.4 µg/L while β-ionone was detected in all wines but not at a quantifiable concentration [1] in the first season. The second season [2] revealed both β-damascenone and β-ionone exceeded perception threshold along with much higher concentrations when compared to previous studies [14] of α-ionone A recent study has shown that β-ionone increased only at the very early stage of berry development [24] and that 100% leaf removal from cluster zones

[17] and ultra-low and low vigour zones [16] resulted in higher concentrations of β-damascenone positively correlating to the increased sunlight.

In more recent studies profiling Oregon Pinot noir wines, β-damascenone received only moderate FD values between 8 and 32 in the acid/water fraction contributing sweet, tea and floral descriptors and FD values between 8 and 16 in the neutral fraction with green apple aromas [18]. The values were below concentrations anticipated by the researchers raising the question of its importance in Pinot noir, despite published literature in which β-damascenone was reported to consistently present the highest AEDA (FD) values in all red wines sampled [28]. β-damascenone has been considered to have a very high odour activity value (OAV) when perception thresholds determined in water or synthetic wines are used. A higher OAV is indicative of a major contribution to wine aroma. The odour activity value is a measure of importance of a specific compound to the odour of a sample. It is calculated as the ratio between the concentration of individual substance in a sample and the threshold concentration of this substance. This suggestion of importance has been challenged with a recent investigation on β-damascenone's actual contribution to red wine aromas [25]. This study determined odour thresholds for β-damascenone to be 850 to 2100 ng/L for model red wine and 7000 ng/L in red wine, which is considerably higher than the aqueous ethanol threshold of 50 ng/L. The group found no overall direct impact on wine aroma but suggested that β-damascenone might act indirectly as an enhancer of red fruit aromas in red wines.

An Oregon study observed increasing concentrations of β-damascenone and β-ionone with grape maturation [19]. Both compounds were present above sensory threshold: β-damascenone (4.5 to 9.4 μg/L) and β-ionone (0.23 μg/L to 0.63 μg/L). A similar trend was seen applying post-harvest dehydration of the grapes, whereby both β-damascenone (6.8 to 7.7 μg/L) and β-ionone (0.36 to 16.6 μg/L) increased with the dehydration treatment [22].

2.1.3 Sulfur compounds with a thiol function

The sulfur compounds in the thiol family (mercaptans) are those compounds which contain a sulfhydryl group (-SH) attached to a carbon atom. This group contains the aromatic varietal thiols 4-mercapto-4-methyl-pental-2-one (4MMP), 3-mercapto-hexan-1-ol (3MH), and 3-mercapto-hexan-1-ol acetate (3MHA). It has been demonstrated that 4MMP and 3MH exist in grapes in the form of non-volatile, cysteine-bound conjugates, which have long been of interest for their release during fermentation by yeasts [29]. However, the actual precursor for 3MH is still a matter of some debate, owing to low conversion rates of cysteine-conjugates to 3MH. There have been four main precursors suggested, S-3-(hexan-1-ol)-L-cysteine [30], (*E*)-2-hexenal[31], S-3-(hexan-1-ol)-glutathione [32] and most recently (*E*)-2-hexenol [33]. However, it has been determined that Cys-3MH and (*E*)-hexen-2-al are not the major precursors [34]. Harsh *et al.* has determined that with the addition of hydrogen sulphide to grape juice, it hugely increases its thiol forming potential. No separate 3MHA precursors have been identified, but it was determined that 3MHA is produced from 3MH by yeast via the ester-forming alcohol acetyltransferase during fermentation [35]. Recent studies show that the conjugated thiol precursors are produced in the grape and concentrations are highest in the skin [36] and that the glutathione can be conjugated to (E)-hex-2-enal [37] but little is known about the biosynthesis.

These varietal thiols play a major role in the characteristic aromas of certain fruits and aromatic plants. Several of these odoriferous compounds have been identified in wine and can contribute to the characteristic aromatic components of Sauvignon blanc [38-41], Merlot and Cabernet Sauvignon [42] and wines made from Alsace grape varieties [43]. It has also been shown that differences in thiol concentrations associated with vineyard sites may contribute to the distinct regional style of NZ Sauvignon blanc wines [44] as well differences in sensory styles from various sites within the region [41].

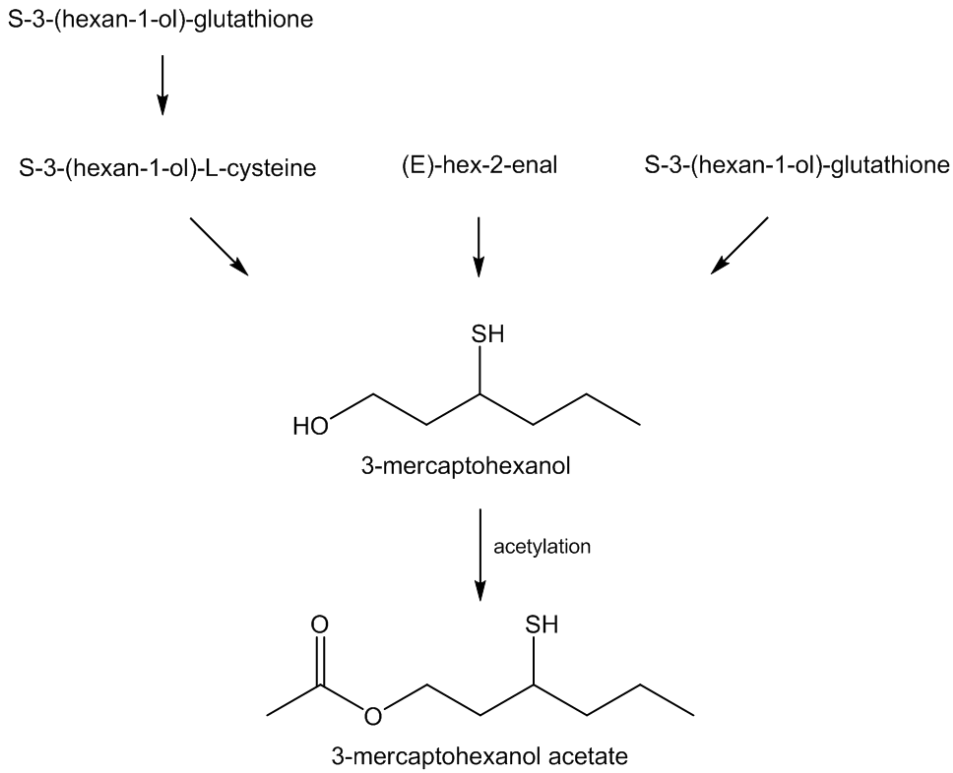

Figure 2.3 Proposed pathways for sulfur compounds with a thiol function

While 3MH has been reported to contribute to fruity aromas of red Bordeaux wines [45] and rosé wines made from Cabernet Sauvignon, Merlot and Grenache varieties [46, 47], there have been only a few studies regarding the role of mercaptans in Pinot noir aromas. The volatile compound, 3MH, was observed at concentrations of approximately 70 ng/L in Pinot noir and Cabernet Sauvignon wines from Oregon [48] although the researchers only gave suggestions to its sensory contribution.

Maharaj [1], on the other hand, reported significantly higher concentrations of 3MH in research NZ Pinot noir wines, ranging from 754 ng/L to 1078 ng/L; no 4MMP or 3MHA was detected. The concentrations of 3MH (mean = 471 ng/L) examined in the second season of the NZ study were lower than the concentrations previously reported (mean = 916 ng/L) for the 2007 vintage [2]. However, both studies revealed noticeably higher concentrations (up to 92%) than those reported by a sole investigation [48] where a mean of 70 ng/L was observed in Oregon Pinot noir

wines. Based on literature investigating the activity and contribution of 3MH in other red varieties [45-47], Maharaj [1] suggested these elevated concetrations of 3MH observed in NZ Pinot noir wines may have an impact on their aromas, contributing a blackcurrant attribute. This proposal was supported by the known perception threshold of 60 ng/L for 3MH in aqueous ethanol. Conversely, determining the perception threshold in a red wine matrix would give a more veritable basis for implications on its contribution to Pinot noir aroma. An additional objective in the second season NZ study included a sensory trial where a trained panel assessed Pinot noir wine samples that had been spiked with 3MH [41]. The results from the discriminative and descriptive tests showed no evidence that an increase of 3MH concentrations from 397 ng/L up to 1200 ng/L significantly changed the overall aroma of Pinot noir. However, omission tests where the compound was completely removed were not performed.

Table 2.3 Sulfur compounds with a thiol function - olfactory descriptions and perception thresholds

Compound	Olfactory description	Olfactory perception threshold (ng/L)
4-mercapto-4-methyl-penta-2-one (4MMP)	Boxwood, broom, cat urine	0.8 [43]
3-mercapto-hexan-1-ol (3MH)	Grapefruit, passion-fruit	60 [43]
3-mercapto-hexan-1-ol acetate (3MHA)	Boxwood, passion-fruit, grapefruit	4 [43]

While NZ is known for its high 3MH concentrations in Sauvignon blanc [49], more research is needed on commercial Pinot noir wines from NZ as well as other countries before considering these high concentrations unique to NZ. Fang *et al*. [18] detected 3MH in the acid-water-soluble fractionated Pinot noir extract using GC-O AEDA with a FD ≥ 16, which they considered high. None of the studies carried out sensorial analyses; consequently, there are no reported aromatic descriptors for 3MH in Pinot noir or red wine. Further investigation into the aromatic

contribution of 3MH to red wine aromas is required before determining its importance. The perception threshold has not been determined yet, but is likely to be considerably higher in red wine than in water (60 ng/L).

2.7.4 C_6 aldehydes and C_6 alcohols

Vegetal tissue, especially stems and leaves, is often included during grape crushing or whole bunch fermenting process during winemaking or can be produced in the grape and be present in the juice. These compounds typically contribute cut grass, cucumber, and herbaceous odours in the wine. The crushing action begins four enzymatic activities resulting in C_6 aldehydes and C_6 alcohols. Firstly, an ecylhydrolase frees the fatty acids from membrane lipids, then the lipoxygenase catalyses the fixation of oxygen on these C_{18} unsaturated fatty acids. The peroxides produced are cleaved into C_6 aldehydes. Some of the aldehydes are reduced to their corresponding alcohols by the alcohol dehydrogenase of the grape [50] (Figure 2.4). There are many factors that determine the concentrations of these compounds in wine such as the level of enzymatic activity, the degree and form of mechanical injury (crushing), presence of inhibitors, pH, temperature, condition of the leaf (young vs. mature), stem ripeness, juice clarification and the amount of oxygen present at the time of crushing [51-53].

Early investigations detected C_6 aldehydes and C_6 alcohols in Pinot noir wines from France [54] and USA [7, 55, 56] using GC-FID and GC-MS. Kwan *et al.* [55] found 1-hexanol to be the most important compound in differentiating French from American Pinot noir wines with higher concentrations observed in the French wines. This was later disputed by Brander *et al.* [7] as a reliable source of discrimination. They suggested the concentration discrepancy could be more related to traditional differences in winemaking technology than on basic compositional differences. This explanation was supported by the biosynthesis mechanisms of hexanol as well as the amount of time and agitation during skin-juice contact. Later investigations quantified concentrations of C_6 aldehydes and C_6 alcohols determining typical concentrations in French

Pinot noir [8] to be 160-560 µg/L of *cis*-3-hexen-1-ol and 10-40 µg/L of *trans*-2-hexen-1-ol. Another study quantified representative concentrations in Italian Pinot noir wines made from various clones in altered environments [12]: hexanol (38-127 µg/L), *cis*-3-hexen-1-ol (11-50 µg/L), and *trans*-2-hexen-1-ol (15-41 µg/L). A recent study has revealed that C_6 alcohols continuously decreased during berry development except at very early stages while the C_6 aldehydes continued to accumulate until reaching harvest when they began to decrease [24].

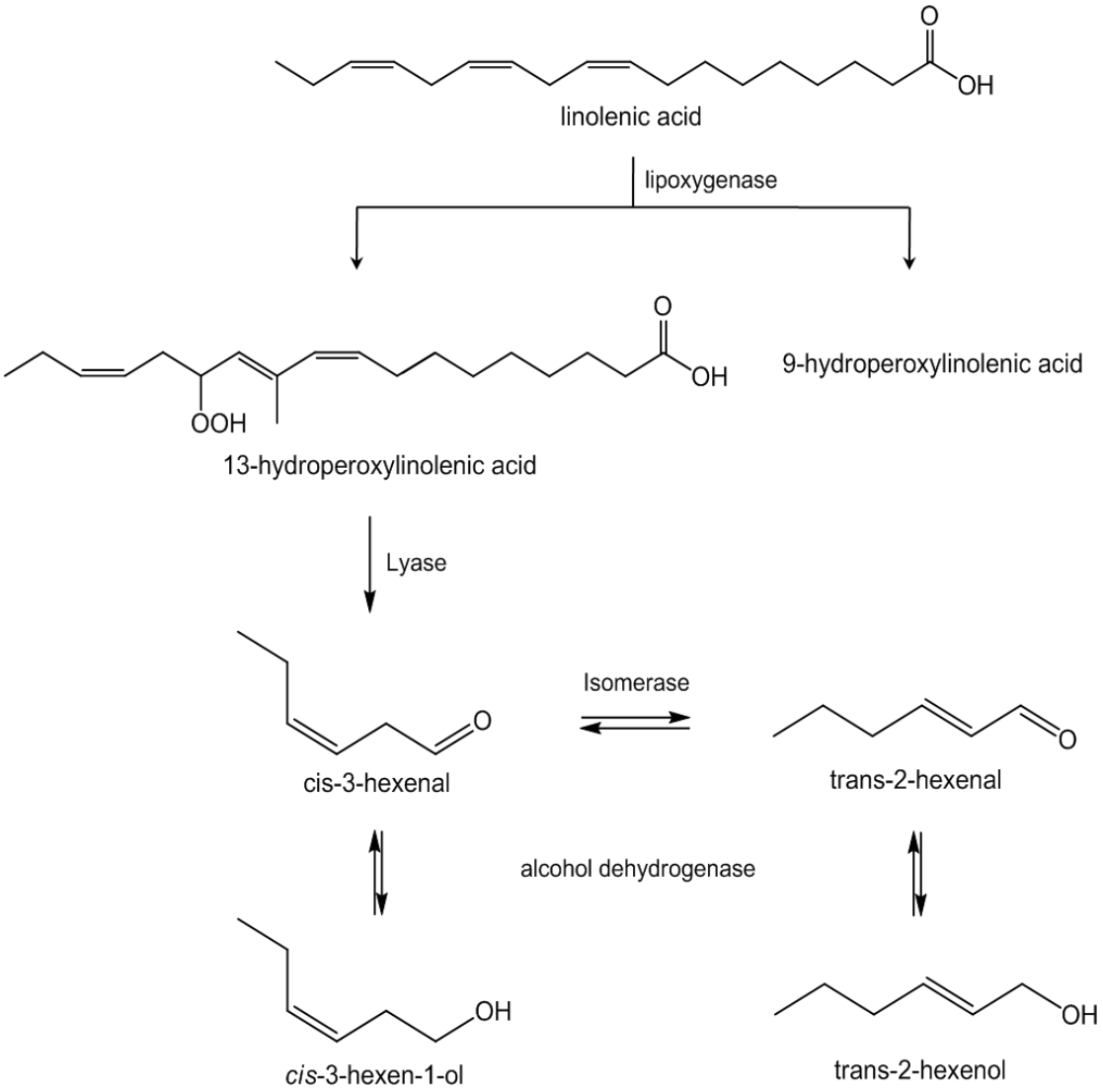

Figure 2.4 Biosynthetic pathway for C_6 aldehydes and C_6 alcohols [11]

The earlier NZ Pinot noir studies observed levels of 1-hexanol, *cis*-3-hexen-1-ol and *trans*-2-hexen-1-ol in both seasons, however, all well below threshold [1, 2]. Fang *et al*. [18] reported *cis*-3-hexenol (fruity, green aromas) and *trans*-3-hexenol (green aromas) to be of potential importance to Pinot noir aroma with moderately high AEDA values (FD≥16) while 1-hexanol (grape juice odour) had low values (FD≤16).

Table 2.4 C_6 alcohols - olfactory descriptions and perception thresholds

Compound	Olfactory description	Olfactory perception threshold (mg/L)
1-hexanol	Resin, floral, green cut grass	8
Cis-3-hexen-1-ol	Green cut grass	0.40
Trans-3-hexen-1-ol	Green cut grass	-

Inoculation with different yeast strains in Pinot noir showed no significant differences in concentrations of 1-hexanol [57], *cis*-3-hexen-1-ol, and *trans*-3-hexen-1-ol [14] amongst the samples. The similarity in the concentrations of these compounds can be supported by their projected pathway which is largely dependent on factors prior to inoculation. Girard *et al.* [14] observed a significant decrease in concentrations of these compounds correlating with an increase in fermentation temperature (from 15 to 30 ^{0}C) and exposure to thermovinification [58] (90^{0}C for 1 min) and (75^{0}C for 20 mins)[59]. These results were credited to the heat inactivation of lipoxygenase enzymes which are essential for the production of these compounds.

2.1.5 Aminobenzoate and phenylpropanoid esters

There are four potent esters that are thought to be important in the odour character of Pinot noir: methyl-2-aminobenzoate (methyl anthranilate), ethyl-2-amino-benzoate (ethyl anthranilate), ethyl-3-phenyl-2-propenoate (ethyl cinnamate), and ethyl-3-phenyl-propanoate (ethyl 2,3-dihydrocinnamte). These compounds are derived from the shikimic pathway. Ethyl- and methyl-anthranilate are esterfications of anthranilic acid which is produced from the cleavage of pyruvate from chorismic acid which is catalysed by anthranilate synthase. Ethyl cinnamate and 2,3-dihydrocinnamate are esterfications of cinnamic acid which is biosynthesized from phenylalanine, one of the three carbo-aromatic amino acids, from the precursor chorismic acid via phenylalanine ammonia-lyase (Figure 2.5).

Table 2.5 Amino benzoates and phenyl propanoids - olfactory descriptions and perception thresholds

Compound	Olfactory description	Olfactory perception threshold (µg/L)
Methyl -2-amino-benzoate (methyl anthranilate)	Grape, fruity, tea	3 [60]
Ethyl-2-amino-benzoate (ethyl anthranilate)	Sweet, fruity, berry	-
Ethyl-3-phenyl-2-propenoate (ethyl cinnamate)	Cherry, plum, cinnamon, floral	1.1 [10]
Ethyl-3-phenyl-propanoate (ethyl 2,3-dihydro-cinnamate)	Fruity, balsamic, floral	1.6 [10]

A group in France investigating aroma compounds that could give Pinot noir its characteristic flavour, developed a method specifically for identifying these compounds [61]. The group identified all four compounds in a Burgundy Pinot noir wine, although not in measurable peaks

in the analytical high resolution GC-MS. GC-O results determined ethyl-2-aminobenzoate (sweet, fruity) to be the most intense odorant followed by ethyl-3-phenyl-2-propenoate (cherry, plum) with ethyl-3-phenyl-propanoate (fruity, balsamic) and methyl-2- aminobenzoate (fruity, grape) contributing less. This was the first time ethyl-3-phenyl-propanoate was identified in the aroma of wine and the first detection of ethyl-2-aminobenzoate and methyl-2-aminobenzoate, in *Vitis vinifera,* although these compounds were previously found in American wines [62, 63] made from *Vitis labrusca*. This same group later quantified these four compounds in 33 Burgundy Pinot noir wines using GC-MS and developed a simpler extraction method. The four esters were detected in all the wines sampled at low concentrations with ethyl-2-aminobenzoate (0.6 to 4.8 μg/L) concentrations varying the most. Ethyl-3-phenyl-propanoate (0.8 to 3.28 μg/L) was observed in some samples above its threshold. The concentrations of ethyl-3-phenyl-2-propenoate (0.5 to 1.6 μg/L) were below or just at threshold and methyl-2-aminobenzoate (0.06 to 0.6 μg/L) concentrations were very low suggesting a limited contribution to wine aroma and flavour. No reports were found for olfactory thresholds of ethyl-2-aminobenzoate.

A later study determined ethyl-2-aminobenzoate (sweet and fruity) and ethyl-3-phenyl-propanoate (fruity) to be of potential importance in the neutral fraction with high AEDA values (FD≥16) while ethyl-3-phenyl-2-propenoate (fruity, floral, and cherry) and methyl-2-aminobenzoate (tea and fruity) exhibited low importance (FD<16)[18]. The group concluded ethyl-2-aminobenzoate to be the more important odorant in Pinot noir wines over methyl-2-aminobenzoate. An additional study quantified three of these esters observing ethyl-2-aminobenzoate, ethyl-3-phenyl-propanoate and ethyl-3-phenyl-2-propenoate concentrations low and to decline with grape maturity [19].

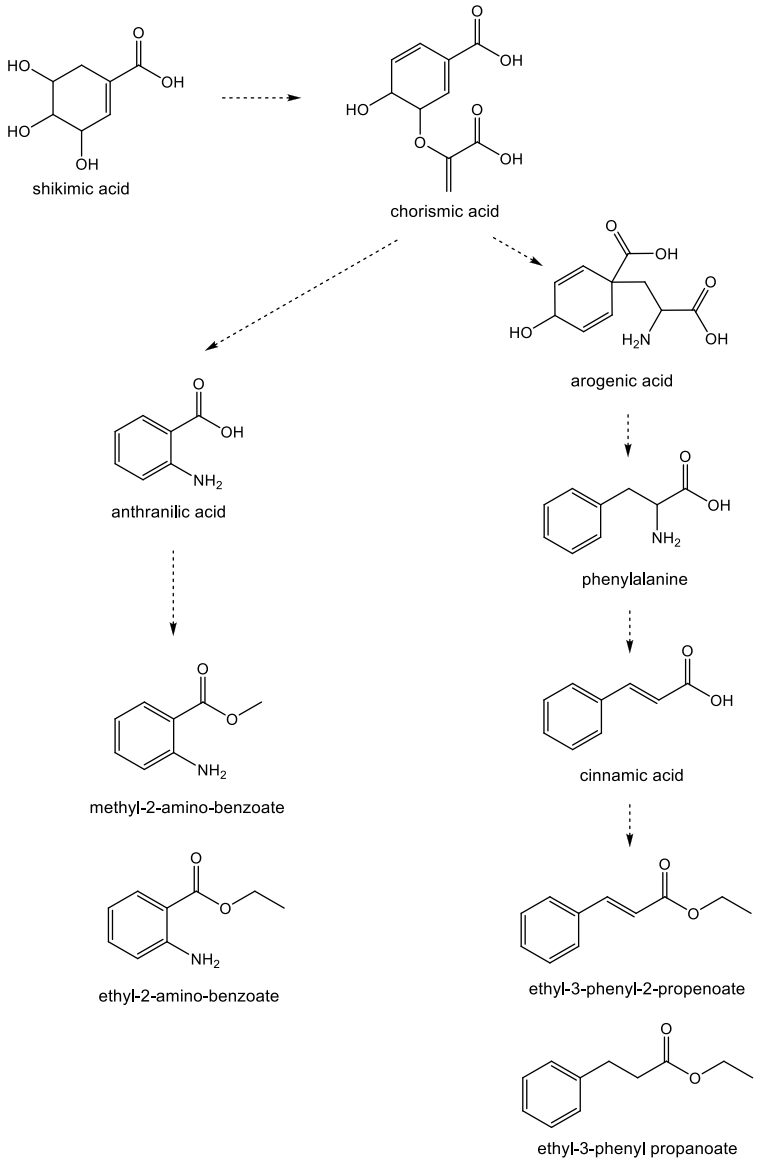

Figure 2.5 Biosynthetic pathway for amino benzoates and phenyl propanoids

They suggest these compounds may act synergistically with each other or with other compounds to contribute to wine aroma. These compounds were quantified in the second season of the NZ Pinot noir study where all were detected in every sample at or above threshold and there were significant differences when comparing regional concentrations[2]. Further research is needed to examine the variables affecting the synthesis and concentrations of these compounds during grape maturity and wine making processes. The possible synergistic role and imply proposed importance in Pinot noir wine aromas should also be considered.

2.2 Aromas formed by biological transformation

Volatile compounds that contribute to a wine's aroma can also be formed during the fermentation and aging processes. The wine's aroma will change rapidly during fermentation and immediately afterwards as a result of numerous chemical reactions (Figure 2.6). As the wine ages and matures, the changes and developments in aroma are slower and more gradual. These chemical reactions typically occur as a result of the enzymatic or acidic hydrolysis of sugars and amino acids during the metabolism of yeast. The primary role of wine yeast is to catalyse the rapid, complete and efficient conversion of grape sugars to ethanol, carbon dioxide and other minor, but sensorial important metabolites [64].

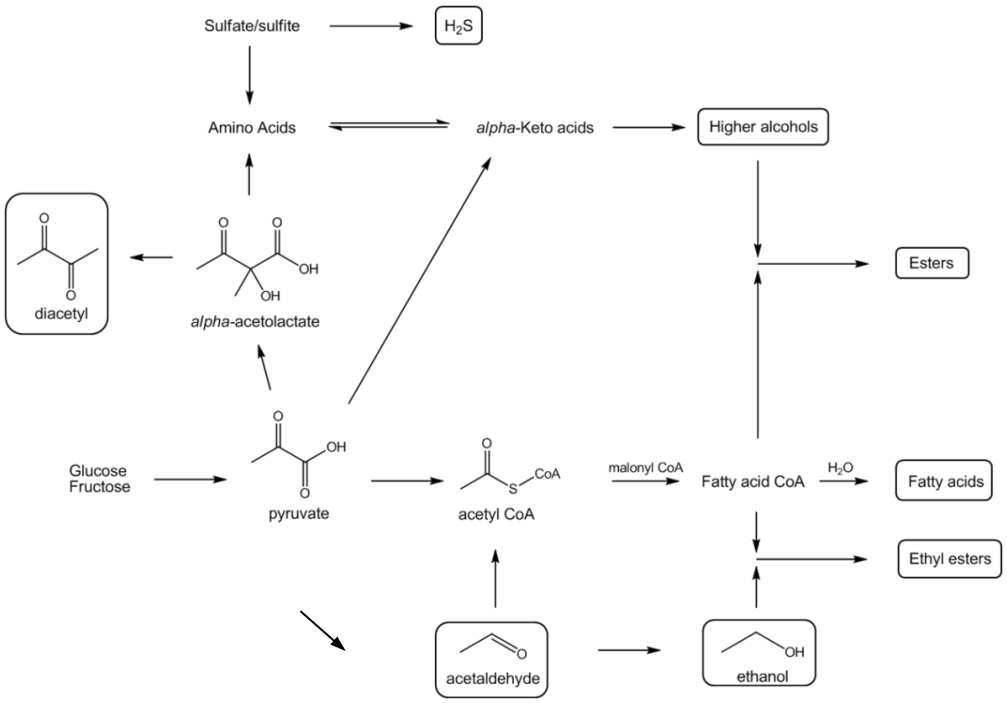

Figure 2.6 Derivation of Yeast Fermentation Flavour Compounds [64]

2.2.1 Higher fermentation alcohols

Alcohols with more than two carbons are more commonly known as fusel or higher alcohols; the straight-chain higher alcohols being the most important in wine. Higher alcohols may originate from grape derived aldehydes by the reductive denitrification of amino acids or directly from sugars as by-products of yeast metabolism. The degradation of certain amino acids, including leucine, isoleucine, valine, methionine, tyrosine, tryptophan and phenylalanine, involves a sequential transamination and decarboxylation. The resulting aldehyde can either be reduced to an alcohol or oxidised to form an acid in a process referred to as the Ehrlich Pathway (Figure 2.7). The classical Ehrlich Pathway, as it is viewed today, was proposed by Neubauer and Fromherz in 1911, and involves a sequential transamination, decarboxylation and reduction. Fusel alcohols may also be derived from the pathway responsible for amino acid biosynthesis. In this situation the α-keto acid, a precursor to amino acid biosynthesis, is decarboxylated and reduced to the corresponding alcohol [64, 65].

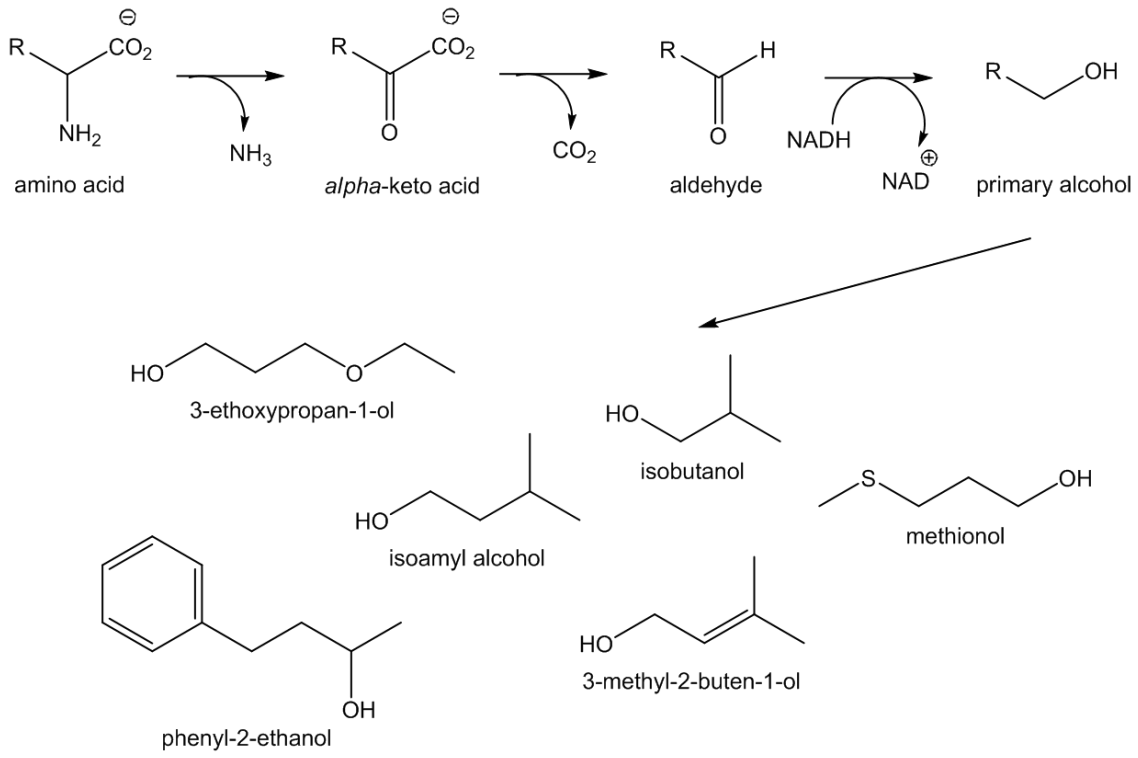

Figure 2.7 Biosynthesis of higher alcohols [11]

In wine, the higher alcohols can reach high concentrations up to 550 mg/L and contribute intense odours to the wine aroma [11]. Higher alcohols account for about fifty percent of the aromatic constituents of wine, excluding ethanol, and their pungent odours can add complexity at lower concentrations(≤ 300 mg/L) while at higher concentrations can produce off and overpowering odours [21]. The formation of these higher alcohols are influenced by many variables during winemaking practices including presence of oxygen, amount of solids in the must, high fermentation temperatures, yeast selection and microbial spoilage from bacteria or yeast. The branch-chain higher alcohols, including isoamyl alcohol and isobutyl alcohol, are synthesized from the branched-chain amino acids and contribute whiskey/malt/burnt and solvent/bitter aromas respectively [66]. The amino acids, including phenylalanine and tyrosine, produce aromatic alcohols, such as phenylethyl alcohol [67, 68] which has a honey/spice/rose/lilac aroma [66].

Table 2.6 Higher alcohols - olfactory descriptions and perception thresholds

Compound	Olfactory description	Olfactory perception threshold (mg/L)
Methyl-2-propan-1-ol (isobutyl alcohol, isobutanol)	Solvent, nail polish	40 [66]
Methyl-3-butan-1-ol (isoamyl alcohol)	Malt, burnt, smoky, spicy, solvent	30 [66]
Phenyl-2-ethanol	Floral, rose, lilac, spice, honey	10 [66]
Methionol	Potato, sweet	1 [66]

Higher alcohols were detected in early aroma studies of Pinot noir wines using various analytical methods [7, 8, 56]. In an endeavour to geographically classify Pinot noir wines from France and USA, Kwan and Kowalski (1980) established phenyl-2-ethanol to be of second importance in separating Pinot noir wines within USA (Pacific north-west vs. California) in the initial

investigation, using GC-FID-MS and pattern recognition techniques. The latter study attempted to make correlations with sensory analyses, finding phenyl-2-ethanol to correlate with the flavour characters of the wine, although the findings were not significant. Vinification techniques, micro-climates, soil and other environmental factors were not considered in this study leaving many variables to be investigated. Brander *et al.* [7] challenged the results stating the findings to be more related to the nitrogen fertilisation history of the vineyards rather than true geographical differences. Recent findings supported this suggestion stating that simple vineyard practices such as nitrogen fertilization can increase nitrogen concentrations in must, subsequently lower concentrations of higher alcohols in wine [69]. A study looking at differing vigour found that wines from the ultra-low vine vigour had the highest concentrations of alcohols [16]. In Oregon Pinot noir wines, phenyl-2-ethanol was found to be important in the acid/water-soluble solution extracts with a very high AEDA value (FD = 8192) contributing rosy attributes [18]. In the same study both methyl-3-butan-1-ol and methyl-2-propan-1-ol, giving nail polish-like odours, had extremely high AEDA values (FD ≥ 4096). Two separate studies investigating Pinot noir grape maturity found no significant differences in phenyl-2-ethanol concentrations (24-37 mg/L) with increasing maturity [19] although it was established that this compound is an important odorant at every maturity level [70]. Maharaj [1] and Rutan [2] both reported that concentrations of isoamyl alcohol and phenyl-2-ethanol exceed perception thresholds for NZ Pinot noir wines.

Additional investigations focusing on various vinification treatments and fruit maturity were conducted in Pinot noir wines. Fischer *et al.* [71] observed (with GC-FID) increased concentrations of fusel alcohols in German Pinot noir when the fermentation process was on the skins and allowed extended contact time. These findings may be related to the increase in must solids causing faster fermentation kinetics and extended contact time, allowing for increased amino acids extractions [58]. A further study compared the effects of two different yeast strains and various fermentation temperatures revealing elevated fusel alcohol concentrations for both strains at lower fermentation temperatures [14, 57]. These results contradict earlier statements of increased fusel alcohol concentrations with higher fermentation temperatures [11, 21].

2.2.2 Fatty acids

Fatty acids are carboxylic acids with long unbranched aliphatic chains which are either saturated or unsaturated. Fatty acid biosynthesis begins in the cytosol with acetyl-CoA (formed by pyruvate decarboxylation) being carboxylated by biotin dependent acetyl-CoA carboxylase to produce malonyl-CoA. The acyl groups of acetyl-CoA and malonyl-CoA are then transferred to separate molecules of acyl carrier protein (ACP) forming acetyl-ACP (a thioester) and malonyl-ACP (a thioester). Condensation of acetyl ACP and malonyl ACP results in the formation of acetoacetyl ACP. The acetoacetyl ACP is reduced by NADPH into D-3-Hydroxybutyryl ACP, which is dehydrated to crotonyl ACP, following a reduction by NADPH into butyryl ACP (a C_4 thioester). This four carbon thioester undergoes condensation with another molecule of malonyl-ACP to repeat the sequence producing a C_6 thioester. Each time this sequence is repeated, two more carbons are added to the chain (Figure 2.8). Once a thioester with the appropriate number of carbon atoms is obtained, it can undergo a transesterfication reaction with water to produce the fatty acid [72].

During alcohol fermentation, yeasts produce short- (< 6 carbons), medium- (6 to 12 carbons), and long- (> 12 carbons) chain fatty acids. The majority of fatty acids produced are long chain; however, these are too large and are non-volatile. The short-chain fatty acid acetic acid accounts for >90% of the volatile fatty acids in wine [65]; however, is typically not found at concentrations to contribute to the overall aroma unless there is significant spoilage. Short-chain fatty acids that have been found to contribute to wine aroma include isobutyric, isovaleric, butyric and propanoic acids[66]. Isobutyric and isovaleric acids contribute sweaty, cheese like aromas and have been noted as markers of *Brettanomyces bruxellensis* spoilage and are thought to be able to mask the "brett character" [73]. The medium-chain fatty acids, caproic (C_6), caprylic (C_8) and capric (C_{10}) acids possess goat-like odours and occur typically at detectable concentrations in wines spoiled by microbes, although their occurrence at or just below the threshold can contribute to the overall complexity of wine aroma [66].

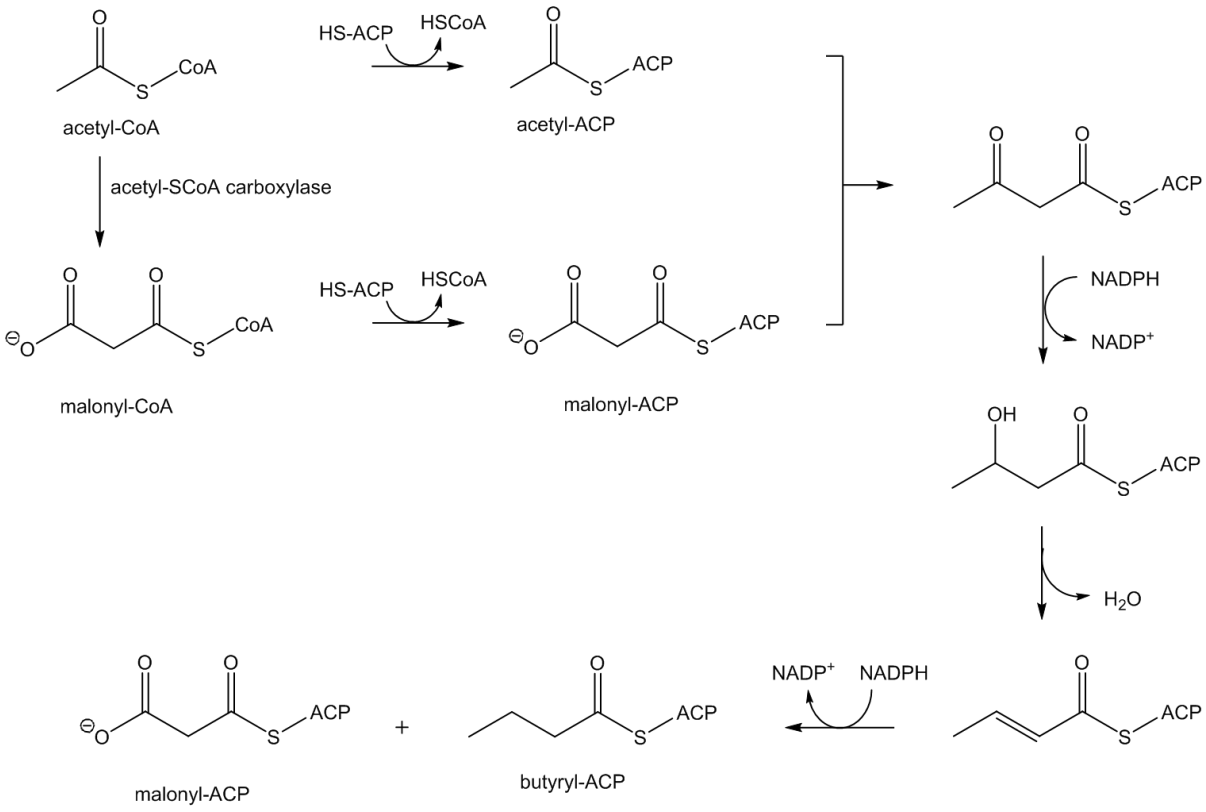

Figure 2.8 Biosynthesis of fatty acids

Their concentrations are dependent on anaerobic growth conditions, must composition, grape cultivar, yeast strain, fermentation temperature and winemaking practices [74, 75]. These acids may also become toxic to *Saccharomyces cerevisiae* and inhibit the fermentation process [11, 76].

There have been few studies solely devoted to (C_6 to C_{10}) fatty acids in Pinot noir aroma reporting only the concentrations quantified with correlating sensory descriptors. An Oregon group, using GC-MS with GC-O, correlated hexanoic acid with fruity, sweet, apple and peach across different grape maturities with little to no difference between samples [70, 77]. As an extension to their previous study, the group detected hexanoic acid again but with tobacco, floral, toasty, herbal, spicy and nutty aromas. Octanoic acid was also detected in one sample having floral, cabbage aromas. All three C_6 to C_{10} acids were detected in an even further investigation which gave descriptors of rancid, fatty, soapy, cheese and oily due to the much higher concentration levels:

hexanoic (11-37 mg/L), octanoic (11-41 mg/L), decanoic (6-34 mg/L)[9]. Hexanoic acid was quantified in NZ Pinot noir research wines at concentrations of 0.02 to 3.0 mg/L [1] and in the second season hexanoic and octanoic acids were observed over threshold [2]. Decanoic was not detected in either season.

Table 2.7 Fatty acids - olfactory descriptions and perception thresholds

Compound	Olfactory description	Olfactory perception threshold (µg/L)
Isobutyric acid	Sweat, cheese	2300 [10]
Isovaleric acid	Sweat, cheese	33 [10]
Butyric acid	Sweat, cheese	173 [10]
Hexanoic acid (caproic acid)	Sweat, cheese, pungent, sour, tobacco, spicy	420 [66]
Octanoic acid (caprylic acid)	Sweat, cheese, rancid, soapy, goaty, fatty	500 [66]
Decanoic acid (capric acid)	Fatty, rancid	1000 [66]

Several studies examined the concentrations of fatty acids in Pinot noir wines applying various fermentation temperatures as well as different yeast strains, but found no significant differences among the samples with exception of Girard *et al.* [14] who reported slightly lower hexanoic concentrations at high fermentation temperature (30^0C) and elevated octanoic concentrations at ambient temperature fermentation (20^0C) for two yeast strains [14, 57-59]. Fischer *et al*. [59], on the other hand, found concentrations of all three (C_6, C_8 and C_{10}) fatty acids significantly higher with thermovinification treatment; possibly due to thermo-enhanced extractions from skin. A

further study showed that fatty acid concentrations decreased with nitrogen additions to ferments with the exception of decanoic aci which increased [78].

Fang *et al.* [18] detected both hexanoic and octanoic acids in the acid/water-soluble fraction, using GC-O AEDA. Hexanoic acid exhibited a strong, sweaty aroma with high FD values (FD=32) and octanoic acid was responsible for goat-like, rancid and cheese aromas with moderately high FD scores (FD ≥8).

2.2.3 Esters

Esters are formed through a process known as esterification where an alcohol function reacts with a carboxylic acid function and a water molecule is eliminated. Esterifications are among the simplest and most often performed organic transformations. Esterification is a reversible reaction limited by hydrolysis which involves adding water, assisted by a catalyst, to an ester to get the sodium salt of the carboxylic acid and alcohol (Figure 2.9). As a result of this reversibility, many esterification reactions are equilibrium reactions. Esters may be grouped into straight-chain (aliphatic) or cyclic (phenolic) categories whereby the aliphatic group is further subdivided into three subgroups according to number and type of group involved: monocarboxylic acid esters, di- or tricarboxylic acid esters, and hydroxyl and oxo acid esters.

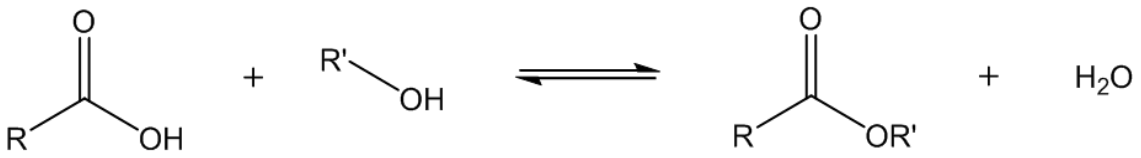

Figure 2.9 Esterification [64]

Considering the constituents of wine (alcohols and acids), esters are formed easily and over 160 have been identified in wine, although most in trace amounts with low volatility and a low to moderate odour impact. Aliphatic esters make up the largest ester group in wine with the monocarboxylic acid esters being the most aromatic. This group of esters are derived from esterifications based on ethanol and various saturated fatty acids, acetic acid and higher alcohols. Overall, esters represent the greatest concentration of volatile compounds in alcoholic beverages and they contribute to and enhance sweet-fruity aromas in wines [79]. The formation of esters in wine and its contributing factors has been well established. Fermentation temperature, yeast strain, nitrogen levels in must, oxygen availability, and grape variety all play important roles in the formation of esters in wine [53, 80].

Acetate esters are produced by yeast from the reaction of acetyl-coA with a higher alcohol. The formation of these compounds is favourable at low temperature, anaerobic fermentations and they are generally present in excess relative to their equilibrium constants. This results in the hydrolysis back to their component alcohols and acetic acid [21]. These esters lend intense unusual odours such as banana, pear-drops, and apple to wines.

The most important esters and acetates in wine are considered to be the fatty acid ethyl esters and acetates which are produced by yeast activity during fermentation. Esters are generally considered to be products of yeast metabolism through lipid and acetyl-CoA metabolism [64] and acetates through ethanolysis of the acetyl-CoA [81] with fatty acid precursor concentrations [82] and alcohol acetyltransferases [83] being the most important factors limiting productivity respectively. The formation of these compounds is lowered considerably during fermentation by the presence of dissolved oxygen and unsaturated fatty acids in wine [79].

Esters can also be produced through bacterial metabolism and chemical modification as well as the reaction of ethanol with di- and tricarboxylic acids (eg. lactic, malic, citric, tartaric, succinic), amino acids (e.g. proline), and phenolic acids (e.g. anthranillic).

Esters were first detected in Pinot noir (France) [54] where over 30 peaks were identified and named, while later analyses of Pinot Noir (USA) [7] revealed over 20 esters. Another group, using GC-FID and GC-MS, quantified 37 esters and detected a further 28, although at low concentrations < 0.01 mg/L [8]. These low concentrations were thought to be the results of an inadequate extraction method and the age of some wines sampled. Maharaj [1] reported the sum of acetate esters in NZ research Pinot noir to range between 820 to 1997 μg/L and the sum of ethyl esters 418 to 543 μg/L and the second season revealed similar if not slightly higher results [2] with only isoamyl acetate and two of the fatty acid esters found to be above threshold. Fang *et al.* [18] reported esters to be the most common odorants in their neutral/basic fraction with many of the ethyl esters having high AEDA values (FD ≥ 64) with tropical fruit aromas. Later examinations, using GC-FID, GC-MS and GC-O, attempted to link odour active peaks with different Pinot noir grape maturities [70, 77]. Esters were quantified at various concentrations in all wines sampled and were reported as contributing to the floral and fruity aromas, although no clear correlations emerged with grape maturity. A further study observed similar trends with the exception of ethyl 2-methylpropanoate and ethyl 3-methylbutanoate, which decreased with grape ripeness [19]. The group reported an overall total concentration of aromatic esters decreased slightly with maturity and noted a decline in fruity aromas.

Research on the effects of ester concentrations from the application of various vinification treatments and inoculation with different yeast strains has contributed useful information. In two separate investigations, Girard *et al.* [58] observed that winemaking conditions have a significant effect on ester concentrations in Pinot noir. In the first study, the group established that the temperature of the fermentation plays a vital role. Total esters were more than doubled for the fermentation with the lowest temperature (15^0C) followed by 20^0C and 30^0C, although the

15^0C had undergone a pre-fermentation heat treatment of 90^0C for one minute. The group's second study revealed similar trends with the lower fermentation temperatures (15^0C) producing the highest concentrations of esters with two yeast strains [14]. These findings were

Table 2.8 Esters - olfactory descriptions and perception thresholds

Compound	Olfactory description	Olfactory perception threshold (µg/L)
Acetates		
2-methylpropyl acetate (isobutyl acetate)	Fruity, floral	1600 [10]
3-methylbutyl acetate (isoamyl acetate)	Banana, fruity, pear	30 [10]
B-phenyl-ethyl acetate	Floral, rose, fruity, honey, tobacco	250 [10]
Cis-3-hexen-1-ol acetate	Sweet green apple, fruity	-
Hexyl acetate	Floral, fruity	700 [64]
Esters of Fatty Acids		
Ethyl-2-methyl propanoate (ethyl isobutyrate)	Sweet, apple, fruity	15 [10]
Ethyl-3-methyl butanoate (ethyl isovalerate)	Sweet apple, pineapple	3 [10]
Ethyl butyrate (butanoate)	Sweet, fruity, peach, apple	20 [10]
Ethyl hexanoate	Sweet, fruity, green apple	14 [10]
Ethyl octanoate	Sweet, soapy, floral, fruity	5 [10]
Ethyl decanoate	Floral, soapy, fruity, sweet	200 [10]
Other esters		
Ethyl lactate	Sweet, floral, fruity	154,000 [10]
Diethyl malate	Brown sugar, sweet, fruity, herbal	10,000 [84]
Diethyl succinate	Floral	200,000 [10]

congruent with earlier literature which supported the suggestion that fermentation temperature effected the formation of esters with fruity esters (acetates) produced in high concentrations at temperatures less than 20^0C and higher boiling esters formed at greater than 20^0C [53, 80]. Another study revealed higher (up to 2-3 times) acetate ester concentrations when thermovinification (75^0C for 20 mins.) was applied [59]. The group proposed that the heat from the thermovinification treatment inactivating certain grape enzymes, such as esterases and transferases. Most esters and acetates were found to be higher in Pinot noir wines that did not undergo pre-fermentation maceration, however, the concentrations of branched chained ester were higher in treatments that had undergone the pre-ferment maceration when yeast were present [23]. In addition, some ester concentrations decreased with the nitrogen additions to the ferments, however, some esters increased significantly when the wines were further artificially aged [78].

A recent sensory study created models using two year Pinot noir wines whereby additions of ethyl octanoate was found to increase the red cherry aroma, 2-phenyl ethanol increased the violet aroma and the combination of ethyl octanoate and ethyl decanoate enhanced the black cherry aroma of the samples [15]. The impact of these esters requires more investigation as some of the models assessed contained higher concentrations typically found in Pinot noir wines after the additions.

2.2.4 Volatile phenols

These potent aroma compounds can be traced back to one of three sources in wine; microbial, oak maturation, and smoke-taint. The most influential of volatile phenols are mainly produced via microbial activity in wine from the precursors, p-coumaric acid and ferulic acid, both cinnamic acids. *Brettanomyces/Dekkera* yeasts are responsible for the decarboxylation of the precursor acids to vinyl phenols via cinnamate decarboxylase. The vinyl phenols are then converted by vinyl phenol reductase to ethyl phenols [85] (Figure 2.11).

The volatile phenols most often found in wine are 4- ethylguaiacol (4- EG), 4- ethylphenol (4- EP), 4-methylguaiacol, vinylphenols, guaiacol, eugenol, and vanillin [85-87]. Each of these compounds has a distinct aroma ranging from sweaty saddle to cloves. The potential sensory impact of these compounds is dramatic due to their low sensory thresholds. For instance, the microbial derived volatile phenols, 4-EP and 4-EG, require only 770 µg/L and 436 µg/L, respectively, to be recognized [88].

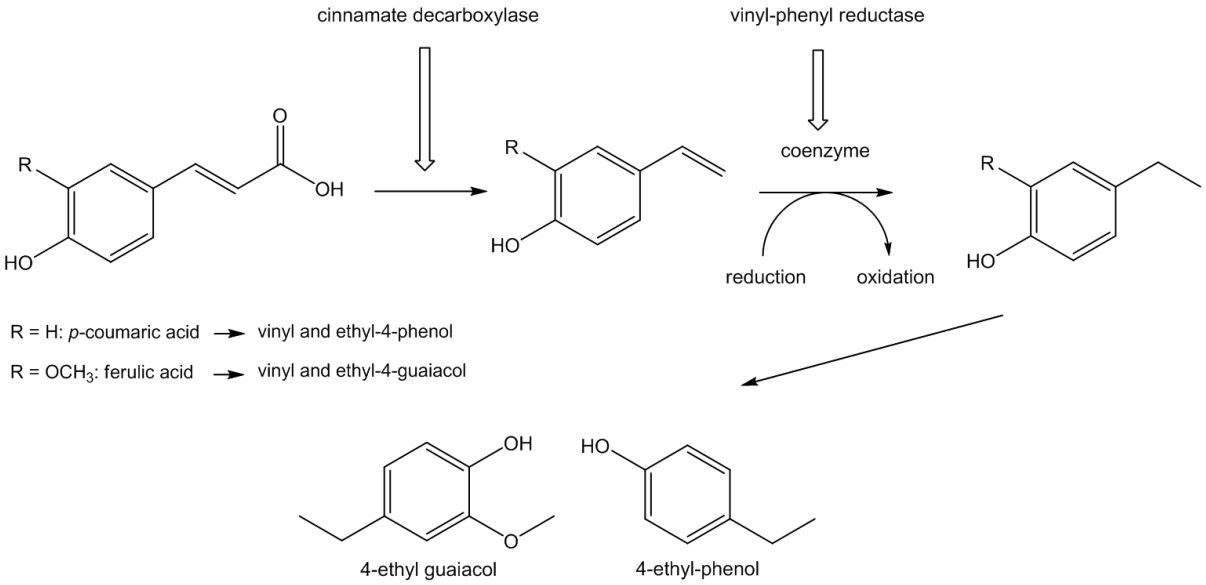

Figure 2.10 Biosynthesis of ethyl phenols [85]

In high concentrations ethyl phenols are considered a fault in red wines and at lower concentrations, these compounds can alter the aroma of red wines simply by masking the other aroma constituents [73]. Red wines typically have a greater proportion of ethyl- to vinyl-phenols while white wines are the opposite. The stoichiometric ratio in which 4-ethylphenol and 4-ethylguaiacol are present can greatly affect the organoleptic properties of the wine [89].

Table 2.9 Volatile phenols including oak derivatives- olfactory descriptions and perception thresholds

Compound	Olfactory description	Olfactory perception threshold (µg/L)
4-ethylphenol	Stables, sweaty saddles, barnyard	600 [85]
4-ethylguaiacol	Phenolic, smoke, spice, clove	110 [85]
Guaiacol	Smoke	9.5 [10]
Eugenol	Clove, medicinal	6 [10]
Fufural	Almond	14100 [85]
Furfuryl alcohol	Toasted bread	15000 [85]
5-Methyl fufural	Smoke	16000 [85]
Vanillin	Vanilla	65 [85]
Acetovanillone	Vanilla	-
γ-Nonalactone	Peaches	30 [10]
(E/Z)-Whiskey lactone	Coconut, wood	67 [10]

There have been few reports of typical concentrations of 4-ethylphenol and 4-ethylguaiacol in Pinot noir wines. A study on Burgundy Pinot noir aroma [8] using GC-FID and GC-MS and a later study on the effects of *Brettanomyces* strains to Pinot noir wine aromas [90] both reported concentrations (0 to 0.12 mg/L for ethylguaiacol, 0 to 0.44 mg/L for ethylphenol) well below perception threshold, although the latter study revealed sensory differences (lower fruity characters) with the increase of 4-ethylphenol; possibly correlating to the previously mentioned masking effect. Rutan [2] reported ethyl phenol in the second season NZ study, however, below

threshold. Fang *et al.* [18] detected (GC-O) 4-ethylguaiacol (phenolic, spicy) in the acid/water-soluble fraction but with very low AEDA values (FD=4). A later study, by the same group [22] revealed 4-ethylguaiacol concentration increased with grape maturity from no detection to 5.6 μg/L. This increase may perhaps be related to the increase in tannins during grape maturation which elevates the number of phenols and subsequently phenolic acids.

2.2.5 Oak derived volatiles

Oak is used in winemaking to vary the colour, flavour, tannin profile and texture of wine. Compounds of note which are imparted by oak include ellagitanins, phenolic aldehydes such as vanillin, volatile phenols such as eugenol, and lactones [91]. The major contributor to extractable oak volatile compounds are β-methyl-γ-octalactones, which is found as both *cis* and *trans* isomers (Figure 2.11). Their presence in oak as well as spirits has led to the colloquial terms of oak or whiskey lactones. The *cis* isomer is the more prevalent form found in wine and has a lower sensory threshold of 0.074 mg/L in red wine compared to 0.32 mg/L for the *trans* isomer [92]. The two isomers while similar have different aromas associated with them, however, both are characterized by coconut aroma when pure. Other volatile aroma compounds can modify the impact of β-methyl-γ-octalactones. In wine they have been described as smelling of vanilla and caramel, as well as woody, spicy, and sweet [91, 93].

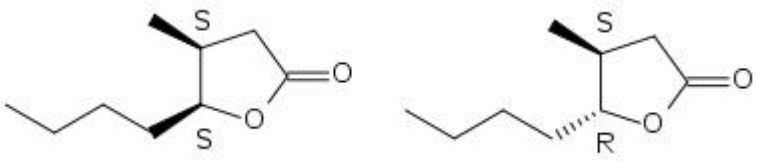

Figure 2.11 Cis **and *trans* isomers of beta-methyl-gamma-octalactone (left and right respectively)**

Differences in oak and oenological procedures can change the concentration of oak lactones in a wine. The species of oak used, generally either *Quercus alba* or *Quercus petrea*, has a large impact on the concentrations of the two isomers. The tree species also influences the ratio of *cis*:*trans* isomers found in the wine, and can be used to identify the species of oak used [94]. Other aspects can also influence the concentration of oak lactones in different sources of wood such as geographic location [95], coopering, and barrel history. As barrels age and are used multiple times, the concentration of oak lactones imparted to wines decreases [96]. The extraction kinetics of oak lactones from barrels can be modified by a wines composition. Notably, hydronium, titratable acid, and alcohol concentrations are negatively correlated with oak lactone concentrations, which could be explained by formation of the acid or ester form of the compounds, rather than lactones.

Furan derivatives including furfural and 5-methylfurfural are formed from pyrolysis of carbohydrates during oak wood toasting and thus extracted into wines during barrel storage [97]. The furfurals contribute toast and caramel aromas to wine, increasing the overall perception of oak intensity, irrespective of their low odour activity values [98]. The concentrations of furans in wine, originating from oak, is dependent on the degree of toasting and oak surface area [99].

Only few studies of Pinot noir aromas have included oak derived compounds. The AEDA study on Oregon Pinot noir wines found whiskey lactone, eugenol and isoeugenol to be of potential importance with FD values ranging from 16 to 64 in the acidic/water-soluble and neutral fractions [18]. Whiskey lactone and γ-nonalactone were detected when using the non-polar column with FD values from 8 to 32. A more recent study found the concentrations of *cis* oak lactone to be the most responsible for aromatic differences when considering oak characteristics in Burgundy Pinot noir wines. The descriptors of woody, vanillin, toasted and coconut increased with higher concentrations [100].

2.3 Sensory analysis

Sensory evaluation of wine on a basic level has historically been a way to establish worth, quality and overall acceptability. Sensorial studies have advanced and go beyond only assessing the drinkability of wine but now provide researchers and the wine industry the knowledge they need for product development and optimisation, viticultural and vinification modifications, cost reduction, quality assurance and most importantly it provides a way to communicate about the properties of wine with others. Wine's complex matrix contains hundreds of volatile compounds yet only a small percentage are known to contribute to its aroma [101]. While the concentrations of these aroma compounds can be quantified in wine using various instrumental analysis (eg. GC-FID, GC-MS), their actual contribution to the aroma is much more complex. Data obtained by these instrumental procedures must be validated against sensory data collected by humans, because no instrument, as of yet, can duplicate the sensory and physiological responses of a human being. While there are numerous sensory techniques, one that can be used to provide analytical and reliable information on sensory perception is that of descriptive analysis. The objective of descriptive analysis is to provide qualitative and quantitative descriptions of products based on the perceptions of a group of trained panellists [102] where the assessments are strictly controlled [103]. There have been several types of descriptive analysis that have been developed over the years with a variety of standardised methods. At the same time, Quantitative Descriptive Analysis (QDA®) is one of the most commonly used techniques in wine research [104].

2.3.1 QDA®

The QDA® method was developed from the necessity of undertaking a statistical analysis of qualitative profile data and exhibits many beneficial features that can be applied to meet different requirements. The method's capability to evaluate multiple products and provide complete word descriptions for all sensory properties makes it ideal for the sensorial analysis of wine, where it

has become standard practice. This method can be utilised to draw a distinction to geographical origin, develop varietal aroma characterisations, or simply evaluate wines that were exposed to a range of viticultural treatments or winemaking styles.

2.3.2 Panellist selection

All descriptive methods require a panel with some degree of training or orientation with a reasonable level of sensory acuity [105]. Selection of panellists should be carried out in a discriminatory manner eliminating prospective subjects that are unfamiliar with or do not use the product at hand. Other important criteria include the subject's capacity to perceive subtle differences in a product consistently, the ability to verbalise these perceptions in clear, descriptive terms, and the willingness to work within a group with sustained motivation and commitment [102, 105, 106]. Finding panellists to meet these requirements can be accomplished using screening procedures which are discussed at length [107], although it has been suggested that personality may play a larger role in the success in selecting a creditable panellist [108]. Wine researchers typically select individuals with professional training in wine tasting such as winemakers, wine judges [14, 109], oenology students and lecturers [110, 111], or individuals with extensive wine tasting experience [112, 113].

2.3.3 Development of descriptors

The success of descriptive sensory analysis techniques depends on the development of a common language that comprehensively and accurately describes the product's attributes. The language must be easily learned and meaningful to the subject in expressing their perceptions about the product [102]. A new panel will typically develop the sensory language themselves with the guidance of an experienced leader, although this overseer is not allowed to directly participate in the assessment of the product. The descriptor selection process requires several sessions, whereby the panellists establish a consensus language. This is accomplished when the panel agrees on a precisely defined list of descriptors that include all attributes that may be

encountered eliminating duplicated, irrelevant, and hedonic terms. The conflicts that occur with the alignment of attributes with their agreed definition can be reduced with the use of reference standards [114]. Reference standards can be made from chemical compounds, food products, or other materials [112, 115] that represent the aroma or flavour being assessed. Reference materials can also be used to assist in determining intensities and anchor the end points of the attribute scales. It has been suggested that the use of reference standards shortens training time in that panellists learn faster when they are not probing for words to describe their perceptions [116]. Wine researchers studying or profiling for particular attributes in wine aromas find recognized descriptors a useful and effective tool for assessing wines in many environments and developing new styles of an existing wine [111, 113, 117].

2.3.4 Training the panel

Individuals visualise, smell, hear, and feel things differently largely due to their various sensory experiences throughout their life. Without training, evaluators use their own, and usually different points of reference to evaluate products, and therefore responses vary widely [114]. It is thought that only through training can panellists develop a common frame of reference allowing them to provide consistent and meaningful qualitative and quantitative responses; however there have been evaluations carried out by wine professionals who had not been specifically trained. It was reported the use of wine professionals as tasters is of interest as they are influential within the industry and provide information to consumers and other wine industry personnel [118, 119]. It is important that a QDA® panellist can detect subtle differences within a product or between products and possess the ability and skill to respond consistently to these differences on repeated occasions. Often a judge will not perceive an aroma while the remainder of the panel can. This judge can be labelled as a non-perceiver for that particular attribute and his or her data will not be included in the statistical analysis.

Quantitative perceptions must be represented through the application of intensity scores. Intensity scores are generated by establishing boundaries or limits that a panellist may use when rating the strength of the perceived attributes. The quantitative frame of reference is fixed by selecting the perception that corresponds to the highest intensity on the chosen scale and establishing that point as the maximum limit [114]. This intensity score becomes the point of reference for all other intensity judgements. When assessing a specific product, the use of a line scale is based on the viewpoint that attribute intensities are rated only within the boundaries of the product category being studied. The panel is trained across several sessions (total 10–15 hours) with references to assist in standardising the use of the scale. Unlike other methods, QDA® assumes that judges will use different parts of the scale to evaluate product attributes. Therefore, it is the relative differences within and among products, not absolute differences that provide the information [105]. Panellists use the line scale by placing a mark on the scale to represent the perceived intensity of the attribute in question. For example if black cherry and clove are the distinguishing characteristic in a particular wine's aroma, they are scored at the highest intensity, affixing the maximum limit of the scale, while an undetected attribute would determine the minimum limit. The highest reference point is then used to score all other attribute intensities that may be present simultaneously in the wine's aroma. These numerical responses are applied to statistical analyses such as means, standard deviations, t- tests, analysis of variance, regression tests, and principal components analysis [104].

2.4 Controlling bias

Physical factors such as noise, external smells, room temperature and lighting are standardised and controlled to reduce the bias in the data collection process, allowing panellists to concentrate on performing their assessments correctly with no outside diversions. The reduction of extraneous audio and visual distractions allows panellists to focus on the product being evaluated, and improves the accuracy of the data obtained from their assessment [106, 120]. Sample size, sample container and sample temperature are also factors that need to be controlled

to reduce bias [121]. For example, if wines are assessed at different temperatures, they will have detectable differences due to the headspace concentrations of volatile compounds. This temperature effect was substantiated in a study assessing the intensity of white wine and red wine aromas [122]. Their results showed that at 4°C, 10°C and 18°C, the aroma intensity of white wine was significantly increased with every increase in serving temperature. However, for red wine served at temperatures of 14°C and 18°C, there were no significant differences in aroma intensity detected; only at temperatures of 23°C were any significant increases noted in the aroma intensity of the red wine samples.

2.4.1 Panellist physiological bias

Panellists' physiological factors need to be considered when controlling bias as they need to have the ability to detect subtle differences, discriminate and respond consistently to similar products on repeated occasions. There are many physiological factors that can make these tasks difficult such as allergies, dental problems, gender or age, with the latter having the most profound effect when performing sensory testing for wine research. Panellists over 60 years old are not normally recruited because as humans age, their sensory organs degrade with the olfactory system deteriorating quite rapidly. By the age of 60, the degree of anosmia increases at such a rapid rate that when an individual reaches age 80, it is very probable that they are going to be completely anosmic [123]. Most sensory panellists are recruited between the ages of 18 and 60. It appears that while olfactory losses are apparent in aging adults, losses in taste sensitivity are less profound and this incongruence can affect how the elderly perceive products in comparison with their younger counterparts.

It is a common thought that there are differences in odour perception found between males and females. Females have been measured to have higher perceived odour intensities, and lower detection thresholds, as well as increased abilities in odour discrimination [124]. Oberg *et.al* [125] discussed research suggesting that women perform better than men in olfactory tasks as well and have a more sensitive sense of smell, as reflected in absolute threshold measurements;

however, goes on to discuss some studies that have failed to find sex differences in odour sensitivity. While this study revealed mixed results after evaluating several olfactory tasks, they concluded that women's superiority in episodic odour memory is largely mediated by their higher proficiency in odour identification. Most physiological factors can typically be identified through a screening questionnaire which typically ask questions about age, gender, food sensitivities, allergies and long term health ailments.

In addition to controlling physical factors, psychological factors should also be considered. As human beings, panellists are affected by certain psychological influences, including Logical Error, Stimulus Error, Contrast Error and Mutual Suggestion Error [120]. The basis of these influences include previous knowledge about the product, predisposition from seeing the product, fatigue from sampling first sample and persuasion from others can impede the accuracy of the tests and generate misleading and false results. Typically these situations can be easily managed by withholding all information about the product, using green lights to distort visual bias, presenting the samples in a random order over multiple sets and prohibiting communication between panellists until testing is completed. In addition to these precautions, keeping panellists motivated is another potential problem as testing can last for several weeks. Intuitively, sensory scientists believe that when panellists are motivated they perform well; however, when they are not motivated they become bored and their performance declines [120]. As a result, panel leaders can use recognition and rewards to ensure that panellists remain motivated.

2.5 Disadvantages of QDA®

A limitation of QDA® is the difficulty in comparing results between panels, among laboratories, and from one session to another [105]. These limitations can lead to inconsistencies in quantitative results and are often correlated with panellist behaviour. The lack of immediate feedback to panellists can result in a lack of motivation and a reduction in opportunity for corrective calibration [120]. Often panels are trained for specific products which limits their

capability to assess different products being inter compared, since each product category has its own intensity boundaries and references. For example a panel trained to assess Pinot noir would need to develop an additional common language and set up new references if Merlot was introduced. This often requires training a completely new panel to avoid confusing one set of descriptors and intensity boundaries with another [114]. This re-training can be costly and time consuming.

2.6 Descriptive analysis of Pinot noir wines

Researchers have utilised descriptive analysis to evaluate Pinot noir wines for many circumstances. A group in Canada [126] interested in the sensory effects of different viticultural practices trained a 12 member panel with references and agreed on 14 aroma descriptors to evaluate wines made from grapes at various cropping levels and canopy treatments . The panel was able to distinguish between the different treatments using more vegetal characters for high shoot density and more fruit aromas for the balanced vines. No differences in sensory were found with varying crop yields. A more recent study by this same group, using a similar sensory method with fewer descriptors, evaluated wines made from a range of Pinot noir clones. The panellists were able to group the wines as having either cherry and berry characteristics or vegetal, grassy, and plum aromas [127].

The effects of aroma profiles on Pinot noir with the application of different fermentation treatments have also prompted researchers to employ the use of descriptive analysis. A reference trained sensory panel rated aroma intensities among wines that were made utilising three cap management treatments, namely extended maceration before plunging, pump over then plunge, and drain then pump over. The referenced trained panellists agreed on six fruity and one vegetal term to assess the different wines. The drain and pump samples were found to possess the highest aromatic and flavour intensities, with plum and stem-like characteristics, while the

extended maceration then plunge treatment received higher intensities of strawberry and cherry. A 12 member trained panel observed differences among wines made with three fermentation temperatures (15^0C with heat treatment, 20^0C, and 30^0C) [58]. The 15^0C with heat treatment ferments produced wines with higher banana, tropical fruit and cooked aromas. This assessment was consistent with the chemical composition of these wines where a fourfold higher concentration of total esters was determined over the other wines, predominantly isoamyl acetate and isoamyl alcohol. A further investigation by this group [14], with the addition of a 15^0C ferment with no heat treatment, observed similar sensory results with the panellists noting fruitier aromas with the lower ferment temperatures (15^0C) opposed to vegetal characters with higher (30^0C) temperatures. Two separate studies investigating the effects of malolactic fermentation on the sensory properties of Pinot noir wines [117, 128] employed the use of both first tier (broad descriptive categories ie. fruity, spicy and savoury) aromatic descriptors as well as specific descriptors (ie. plums, vanilla) to evaluate the samples. The first study determined 20 out 33 terms to show significance among the wines while the second examination's panellists only found minor differences. However, both studies revealed a loss of fruity aromas post malolactic fermentation.

Several investigations have utilised descriptive analysis to gauge the correlation of the chemical constituents of wine with its sensory attributes. Kwan et al. [55] in an early study revealed high correlations, via regression analysis, among sensory panel scores for several GC quantified compounds. A later study, examining wines from different grape maturities, employed two separately trained panels to evaluate Pinot noir wines as well as utilising GC-FID for chemical analysis. The group observed odour response to vary significantly among the samples while the FID chromatogram peaks were similar. However, the aroma compounds were not quantified [70, 77].

Descriptive analysis was applied to 28 Californian Pinot noir wines from three prominent wine growing regions, Carneros, Napa, and Sonoma, in an endeavour to define distinct sensory

properties for each region. A panel of seven referenced trained judges using 13 aroma descriptors determined the Carneros region's unique sensory attributes. The wines were characterised by fresh berry, berry jam, cherry, and spicy unlike the vegetal, leather, and smoke-tar aromas observed in the other two regions [112]. Significance ($p < 0.001$) amongst the judges was reported to be a sizeable source of variation.

Sensory analysis that included multidimensional scaling and descriptive analysis was undertaken evaluating the influence that water status might have on Pinot noir wines from Ontario [129]. This study observed differences between vineyards with varying water status levels across two growing seasons with respect to particular wine descriptors. The data indicated that Pinot noir is responsive to vine water status on a micro-terroir scale, but is highly dependent upon vintage. Another study using multidimensional scaling analysis descriptive analysis was used to differentiate NZ Pinot noir wines that had undergone differing levels of DAP supplementation [78] and Oregon Pinot noir wines fermented using different yeast strains that produced both more and less fruity notes [130]. Descriptive analysis and canonical variate analysis has also been used to differentiate Pinot noir wines from four major wine growing regions in NZ [15]. Results showed the four regions as being stylistically different and that experienced but untrained wine professionals can produce reliable results.

2.7 Identification of key aroma compounds of Pinot noir wines by means of linking chemistry with sensory

The aroma of wine is very complex as there are many different wines showing quite diverse aromas and the aromas of even a single wine change with time as it is being stored in the bottle or while it is in the glass waiting to be consumed. In general, wines do not have a simple characteristics aroma but rather a palette of subtle aromas which are difficult to define and are perceived differently by various people [131]. A valid impact compound is thought to be when

a molecule is able to transmit its entire sensory descriptors to a product to the point that the odour of the product is closely related to the odour of such chemical [132]. Due to the large number of volatiles in wine, it is thought that most wines do not have genuine impact compounds but rather depend on several active odorants that contribute to an overall distinct aroma. Some of these active volatiles are commonly referred to as key or primary aroma compounds or simply potential impact compounds. To understand and get a better understanding of the role each compound may play in the complex matrix of different wine aroma nuances, several structured steps should be undertaken [131]. These steps include the screening of aroma molecules via gas chromatography-olfactory (GC-O) followed by the isolation, identification and quantitative determination of the odorants. The final step is carried out using sensory tools to assess the potential importance and role played by each odorant.

2.7.1 GC-O

Gas chromatography with olfactometric detection is based on sensory evaluation of the eluate from the chromatographic column. This process uses humans as the assessors, whereby, they sniff the volatile compounds eluting from GC separation via a specifically designed odour port. For each separated compound, the assessor has the potential to detect its presence, measure the duration of odour activity, describe the quality of the odour and quantify the intensity of the odour [133]. In general, the main purpose of the GC-O evaluations is to list and rank the aroma compounds present in the product according to their potential importance in the overall flavour and smell [131]. The techniques developed to carry out these evaluations can typically be classified into three categories, namely, detection frequency, dilution to threshold and direct intensity.

2.7.1.1 Detection frequency

The detection frequency method requires 6-12 participants who each carry GC-O on the same extract. The proportion of the panel that is able to detect an odorant at a particular retention time

is counted [134-136]. Compounds that are detected more frequently are concluded to have a greater relative importance and this is assumed to relate to actual odour intensity perceived at the concentration of compound present in the extract [133]. The theoretical background and applications of this technique are well documented in the scientific literature [131, 134, 137].

The advantage of this method is that assessors do not need to be trained and the process is quite simple. In theory, the method accounts for the variable sensitivities of assessors and is repeatable [133, 134, 138]. A limitation to this method relates to the scale of measurement. It is assumed that detection frequency is related to actual odour intensity perceived [135].

2.7.1.2 Dilution to threshold

Dilution to threshold methods are used to quantify the odour potency of a compound, based upon the ratio of its concentration to its odour threshold in air [133]. A dilution series of an extract is prepared by a factor of two or three and each dilution is assessed by GC-O [139]. One to two assessors record when they detect an odour and record an odour description. The most frequently reported dilution methods are CharmAnalysis™ [139] and Aroma Extraction Dilution Analysis (AEDA)[140, 141] of which are reproducible and have been clearly established [47].

AEDA measures the maximum dilution of an extract that an odour is perceived in, and reports this as the flavour dilution (FD) factor [140-142]. CharmAnalysis™, on the other hand, records the duration of the odours from start to end and generates chromatographic peaks which are expressed as unit-less "Charm" values [139]. Overall, CharmAnalysis™ has more discriminating power than AEDA, bit it results in greater variation [143]. It should be known that neither method measures the odour intensity at any of the concentrations evaluated.

The major disadvantages of dilution methods is the length of time required to complete the analyses on each dilution for a single extract. While only one to two assessors are needed, this low number of assessors makes the results highly susceptible to the large variation in individual sensitivities. A robust selection process should be in place when choosing assessors to eliminate

this problem. In addition, the results depend on the sensory detection threshold of the analytes rather than on the realistic intensity of the analyte odour in a given sample [144]. The underlying assumption that the odour intensity increases with the concentration for all odour components in a sample also draws much criticism for dilution methods [145].

2.7.1.3 Direct intensity

Direct intensity methods require the assessor to use a scale to measure the perceived intensity of the compound as it elutes. This method can include a single time-averaged measure such as posterior intensity where assessors only rate the maximum odour intensity once the compound has eluted, or it can be dynamic such as the OSME method [77], whereby, the onset, maximum intensity and decay of the eluting odour is recorded continuously. These methods typically require a panel of 3-10 assessors and several repetitions per sample [133].

One drawback of the direct-intensity methods is the substantial amount of training that assessors require in order to obtain individual reproducibility and agreement with one another. This method is a more complex task than the others for collecting data as a unit of measurement or a scale needs to be created often creating the need for a specific software or physical apparatus [133].

2.7.2 Isolation and identification

The preparation of the extracts is an important and critical issue in GC-O and can have significant effects on the outcomes and success of research projects. There is no single perfect solution, therefore, the preservation of the compounds of interest is of utmost importance when choosing an isolation method. In general there are three main categories of extraction methods, namely, liquid extraction, static and dynamic headspace and finally, solid-phase micro extraction (SPME). There is an abundance of information about all of these methods and when considering isolation of compounds from wine, all are well documented in extensive reviews [131, 133, 146, 147].

Given the different concentration that wine active odorants are found, the most favourable results are typically when an array of analytical methods are used.

Following isolation and GC-O, the results are hierarchical lists of odour zones ranked by their potential sensory importance according to their respective values depending on GC-O method of choice as discussed earlier. The only indications of identification from these lists is the nature of the odour and its retention time, typically from a single GC column. This next step of identifying the odorants is critical and often a difficult task that requires precision and determination. General guidelines on this process, as discussed below, have been outlined and should be undertaken [131, 148].

The first step is the standardization of retention times which have to be normalised using the *n*-alkanes as standards. This mixture of alkanes is injected under the same conditions as the GC-O experiment. This process allows for odour zones to be determined and subsequently the Liner Retention index to be created. This process and data should be collected with columns of different polarities. The next step is to run the extracted sample on a GC-MS system in scan mode using the same column as the GC-O experiments. The n-alkane mixture should also be injected under the same conditions allowing a MS spectrum to be obtained. Once this data is obtained, the retention times and mass spectra can be compiled and in order to propose candidates for identification. There are several complete lists of the odorant composition of many wines in the literature [149-151]. Confirmation of the candidates can then be completed by simply injecting pure standards in the two columns in the GC-O system and the GC-MS system. This process should ensure the confirmation of most odorants present.

For odorants that could not be identified using this process, there are several questions to ask to assist in obtaining a more refined and concentrated fraction for identification. The compound could be quite heavy and/or polar, therefore, making the targeted odour zone come out at the end of the chromatogram. The targeted odour zone could correspond to an exceedingly volatile compound causing the peaks to overlap. The targeted odour zone may correspond with an ultra-

trace odorant belonging to the families of polyfunctional mercaptans or methoxypyrazines or the target zone may simply be a nonpolar compound of average to low volatility. All of these scenarios must be considered and researched for solutions.

2.7.3 Quantitative determination of the odorants

The next step in the process is quantifying the identified compounds and due to the complexity of the volatile fraction and the large differences in concentration, one must keep in mind that all sample preparation techniques will result in some selectivity and/or analyte losses during extraction [152]. GC-MS detectors are considered universal indicators responding to all chemical masses, but differences in analyte ionisation and fragmentation efficiencies can result in different response ratios and therefore should be carefully calibrated. Reviews of different calibration methods are widely available in most analytical chemistry textbooks. Typically internal standards are widely used for correcting for matrix effects, sample preparation losses and/or injection variability [147].

Once concentrations of all the known volatiles in the sample have been determined the, odour activity value (OAV) can be revealed as a ratio of the measured analyte concentration in the sample to the analyte sensory threshold. In general thresholds are simple values that are widely available in the literature. The threshold value, along with the odour description are typically the only parameters defining the behaviour of a given aromatic compound which are used and tabulated [132]. As a first approximation, it is expected that a compound above threshold (OAV > 1) is necessary to contribute to the overall aroma. However, this is lacking particularly in complex products as was demonstrated when a group of compounds with OAVs less than one were required to reconstruct the aroma of a dearomatised Maccabeo wine during a reconstitution study [153]. It is also presumed that all aroma chemicals present in a product at a concentration one order of magnitude below the threshold (OAV < 0.1) are irrelevant to the aroma of that

product [132]. Those compounds with high OAVs would be expected to most directly impact the overall aroma of the sample [154].

The shortcomings of OAVs are that the thresholds of various aroma compounds are determined in various mediums such as water, ethanol, water-ethanol solutions, white wine and red wine. This approach of determining thresholds does not account for the complex interactions that occur in perception of mixtures. In addition, the relationship between perceived intensity and concentration at suprathreshold concentrations cannot be predicted from the threshold concentration as often perceived intensity plotted against concentration is different for different compounds. These limitations should be considered when using OAVs as indicators of the contribution of individual odorants and its perceived intensity when in a mixture with other compounds [155].

2.7.4 Reconstitution studies

In previous studies, the final step in determining the impact of particular odorants in a sample was to use the obtained analytical data and prepare a synthetic blend or aroma model in an attempt to reconstruct the product. The odorants are mixed in a synthetic medium to evaluate the degree of similarity between the model and the original product. Often at this stage, compounds showing OAVs greater than a determined amount are used for the model. Some of the earlier attempts to prepare aroma models of wine proved to be difficult to use rigorous guidelines as such when the model turned out to unsatisfactory when only compounds with OAVs ≥ 10 were used. However, when a model was completed with odorants having OAVs of 1-9, the aroma matched very well that of the original [156]. In contrast when an additional 13 odorants with OAVs < 1 were added, there was no change to the model. These experiments led to the next step where additional models are prepared where some of the odorants have been omitted. The effect of these omissions are then evaluated. Obviously, the most important odorants are those whose omission causes the highest impact. Studies using this technique have found that the

omission of specific compounds can have drastic effects on the aroma perceptions of the model [156] or by contrast moderate to little effects when there are no true impact compounds [47, 153].

These initial experiments led to several conclusions and new ways of looking at the preparation of aroma models for reconstitution studies. Compounds showing similar aromas should be considered together as members of a family particularly if they are formed along similar biochemical pathways [131]. These compound families are typically always present in wine together, therefore, treating them as one will save time and give more accurate results. The impact of a compound should never be determined by its OAV but is the result of the interaction of the odorant with other odorants in the mixture. The reconstitution study aroma models should closely represent the original wine matrix as odorants in the blend interact and have been shown to exhibit suppressive and amplifying effects when the intensity of specific aroma attributes of the blend were compared to the original [157]. There is extensive literature to support that matrix components including ethanol, tannins, phenolics, polysaccharides and proteins can interact with volatiles affecting their volatility. Finally, experimenting with several wines (4-5) simultaneously instead of one wine can give better results [131].

2.7.5 Roles odorants can play in wine

Aromatic compounds in wines can play many roles dependent upon their concentration ratio to other odorants in the matrix, interaction with other non-volatile components in the mixture and the variety of wine. There are clearly identifiable roles in the way in which aroma compounds contribute to the overall nuances of a wine according to Ferreira and his group in Spain whom after extensive research have completed a framework for classifying volatile aromas in a wine matrix [131]. All these different roles can be categorised making it possible to organise the aroma compounds according to the role they may play in a given wine [132].

Genuine impact compounds are individual compounds which are at concentration high enough to transmit their specific aroma nuances to a given wine and is recognizable. A major contributor is played by individual compounds or by families of compounds that are present at a concentration high enough to transmit a primary generic descriptor of its aroma but not the specific descriptor of the compound. If these compounds are removed, there would be intense quantitative and qualitative changes to the overall aroma. Net contributors is a role played by individual of by families of compounds that are at a concentration high enough to transmit a generic descriptor. This descriptor is also transmitted by other compounds, therefore, if these compounds are removed there is a significant decrease in the intensity of this nuance but no change to the qualitative aroma profile is noted. Secondary or subtle contributors are played by individual compounds that are present in the wine at a concentration below that required to transmit individually a generic descriptor. If these compounds are removed from the wine, the overall effect is weak or null. Aroma enhancer is an individual or family of compounds that fail to transmit their specific or generic descriptors but instead enhance the specific aroma of some other molecule or group of molecules. If removed, there is a decrease in the intensity of the aroma nuance that it was enhancing. An aroma depressor is a compound or family of compounds whose presence causes a decrease in the intensity of an odour note. If removed, then there is an increase in the intensity of the depressed nuance [132].

2.8 Influences on aroma compound formation and their roles in wine

Wine aroma is derived from multiple sources and processes including grape-derived, microbially derived, oak-derived, chemical changes associated with acid and chemical modifications associated with oxidative processes. On the other hand, wine aroma can be influenced by environmental conditions and human intervention. A recent study has identified that approximately 18% of genes in grapevines can be impacted by environmental conditions and that

climate has the greatest effect on gene expression at veraison [158]. Environmental influences include vintage, sunlight, water and canopy management, grape maturity and fungal infections. Robinson *et al.* [147] has presented a review covering all of these influences. Understanding these interrelationships may become even more important as the impacts of climate change on grapevine metabolism, water quality and availability, grape berry maturation and other parameters become more apparent [159, 160].

Human intervention can also have great influence on compound formation and their subsequent roles in wine. There is an abundance of literature supporting the concept of how different yeasts, ferment temperatures, cap management, additives and barrel storage, to name a few, can influence have dramatic effects over the overall aroma. In contrast, the understanding of the role viticulture plays in the evolution of aroma compounds remains limited. One area that was of interest in this study was the role of crop thinning for yield management.

Cluster thinning is one way to control the crop load of a vine by simply removing whole clusters after the berries have set to achieve the desired yield. Despite the economic impact from increased human labor and loss of revenue from less yield, this method is widely used in many regions and is thought to provide more favorable conditions for the retained clusters by changing the ratio between leaf area and total fruit weight per plant. This increase in leaf area promotes berry development and maturation by providing a more beneficial situation for photosynthesis to occur [161]. It has been clearly demonstrated that cluster thinning advances grape maturity [162] improves phenolic grape composition [163] diminishes acidity and increases soluble solids and pH in juice [164]. These positive effects are thought to be vintage related [165] and can be altered by the vine water regime [166]. In contrast, cluster thinning usually increases the grape size which decreases the skin-to-pulp ratio and subsequently diminishes the positive effects on wine quality [167].

There have been few if any studies that have focused on the effects cluster thinning may have on the aromatic composition of wine and the consequent sensory effects. Most have centered their

attention on consumer perception of overall wine quality. It has been demonstrated that cluster thinning increased the herbaceousness in Chardonnay Musqué [168] and vegetal aromas in Cabernet Sauvignon [169] and decreased the consumer quality ratings in Sauvignon blanc [163, 170]. Conversely, cluster thinning had no effects on consumer perception of wine quality in other varieties [171-173].

2.9 Summary

Wine aroma has been studied, through chemical and sensorial evaluations, extensively over the last few decades. It is apparent that the aroma of wine is dependent not on a particular compound but on the profile and interactions of many odour-active compounds that are present. The potential aroma of wine is largely dependent on the release of aroma compounds from their odourless precursors during grape processing and wine maturation and the formation and modification of volatiles due to chemical changes during fermentation and aging. The developments in analytical, sensory, and statistical analysis have been significant for understanding the relationships between grape and wine composition and sensory perception. It is still not possible to fully predict aroma quality based on chemical composition alone and there have been few studies encompassing both chemical and sensory evaluations.

Despite the significant research on wine composition, summarized in this review, there is still much to be studied about the biochemical and chemical origins of wine volatiles and the effects of climate and viticultural practices on the concentrations of these compounds. Understanding the source of wine volatile compounds and the mechanisms that influence their formation through grape growing and winemaking is essential to developing strategies for production of wines with specific sensory attributes. Ultimately, multidisciplinary studies, using numerous approaches, will generate essential knowledge and be vital in the advancement and future discoveries in wine aroma research.

Chapter 3

Survey of major aroma compounds and phenolics in Pinot noir wines from New Zealand, France, Australia and the U.S.A.

Tanya E. Rutan[1], Mandy Herbst-Johnstone[1], Gwénaëlle Haese[2], Marion Piquet[2], Paul A. Kilmartin[1]*

[1]Wine Science Program, School of Chemical Sciences, University of Auckland, Private Bag 92019, Auckland, New Zealand

[2] Institut supérieur des sciences agronomiques, agroalimentaires, horticoles et du paysage. 65 Rue de Saint-Brieuc, 35000 Rennes, France

*Corresponding author (email: p.kilmartin@auckland.ac.nz)

Prepared for submission to the Australian Journal of Grape and Wine Research

Abstract

The chemical aroma, phenolic, tannin composition and colour properties of 105 Pinot noir wines from NZ, Australia, France and USA were determined using several analytical methods across two vintages. Perception threshold values were exceeded for several C_{13}- norisoprenoids, higher alcohols, esters from isoacids and fatty acids and the varietal thiol 3mercaptohexanol (3MH). The NZ wines contained higher concentrations of α-ionone and 3MH, on average, while the French wines had considerably higher concentrations of βdamascenone, higher alcohols, esters and volatile phenols. The concentrations of both flavonoids and non-flavonoids covered a broad range and no notable differences were seen. Overall, the French wines displayed the highest tannin concentrations for both survey years and slightly higher values for total phenols. When Principle Component Analysis (PCA) was applied the wines grouped regionally in both years. In the first year the NZ and Australian wines grouped closely, while the French wines were slightly more variable. In the second year the French and NZ wines grouped more closely while the wines from USA were more variable. Overall, the French wines had higher concentrations on average of the majority of the compounds considered in this study.

Keywords: Pinot noir, aroma survey, phenolics, tannin, phenolics

3.1 Introduction

Pinot noir is a difficult grape variety to cultivate and is greatly influenced by the local climate, topography of the land and soil type. It is a common belief that the flavours, aromas and textures of Pinot noir wines are directly related to the location where the grapes are grown and to the practices of the winemaker, more so than other red wines. The marketing success of place of origin labelling while dependent, upon the product [174], has been successful for the wine industry and is often used to differentiate wines in foreign markets. It has been used as a marker of quality and to influence consumer purchasing decisions.

Like several other red wines, there have been no genuine impact compounds identified in Pinot noir to date [132]. Pinot noir wines, despite their origin, are quite capricious and can encompass a range of flavours often depending on vintage. However, generalizations are regularly made with regards to wine flavours and textures, and it is often presumed that wines from the same country or region will have similar sensory properties and therefore a similar composition of volatile and non-volatile compounds.

Reports on the aroma content of Pinot noir wines date back to the early 1980s, when gas chromatographic (GC) methods coupled with mass spectroscopy (MS) detection were first applied to identify the compounds present. Over 60 GC peaks were seen with Burgundy [54] wines, and in Pinot noir wines from USA [7], many esters and alcohols were identified. Improved extraction methods later allowed 65 volatile compounds to be quantified and identified, and a further 45 compounds at concentrations less than 0.01 mg/L in Burgundy wines, including esters, alcohols, carbonyls and various other compounds [8]. In further studies, over 90 GC peaks were detected for Pinot noir wines from France and USA, enabling the wines to be grouped by geographic origin, and related to sensory quality scores [55, 56].

Specific groups of chemicals in Pinot noir wines were later investigated by two research groups [60, 61], where potent anthranilate and cinnamate compounds in Burgundy wines were detected. The chemical composition of Pinot noir wines has been monitored in response to different

viticultural and winemaking interventions. Different vinification techniques [58] and yeasts [14, 175] were applied to Canadian and South African Pinot noir wines, where the concentrations of a variety of aroma compounds were determined using GC-MS. Trends in a range of aroma classes were assessed for Oregon wines made from grapes harvested at different levels of maturity across two vintages [19].

These reports revealed substantial information about the chemical components of Pinot noir wines from many of the major Pinot noir growing regions around the world. Several studies made connections between the chemical data and flavour and mouth feel attributes collected through additional sensory studies. While informative, these studies typically considered only a few wines from one region, leaving open the question of how the chemical components of Pinot noir wines from international regions compare, using the same methodology for extraction and compound quantification. The aim of this study was to investigate the chemical composition of Pinot noir wines from several different regions by quantifying the concentrations of major aroma compounds, tannins and small phenolics, and colour properties. The data from this comparative analysis can provide a better understanding of the typical chemical makeup of Pinot noir wines, and the potential to differentiate between global winemaking regions.

3.2 Materials and methods

3.2.1 Wine samples

Seventy five Pinot noir wines from NZ, Australia and France from the 2005-2006 vintage were selected for analyses. This selection contained wines from eight different regions in NZ (n = 59): Martinborough, Nelson, Marlborough, Waipara, Hawkes Bay,

Coromandel, Auckland and Central Otago; three different regions of Australia (n = 6): Clare Valley, Hunter Valley and Yara Valley; and from the Burgundy region of France (n = 10). The following vintage (2006-2007), an additional 10 wines from NZ (Martinborough, Marlborough and Central

Otago), 10 wines from France (Burgundy) and 10 wines from Oregon, USA (Willamette Valley) were selected for analyses for a total of 105 samples across two consecutive years.

3.2.2 Aroma extraction and analysis

The extraction of the varietal thiol 3MH was carried out for all wine samples in duplicate using 50 mL samples spiked with 4-methoxy-2-methyl-2mercaptobutane (4M2M2MB) and deuterated standards (d3MH and d3MHA) [40]. The varietal thiols were reversibly combined with p-hydroxymercuribe oate (Sigma-Aldrich) and absorbed onto an anion exchange column. The addition of L-cysteine (Sigma-Aldrich) released the thiols from the complex. The eluate was extracted twice using dichloromethane (Merck). The recovered organic phase was dried with anhydrous sodium sulfate, filtered through glass wool, and concentrated under nitrogen gas flow to 25 µL in Agilent crimp top vials for immediate injection into an Agilent 6890N GC coupled with an Agilent MS 5973. A split injection (5:1) with a split flow of 4.9 mL/min and total flow of 8.8 mL/min was delivered with helium as a gas carrier onto an Agilent 19091N-136 HP-INNOWAX capillary column (0.252 mm x 60 m x 0.25 µm). The oven parameters were an initial temperature of 50°C and maximum temperature of 255 °C. The GCMS was operated in selective ion monitoring (SIM) mode with a total run time of 77.31 minutes.

The extraction of all other volatile compounds including monoterpenes, C_{13}-norisoprenoids, C_6-alcohols, higher alcohols, fatty acids, esters, and volatile phenols was performed in triplicate according to an established method [167]. This method was developed specifically for the quantification of methoxypyrazines in red wine but was modified to accommodate the quantifications of other compounds in this study by adding different internal standards and taking extracts from the organic phase at different steps of the extract concentration under nitrogen flow. A 200 mL wine sample was spiked with internal standards: 100 µL of DL-3-octanol (1830 mg/L) (99%, Lancaster, Ward Mill, MA) and 50 µL of 4-decanol (123.7 mg/L) (98%, Lancaster). The sample was adjusted to pH 8 and volatile compounds were purified using a triple

extraction with ether: hexane (1/1, v/v). The organic phase (~15 mL) was dried over anhydrous sodium sulfate, filtered through glass wool, and concentrated under nitrogen. Once the extract reached 1 mL, 200 µL was transferred into a GC vial for immediate injection onto a GC-FID (Agilent G1531 60010). The remainder of the sample was allowed to concentrate to 0.1 ml before being transferred into a GC vial for injection onto a Agilent 6890N GC coupled with an Agilent MS 5973 for quantification of ethyl cinnamate, ethyl 2,3-dihydrocinnamate, ethyl- and methyl anthranilate, and β-damascenone. A pulsed splitless injection with a pulse and purge time at 1.00 minute, purge flow of 12.0 mL/min, and total flow of 16.6 mL/min was delivered with helium as a gas carrier onto an Agilent 19091N-136 HP-INNOWax polyethylene glycol capillary column (0.25mm x 60m x 0.25µm). The oven parameters included an initial temperature of 60°C and maximum temperature of 250°C. The GC-MS was operated in selective ion monitoring (SIM) mode with a total run time of 86.46 min. GC-FID parameters included a splitless injection with a purge time at 1.00 minute, a purge flow of 12.0 mL/min, and a total flow of 15.7 mL/min was delivered with helium as a gas carrier onto an Agilent 19091N-136 HP-INNOWax polyethylene glycol capillary column (0.25mm x 60m x 0.25µm). The oven parameters had an initial temperature of 40°C and a maximum of 250°C. Total run time was 108.55 minutes.

Stock solutions of individual compounds (Table 3.1) were made by dissolving the compounds in absolute ethanol (Univar). Serial dilutions of these stock solutions were prepared, filtered and injected onto the GC-MS and GC-FID employing the same method and parameters as previously discussed. Eight point calibration curves were attained for each compound and respectively used for quantification of wine samples. A calibration curve was created for each volatile compound by spiking a deodorised commercial Pinot noir with increasing concentrations of each compound. The concentration ranges, peak ions, and calibration equations can be found in Table 1b. The calibration curves for compounds quantified on the GC-FID were generated using the internal standard 4-decanol due to the co-elution of cis-3-hexen-1-ol acetate with DL-3-octanol.

3.2.3 Phenolic analyses

The analysis of individual wine phenolics was conducted using Reversed Phased HPLC [176]. The samples were injected onto a Hewlett-Packard (Palo Alto, CA, USA) Agilent 1100 series instrument coupled to a Diode Array Detector (G1315B)a Column Heater (G1316A), an Auto-sampler (G1313A), a Quaternary Pump (G1311A) and a Degasser (G1379A). The column used was a Phenomenex Luna C18 (250 mm x 4.6 mm; pore size of 5 microns; Agilent). Milli-Q water (Barnstead; Thermo Scientific), acetonitrile, and a 5 % (v/v) acetic acid solution were used for the mobile phase. The column temperature was set at 25°C with a maximum column pressure of 259 bars and minimum pressure of 10 bars. The injection volume was set at 20 µL and a flow rate of 0.8 mL/minute. These parameters were constant for each sample with a run time of 150 minutes. Samples were filtered with a Sartorius Minisart (RC 15) 0.45 micron polytetrafluoroethylene syringe filters (Agilent) into Grace Ram 12 x 32 mm amber vials (Agilent). After degassing with argon, vials were sealed with Sun 9 mm silicon septas and screw caps (Agilent) for direct injection.

Wine phenolics were monitored at four different wavelengths, namely 280 nm, 320 nm, 365 nm, and 520 nm, matching the absorbance maxima of the various phenolic compounds, and permitting simultaneous quantification of various compounds in a single HPLC run. The quantification of phenolics was carried out using external standards, with the stock solutions being made up in absolute ethanol, except for quercetin which was prepared in methanol. Serial dilutions of stock solutions in model wine (5 g/L tartaric acid, 12 % v/v aqueous ethanol, pH adjusted to 3.6) were then performed to obtain different phenolic concentrations, chosen to be in the range of these compounds usually found in red wines (Table 3.2). The second year of wines were not able to be analysed in a timely manner due to equipment failure.

3.2.3.1 Tannin analyses

The tannin content was quantified in triplicate with 0.25 ml of sample using the Methyl Cellulose Precipitation (MCP) tannin assay [177]. Quantification is based on subtracting the absorbance values at 280 nm (A280) of solutions with and without precipitation, and the subtractive value is expressed as epicatechin equivalents. Spectrophotometric absorbance values were obtained using a Genesys ™ 10 Thermospectronic and Eppendorf disposable cuvettes (2 mm pathway). Centrifugation was performed using an Eppendorf Centrifuge 5804. The calibration curve was constructed by serial dilution using nine known concentrations of (-)-epicatechin (Sigma-Aldrich, St. Louis, MO) ranging from 5 to 250 mg/L in Milli-Q water. The absorbance values measured at 280 nm for these standard solutions were plotted against their corresponding concentrations to construct the calibration curve, which was used to report results of MCP tannin in epicatechin equivalents (R2 = 0.996).

3.2.3.2 Colour analyses

Each wine sample (50 ml) underwent spectrophotometric analyses in triplicate on a Genesys™ 10 Thermospectronic (Waltham, M.A., USA) using Eppendorf cuvettes (Hamburg, Germany) according to established procedures [164]. Wine colour density was measured at absorbance (A) 420 nm + A520 nm and wine hue was A420/A520. Total phenols was measured at A280.

3.2.4 Reagents

All the chemicals used were of analytical grade. Sodium hydroxide, sodium acetate, diethyl ether, sodium sulfate, sodium dihydrogen phosphate dihydrate, hexane (96%), absolute methanol (99%), acetic acid glacial (99%), acetonitrile (gradient grade) and hydrochloric acid were from Scharlau (Sentmenat, Spain). Ammonium sulfate was from Univar (Redmond, WA). Methyl cellulose and tartaric acid were from Sigma-Aldrich (St. Louis, MO). Dichloromethane was from Merck (Darmstadt, Germany). Potassium metabisulfite was from Redox Pty Ltd (Auckland, NZ) and absolute ethanol (99%) from ECP Ltd. (Auckland, NZ).

3.2.5 Statistical analysis

Principal component analysis (PCA) was conducted on the mean concentrations of each compound for individual samples using MultiBase 2012 (Numerical Dynamics, Chiba-ken, Iso, Japan).

3.3 Results and discussion

3.3.1 Aroma compounds

Of the aromas compounds, the average values for all of the samples (Table 3.3) exceeded the respective perception threshold for many of the C_{13}- norisoprenoids, higher alcohols, esters from isoacids and fatty acids and the varietal thiol 3-mercaptohexanol (3MH). The average concentrations of α-ionone, which imparts woody and floral aromas, were noticeably higher in the NZ wines, while the French Pinot noir wines had considerably higher concentrations of β-damascenone. This compound exhibits varying odours depending on its concentration from lemon balm at low levels, to apple, rose and honey in higher amounts [59]. Historically, β-damascenone was consistently perceived as having high odour activity values (OAV) values in all red wines sampled [28], and was therefore considered to be a major contributor to wine aroma. This suggestion was challenged when a study [25] determined the perception threshold of β-damascenone to be considerably higher in red wine (7 μg/L) than that previously reported in an ethanol/water base (0.05μg/L) [10]. Norisoprenoids, an important enhancer of fruity aromas in red wines [47], often vary in concentration between vintages [178], and have been shown by various studies to be affected by sunlight, water and canopy management [147].

The varietal thiols 4MMP and 3MHA were not detected in any of the samples, while the NZ wines had slightly higher concentrations of 3MH, which has been reported to contribute to fruity aromas in some red wines [48], and to provide a general lift aromas in many wines [131]. These varietal

compounds are highly influenced by vintage, climate and canopy management in varieties such as Sauvignon blanc [41].

Of the higher alcohols, isoamyl alcohol was of interest in both surveys, as it was present at concentrations well above the perception threshold in all of the wines tested. The concentrations of isoamyl acetate, derived from isoamyl alcohol, also exceeded perception threshold in all of the samples. The wines from France were higher on average in isoamyl alcohol concentrations for both years, a trend also seen with isobutanol, β-phenyl ethanol, and for the 2006 wines, methionol. The higher alcohols are formed by yeast activity from grape sugars and amino acids and contribute to wine aroma through their intense odors, even at low concentrations. Their concentrations are largely influenced by fermentation temperatures along with varying wine making styles [21].

All of the ethyl esters derived from isoacids, fatty acids and cinnamic acids were present at concentrations above their perception thresholds in the surveys, with the exception of ethyl decanoate and ethyl hydrocinnamate. The average concentrations of the esters were similar across all samples in both surveys, with the French wines having higher concentrations of esters from isoacids in both years, and a considerably higher concentration of ethyl cinnamate. Esters, known to contribute fruity aromas to wine, have been reported at a wide range of concentrations in Pinot noir wines [8, 57, 90, 175, 179], and their formation depends largely on fermentation temperature and yeast strain chosen [14, 58]. These important volatile compounds have been shown to decrease with aging [19, 70, 77]. Additional esters were quantified during the second survey, although none of the samples exceeded the respective perception thresholds, with the exception of ethyl lactate, whose concentration has been directly linked to the concentration of lactic acid produced though malolactic fermentation [147].

Fatty acids (C_6 to C_{10}) typically occur at detectable concentrations in wines spoiled by micro-organisms, although their occurrence at or just below the threshold can contribute to the overall

complexity of wine aroma. Many of the wines had concentrations of hexanoic acid and octanoic acid above perception threshold, but with decanoic acid the concentrations well below threshold.

At high concentrations ethyl phenols are considered a fault in red wines, lending aromas of sweaty saddles, stables, and smoky. At lower concentrations, these compounds can add complexity to the aroma of red wines, or alter the aroma by masking the other volatile constituents [73]. The concentration of these compounds in Pinot noir wines have been reported over a broad range [8, 19, 90, 180]. In this study, the concentrations of 4-ethyl phenol and 4-ethyl guaiacol were the lowest in the NZ wines, only slightly over threshold in the USA wines, while noticeably higher values, well over perception thresholds, were observed in the wines from France.

The concentrations of monoterpenes, like the norisoprenoids, vary considerably amongst cultivars with some varieties depending on them for cultivar distinction. In general, the role of terpenes in red wine, particularly in Pinot noir, is still largely unknown. The values found in both surveys fell well below perception thresholds, with the exception of geraniol in some NZ wines, where it just reached threshold. These finding were consistent with earlier studies where monoterpenes were quantified in Pinot noir wines [12, 13]. The overall low concentrations of these compounds suggest a minimal importance in Pinot noir aromas; although the possibility of a synergistic function in the red wine matrix has been reported in an aroma study on Touriga Nacional, a Portuguese red variety [181]. The C_6 alcohols were found in all samples at similar levels, although below perception thresholds.

3.3.2 Phenolics

Phenolics are a large and complex group of compounds that affect the appearance, taste, mouth-feel, and anti-microbial properties of wine. The concentrations of both flavonoids and non-flavonoids in this survey (Table 3.3) fell into broad ranges and no notable differences were seen. The French wines presented the lowest values on average for all of the flavonoids, but displayed slightly higher values for many of the non-flavonoids. The concentration of phenolics in wines

are altered by environmental factors including vine water status, heat, sunlight and vine vigour [182, 183] as well as winemaking techniques [184].

The concentrations of tannins observed (Table 3.3) were similar to a previous study where values ranged from 30-998 mg/L EC in Pinot noir wines [185]. Overall, the French wines displayed the highest tannin concentrations for both surveyed years.

Spectroscopic colour absorbencies provide a range of information such as levels of red and brown coloured pigments and total phenols. Of the properties tested, there were no exceptional differences on average across both years (Table 3.3). The French wines had slightly higher concentrations for total phenolics for both surveyed years.

3.3.3 Principal component analysis (PCA)

The representative wines from each region and the relationships with the chemical parameters are shown in PCA plots providing graphic representations for both surveyed years. For the 2005-2006 survey the first PCA (Fig. 3.1a) shows the relations between the chemical parameters, and the second PCA (Fig. 3.1b) shows the position of the wines. The PCs represent 46% of the variation in the data set with 23 and 12% explained by PC1 and PC2, respectively, and 11% by PC3. The probable reason for not having a higher explained variance was the fact that the number of samples from NZ was significantly higher than the other two regions. Component 1 has positive loadings for higher alcohols and esters and negative loadings for the flavonols, which were also negative in PC2. Component 2 displayed positive loadings for acetates and monoterpenes. The concentrations for each wine for the two components are projected in Fig. 3.1b. The first component has positive loadings for the French wines and varied loadings for the NZ wines. The Australian wines were positive for both components. The wines grouped by country and were separated by families of volatile compounds. The French wines on average had higher concentrations of alcohols and esters while the NZ wines were separated by higher concentrations of the flavonols, and the Australian wines by acetate esters. The NZ and Australian wines grouped very closely together, while the French wines were slightly more variable.

Figures 3.2a and b correspond to the 2006-2007 survey. The PCs represent 67% of the variation in the data set with 37 and 23% explained by PC1 and PC2, respectively, and 7% by PC3. When considering the chemical parameters (Fig. 3.2a), component 1 has positive loadings for total phenols, tannins, volatile phenols, two esters of isoacids, four miscellaneous esters, three alcohols, two acetates, α-terpineol, ethyl cinnamate and β-damascenone and negative loadings for hexanol, αionone and one colour property, which was also negative in PC2. Component 2 showed positive loadings for several monoterpenes, isoamyl and β-phenylethyl acetates, methionol, fatty acids and their esters, and negligible negative loadings for 3MH and ethyl hydrocinnamate. When considering the concentrations for each wine (Fig. 3.2b), all the French wines, except one, had moderately positive loadings in PC1, while most of the wines from USA and NZ loaded negatively. In PC2, most of the wines from USA had positive loadings and most of the French and NZ wines loaded negatively. The wines grouped regionally, as in the first survey, with the French wines having closer associations with the majority of the compounds considered in this study. In view of the aroma families, the French wines had higher mean concentrations of tannins, volatile phenols, esters and alcohols. Many of the wines from USA were separated by monoterpenes, fatty acids and their esters and the NZ wines by wine colour density. The French and NZ wines tended to group more closely, while the wines from USA were more variable.

3.4 Conclusions

The chemical constituents of Pinot Noir wines can vary both between and within the different growing regions. There were several compound families where perception thresholds were exceeded for most of the wine samples, including C_{13} norisoprenoids, higher alcohols, esters of isoacids and fatty acids and cinnamic esters, and can be considered of importance to the overall varietal aromas of Pinot Noir wines. Both ethyl phenols and tannins were consistently higher in the French wines, however, no other consistent trends were observed. When considering the

data using a different approach, via PCA, the NZ and Australian wines grouped closely. By contrast the French wines were slightly more variable in the first year, and in the second year the French and NZ wines grouped more closely while the wines from USA were more variable. Overall, this data provided insight into the typical concentrations of various aroma compounds found in Pinot noir wines from an international perspective. The groupings displayed in the PCA data revealed that while the wines shared common aromatic compounds, the ratios of these compounds were distinct when considering the different regions. The clustering of wines from the same region helps explain why Pinot noir wines from a particular place can often have similar sensory profiles and perceived characteristics that originate from climate and viticultural and winemaking practices of a distinct area.

Table 3.1 Supplier, concentration range, instrument employed, ions, retention times, and R^2 values for the aroma compounds (µg/L) used in this study

	Supplier	Conc. Range (µg/L)	Instrument	Wavelength or Ions (m/z)	Retention Time (min) 2005-06/2006-07	R^2 2005-06	R^2 2006-07
C_{13} norisoprenoids							
α-ionone	Sigma-Aldrich	1.8 - 36.2	GC-FID		49.5		0.989
β-ionone	Sigma-Aldrich	1.9 - 39.4	GC-FID		52.3		0.992
β-damascenone	Sigma-Aldrich	0.9 - 18	GC-MS	190/121/175	25.78		0.996
Terpenes							
Linalool	Acros	1.6 - 33	GC-MS/FID	71/93/121	38.1/37.9	0.994	0.996
Nerol	Sigma-Aldrich	0.53 - 11	GC-FID		47.12		0.993
Geraniol	Acros	0.55 - 11	GC-FID		48.8		0.992
Citronellol	Acros	0.76 - 15	GC-FID		46.0		0.993
A-terpineol	Acros	1.6 - 31.5	GC-MS/FID	93/121/136	44.4/43.7	0.998	0.990
C_6 alcohols							
1-hexanol	Acros	273 - 5468	GC-MS/FID	69/56	30.4/29.3	0.998	0.994
Cis-3-hexen-1-ol	Sigma-Aldrich	53 - 1066	GC-FID		30.5		0.995
Trans-3-hexen-1-ol	Lancaster	36 - 714	GC-FID		29.5		0.997
Alcohols							
Isoamyl alcohol	Panreac	34000 - 286000	GC-MS/FID	70/55	22.1/21.8	0.983	0.994
Isobutanol	Scharlau	9023 - 247000	GC-FID		15.3		0.994
Methionol	Acros	104 - 6086	GC-MS/FID	106/73/59	45.1/43.9	0.998	0.991
β-phenyl ethanol	Acros	5020 - 25568	GC-MS/FID	122/91/92	54.2/50.2	0.999	0.986
Fatty acids							
Hexanoic acid	Acros	530 - 10000	GC-FID		48.6		0.992
Octanoic acid	Acros	600 - 12000	GC-FID		55.9		0.993
Decanoic acid	Acros	560 - 11100	GC-FID		62.6		0.995
Varietal thiols							
3MH	Acros	0.514 – 12.3	GC-MS	134/100	37.9		0.994

3MHA	Oxford	0.052 – 1.7	GC-MS	116/101	34.5		0.993
4MMP	Interchim	0.0054 – 0.17	GC-MS	132/75	24.8		0.993
Ethyl esters of isoacids							
Ethyl isobutyrate	Sigma-Aldrich	45 - 909	GC-MS/FID	73/71	9.5/9.9	0.989	0.990
Ethyl butyrate	Acros	47 - 947	GC-MS/FID	60/71	12.8/13.9	0.995	0.993
Ethyl isovalerate	Fluka	5 - 99	GC-MS/FID	60/70/85	13.4/14.7	0.989	0.990
Ethyl esters of fatty acids							
Ethyl hexanoate	Fluka	48 - 960	GC-MS/FID	101/88/99	23.8/23.7	0.998	0.993
Ethyl decanoate	Sigma-Aldrich	9 - 170	GC-MS/FID	70//88/101	42.3/41.8	0.991	0.993
Ethyl octanoate	Acros	86 - 1728	GC-MS/FID	101/88	35.3/33.5	0.994	0.995
Cinnamic esters							
Ethyl cinnamate	Sigma-Aldrich	0.72 - 15	GC-MS	176/131/103/148	33.4		0.992
Ethyl hydrocinnamate	Sigma-Aldrich	0.84 - 17	GC-MS	178/104/107/133	27.3		0.991
Miscellaneous esters							
Ethyl lactate	Acros	6000 - 98000	GC-FID		28.4		0.994
Ethyl-2-furoate	Sigma-Aldrich	15 - 305	GC-FID		40.4		0.996
Ethyl anthranilate	Sigma-Aldrich	0.23 - 15	GS-MS	165/119/120/137	36.3		0.995
Methyl anthranilate	Sigma-Aldrich	0.24 - 9	GC-MS	151/119/120	35.5		0.992
Diethylmalate	Sigma-Aldrich	5000 - 108000	GC-FID		54.1		0.992
Diethyl succinate	Acros	5100 - 10000	GC-FID		42.6		0.993
Acetates							
Isobutyl acetate	Fluka	19 - 376	GC-MS/FID	73	11.6/11.9	0.982	0.997
Isoamyl acetate	Univar	272 - 5445	GC-MS/FID	87/55/70	17.8/17.6	0.997	0.995
Hexyl acetate	Acros	34 - 683	GC-MS/FID	61/56/69	25.8/27.6	0.997	0.994
Phenylethyl acetate	Acros	27 - 543	GC-MS/FID	91/104/105	48.7/47.7	0.997	0.993
Volatilephenols							
4-ethylphenol	Sigma-Aldrich	11 - 424	GC-MS		59.0		0.998
4-ethylguaiacol	Sigma-Aldrich	21 - 853	GC-MS		53.7		0.994

Table 3.2 Supplier, concentration range, instrument employed, ions, retention times, and R^2 values for the phenolic (mg/L) used in this study

	Supplier	**Conc. (mg/L)**	**Instrument**	**Wavelength or Ions (m/z)**	**Retention Time (min) 2005-06**	**R^2 2005-06**
Flavonols						
Gallic acid	Sigma-Aldrich	1.5 – 200	RP-HPLC	280 nm	9.4	0.999
Syringic acid	Sigma-Aldrich	0.4 – 50	RP-HPLC	280 nm	53.8	0.999
Caftaric acid	Sigma-Aldrich	0.5 - 60	RP-HPLC	320 nm	24.0	0.999
Cis-coutaric acid	Sigma-Aldrich	0.5 – 60	RP-HPLC	320 nm	35.0	0.998
Trans-coutaric acid	Sigma-Aldrich	0.5 - 60	RP-HPLC	320 nm	37.0	0.998
Caffeic acid	Sigma-Aldrich	0.5 – 50	RP-HPLC	320 nm	47.6	0.999
Trans-resveratrol	Sigma-Aldrich	0.5 – 60	RP-HPLC	320 nm	89.7	0.999
Non-flavonols						
Catechin	Sigma-Aldrich	3.0 – 350	RP-HPLC	280 nm	38.3	0.999
Epicatechin	Sigma-Aldrich	2.0 - 300	RP-HPLC	280 nm	54.5	0.999
Quercetin-3-glucoside	Sigma-Aldrich	0.6 - 85	RP-HPLC	365 nm	77.2	0.998
Delphinidin-3-glucoside	Sigma-Aldrich	4.0 - 1000	RP-HPLC	520 nm	50.9	0.998
Malvidin-3-glucoside	Sigma-Aldrich	4.0 - 1000	RP-HPLC	520 nm	62.9	0.998

Table 3.3 Mean concentrations of aroma compounds (µg/L), phenolics (mg/L), tannins (mg/L) and colour properties (absorbances) for the 2005-06 and 2006-07 surveys. Minimum and maximum range includes both surveys

	NZ 2006	FR 2005	AU 2006	NZ 2007	FR 2006	USA 2006	Range min - max	Perception Threshold
C_{13}norisoprenoids								
α-ionone				0.31 (± 0.12)	0.14 (± 0.02)	0.19 (± 0.08)	0.17 - 1.62	2.6 [10]
β-ionone				0.63 (± 0.19)	0.58 (± 0.15)	0.73 (± 0.39)	0.31 - 0.81	0.09 [10]
β-damascenone				2.7 (± 1.1)	12.4 (± 1.9)	4.3 (± 2.5)	0.80 - 14	0.8, 7 [10, 25]
Terpenes								
Linalool	3.1 (± 1.6)	2.1 (± 1.3)	4.6 (± 3.6)	2.4 (± 0.9)	1.9 (± 0.6)	3.5 (± 1.5)	1.7 - 11	25 [10]
Nerol				3.7 (± 1.7)	3.3 (± 0.7)	6.1 (± 3.2)	1.9 - 13	400 [41]
Geraniol				20 (± 9)	7.4 (± 3.9)	11(± 7)	2.6 - 39	20 [10]
Citronellol				9.9 (± 4.6)	4.6 (± 2.3)	6.8 (± 1.1)	nd - 14	100 [10]
α-terpineol	4.5 (± 3.6)	5.9 (± 1.8)	4.8 (± 2.6)	3.1 (± 1.5)	8.5 (± 2.4)	4.7 (± 1.7)	1.6 - 18	250 [10]
C_6 alcohols								
1-hexanol	1159 (± 308)	824 (± 226)	1032 (± 378)	835 (± 195)	475 (± 103)	619 (± 181)	319 - 2041	8000 [10]
Cis-3-hexen-1-ol				53 (± 10)	43 (± 8)	34 (± 7)	26 - 58	400 [10]
Trans-3-hexen-1-ol				35 (± 11)	25 (± 16)	26 (± 12)	5.2 - 57	1000 [41]
Alcohols								
Isoamyl alcohol	95720 (± 26387)	150236 (± 32300)	117305 (± 25440)	114171 (± 12992)	185422 (± 26917)	146505 (± 29026)	45389 - 240839	30000 [66]
Isobutanol				12654 (± 2981)	32456 (± 8610)	15655 (± 3712)	9500 - 51500	40000 [66]
Methionol	560 (± 108)	1298 (± 302)	885 (± 185)	1595 (± 543)	1643 (± 369)	2349 (± 1520)	173 - 5304	1000 [66]
β-phenyl ethanol	15692 (± 7090)	24407 (± 6320)	21690 (± 4818)	69113 (± 1792)	115342 (± 3010)	97702 (± 1483)	5321 - 173321	14000 [28]
Fattyacids								
Hexanoic acid				710 (± 260)	380 (± 54)	570(± 221)	170 - 1100	420 [28]
Octanoic acid				335 (± 120)	401 (± 71)	605 (± 188)	190 - 1010	500 [28]
Decanoic acid				135 (± 100)	161 (± 122)	197 (± 184)	nd - 580	1000 [10]
Varietal thiols								
3mh				764 (± 294)	624 (± 216)	555 (± 244)	325 - 1414	0.06 [43]
Ethyl esters of isoacids								
Ethyl isobutyrate	84 (± 51)	189 (± 66)	105(± 72)	70 (± 19)	161 (± 42)	86 (± 18)	61 - 295	15 [10]
Ethyl isovalerate	39 (± 4)	51 (± 5)	20 (± 14)	26(± 8)	41 (± 11)	28 (± 18)	18 - 61	3 [10]
Ethyl esters of fatty acids								
Ethyl butyrate	97 (± 46)	133 (± 54)	157 (± 33)	167 (± 54)	173 (± 69)	195 (± 70)	88 - 263	20 [10]

Ethyl hexanoate	168 (± 62)	203(± 99)	256 (± 93)	143 (± 79)	166 (±72)	283 (± 134)	125 - 315	14 [10]
Ethyl decanoate	146 (± 47)	121 (± 22)	187 (± 50)	137(± 55)	122 (± 74)	154 (± 72)	102 - 201	200 [10]
Ethyl octanoate	341 (± 74)	346 (± 182)	383 (± 151)	363 (± 147)	267 (± 92)	425(± 162)	219 - 499	5 [10]
Cinnamic esters								
Ethyl cinnamate				2.7 (± 0.7)	8.0 (± 1.1)	1.7 (± 0.6)	0.93 - 9.0	1.1 [10]
Ethyl hydrocinnamate				1.5 (± 1.4)	1.1 (± 1.0)	nd	nd - 4.1	1.6 [10]
Miscellaneous esters								
Ethyl lactate				137793 (± 43019)	191494 (± 33307)	145543 (± 44025)	79628-258643	154000 [10]
Ethyl-2-furoate				45 (± 17)	24 (± 14)	21 (± 5)	11.2 - 74.3	16000 [10]
Ethyl anthranilate				0.8 (± 0.3)	4.3(± 1.6)	1.5 (± 1.1)	0.39 - 6.56	
Methyl anthranilate				0.2 (± 0.2)	1.3 (± 1.0)	0.5 (± 0.6)	nd - 2.03	3 [60]
Diethylmalate				69 (± 22)	115 (± 32)	36 (± 17)	11.2 - 166	10000 [66]
Diethyl succinate				9473 (± 4211)	1047 (± 1851)	18912(± 9521)	3544 - 34026	200000 [10]
Acetates								
Isobutyl acetate	29 (± 14)	44 (± 18)	28 (± 11)	28 (± 9)	66(± 15)	54 (± 15)	12.2 - 79.7	1600 [10]
Isoamyl acetate	152 (± 64)	193(± 51)	476 (± 317)	223 (± 27)	275 (± 46)	330 (± 43)	55.8 - 843	30 [10]
Hexyl acetate	10 (± 14)	5 (± 3)	12 (± 6)	18 (± 4)	23 (± 5)	20(± 4)	4.51 - 102	700 [66]
Phenylethyl acetate	17 (± 28)	13 (± 7)	31 (± 24)	10 (± 2)	13 (± 2)	14 (± 1)	2.12 - 202	250 [10]
Volatile phenols								
4-ethylphenol				420 (± 126)	1859 (± 741)	631 (± 402)	281 - 3212	600 [85]
4-ethylguaiacol				Nd	489 (± 220)	23 (± 73)	nd - 851	110 [85]
Phenolics								
Flavonols								
Gallic acid	65 (± 11)	53 (± 26)	58 (± 12)				10.8 - 115	
Syringic acid	10 (± 3)	4 (± 3)	10 (± 1)				1.32 - 15.9	
Caftaric acid	19 (± 1)	57 (± 20)	20 (± 10)				1.58 - 23.8	
Cis-coutaric acid	1.4 (± 0.8)	2.8 (± 0.6)	1.5 (± 0.6)				0.24 - 3.40	
Trans-coutaric acid	5 (± 10)	14 (± 6)	5 (± 3)				0.81 - 18.8	
Caffeic acid	14.3 (± 0.3)	13.4(± 4.8)	12.4 (± 6.9)				0.91 - 36.1	
Trans-resveratrol	5.3 (± 2.2)	7.4 (± 8.3)	5.0 (± 0.3)				2.19 - 30.9	
Tannins	828 (± 109)	1275 (± 461)	1013 (± 139)	757 (± 124)	1926 (± 429)	1110 (± 329)	160 - 2659	
Non-flavonols								
Catechin	161 (± 37)	83 (± 37)	129 (± 52)				22.6 - 353	
Epicatechin	94 (± 34)	65(± 50)	67 (± 30)				21.1 - 184	
Quercetin-3-glucoside	6.3(± 3.1)	5.4 (± 3.1)	8.1 (± 6.5)				2.03 - 22.6	
Delphinidin-3-glucoside	2.9 (± 0.7)	0.34 (± 0.74)	2.6 (± 2.9)				nd - 17.1	
Malvidin-3-glucoside	65 (± 1)	36 (± 27)	64 (± 55)				8.78 - 167	
Colour properties								
Wine colour density	4.3 (± 0.6)	4.6 (± 0.8)	4.4 (± 0.8)	5.9 (± 1.8)	3.8 (± 0.5)	3.9 (± 0.8)	2.51 - 10.3	
Wine hue	0.86 (± 0.06)	0.77 (± 0.07)	0.94 (± 0.16)	0.78 (± 0.09)	0.90 (± 0.04)	0.89 (± 0.09)	0.28 - 1.15	

Total phenols	57 (± 11)	60 (± 14)	53 (± 7)	39 (± 16)	53 (± 6)	32 (± 9)	9.64 - 100

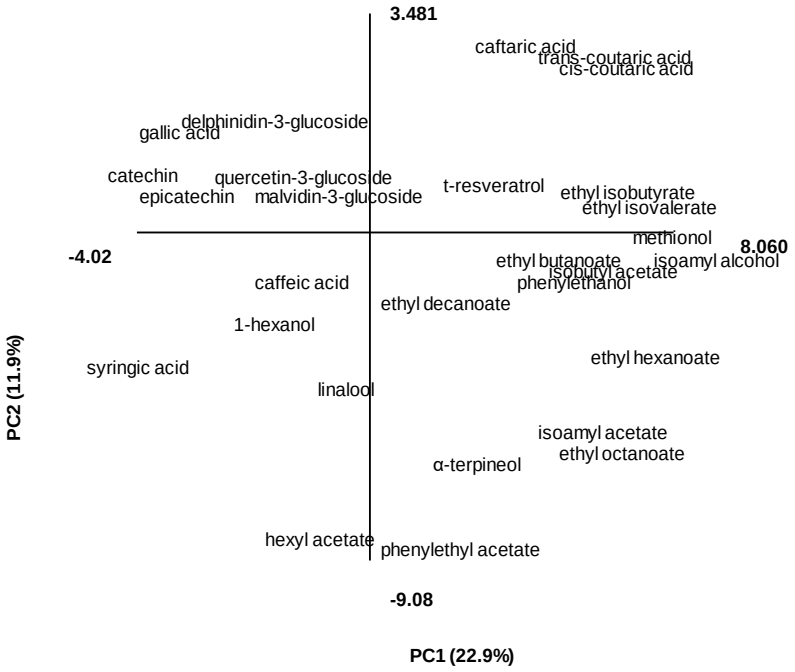

Figure 3.1a Principal component analysis showing the relations between the chemical parameters for the 2005-2006 survey

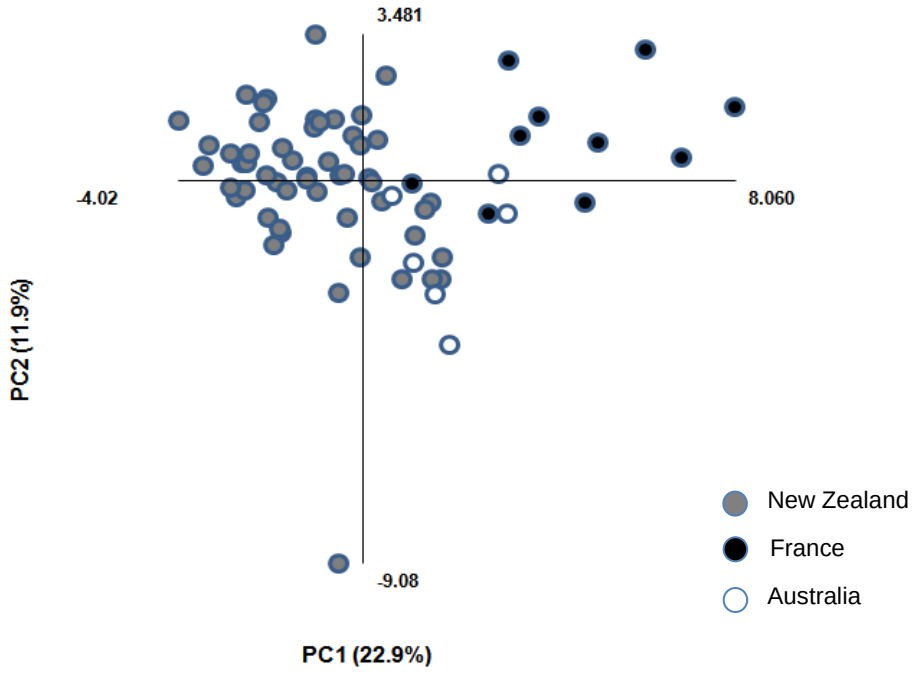

Figure 3.1b Principal component analysis showing the position of the wines in relation to the chemical parameters for the 2005-2006 survey

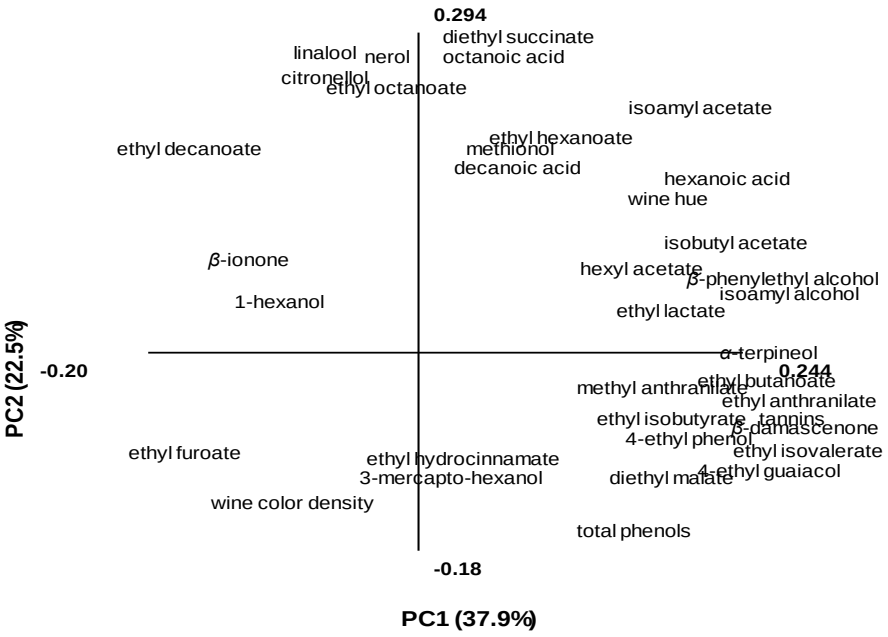

Figure 3.2a Principal component analysis showing the relations between the chemical parameters for the 2006-2007survey

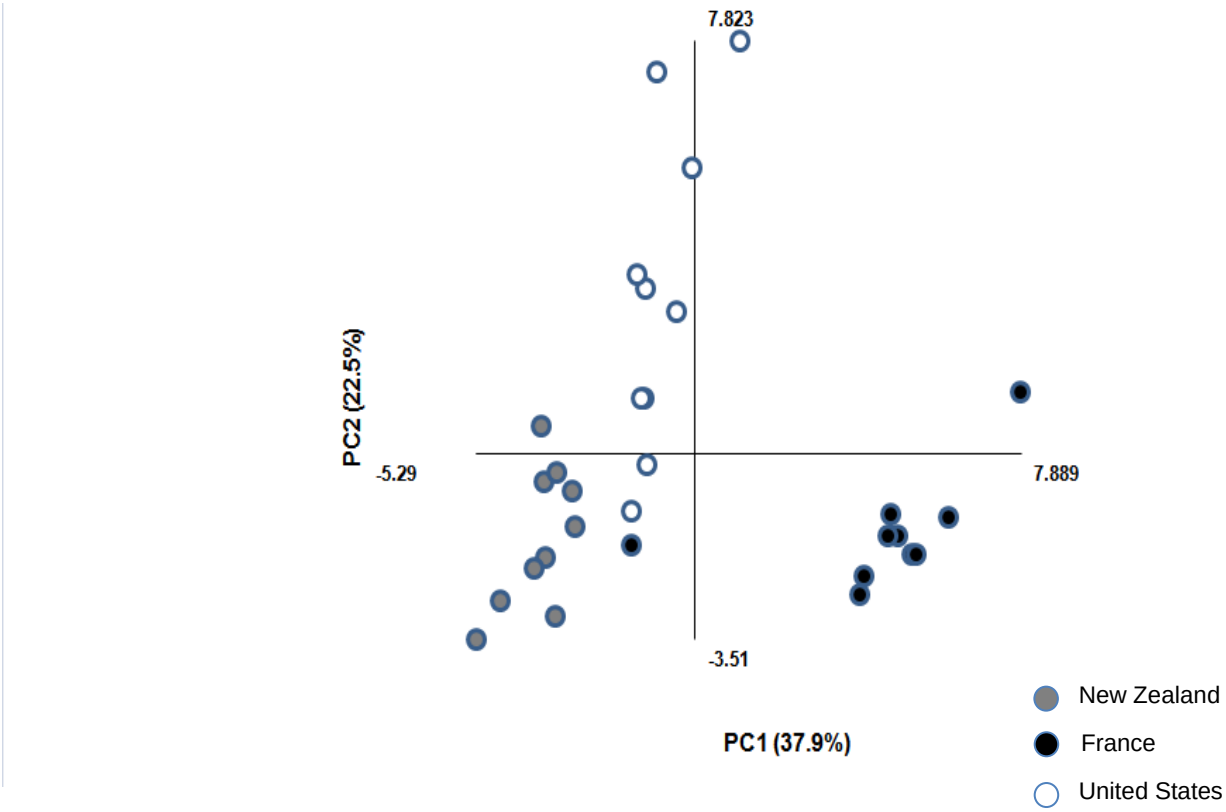

Figure 3.2b Principal component analysis showing the position of the wines in relation to the chemical parameters for the 2006-2007 survey

Chapter 4

Characterisation of the aroma of Central Otago Pinot noir wines using sensory reconstitution studies

Tanya Rutan[1], Mandy Herbst-Johnstone[1], Benedicte Pineau[2] and Paul A. Kilmartin[1]*

[1] Wine Science Programme, School of Chemical Sciences, The University of Auckland, Private Bag 92019, Auckland 1142, New Zealand; [2]Plant & Food Research Mt. Albert, Private Bag 92169, Auckland 1142, New Zealand

Published in American Journal of Enology and Viticulture 2014

Key Words: Pinot noir, wine aroma, OAV, AEDA, aroma reconstitution, impact odorants

Acknowledgements: This research was funded by The New Zealand Ministry of Business, Innovation and Employment, and New Zealand Wine Growers. We thank Mt. Difficulty Winery for the supply of wines.

Abstract

The aroma of two representative Pinot noir wines over two consecutive vintages from the Central Otago region of NZ underwent gas chromatography olfactory analysis. Forty-two odorants were identified in the Aroma Extract Dilution Analysis study with flavourdilution (FD) factors ranging from 3 to 19683, with over 20 having FD > 81. The highest FDs were for phenylethyl alcohol (rose), ethyl isobutyrate (strawberry), *β*-damascenone (tea, floral), isovaleric acid (cheese), ethyl isovalerate (fruit, cherry), ethyl cinnamate (honey, cinnamon), and guaiacol (medicinal, smoky). Following AEDA studies, 51 compounds were quantified via GC-MS following liquid-liquid extractions, of which 22 were present at concentrations above perception threshold. The odorants with the highest odouractivity values were *β*-damascenone, ethyl octanoate, ethyl hexanoate, ethyl isovalerate, isovaleric acid, and 3-mercapto-hexanol with values ranging from 17 to 95. Using the data from these screening methods, aroma models were prepared by deodorizing two Pinot noir wines with LiChrolut EN resin and reconstituting the wines using purified aroma standards. Five further models for each wine were prepared in the same manner but with the omission of one major aroma family, and the models were presented to a trained panel. When compared to the fully reconstituted model, the omission treatments altered certain sensory descriptors, although overall no profound differences were observed and no genuine impact compounds were revealed.

4.1 Introduction

Central Otago is a relatively young and small wine growing region of NZ, releasing its first commercial Pinot noir wine in 1987. The area has rapidly expanded to 120 wineries and has over 1500 planted hectares, with Pinot noir accounting for 80%. This winegrowing region is known for producing particular styles of wines [186], and relies on rationality as its primary marketing plan. Informally, Central Otago Pinot noir wines are noted as having a recognized typical style when compared to other NZ Pinot Noir wines, and more recently these regional stylistic differences have been supported by the study conducted by Tomasino *et al*. [187].

To understand stylistic differences, there have been various studies conducted to characterize wines from different regions with the aim of identifying impact odorants that contribute to the typical aroma of a specific variety, while also attempting to promote its place of origin [41, 149, 153, 188]. While every wine contains hundreds of volatile compounds, most are not relevant as they fall well below their respective sensory perception thresholds. However, when an individual compound is at a concentration high enough to transmit to that wine specific aroma nuances, i.e. the aroma of the compound can be recognized in the wine, the compound is considered to be a genuine impact compound [132]. Impact odorants do not include just varietal or grape derived compounds but can also include fermentative and age-related odorants.

The aim of this research was to characterize and identify the primary odorants that contribute to the typical aromatic profile of Central Otago Pinot noir using chemical and sensory approaches. Gas Chromatography with Olfactometry (GC/O) studies based on aroma extract dilution analysis (AEDA) followed by quantification and determination of OAVs was useful for separating odor-active compounds from the many non-volatiles found in the wine matrices. This provided an effective screening method for determining important compounds that were later included in sensory recombinant studies. Recombinant studies are a more definitive way to establish the importance of an odorant in a complex mixture such as red wine. Previously, reconstitution studies were often conducted using a synthetic model that mimicked the matrix of the wine being tested [47, 189], while very few studies have used the original wine matrix [190]. However, in

this study, aroma models were made by deodorizing the authentic wines, thus keeping their natural non-volatile matrix intact. Additionally, aroma families, as opposed to individual odorants, were considered because of previous demonstrations that in complex matrices, such as red wines, omitting one compound at a time often does not have a relevant sensory consequence [153].

There have been several studies on Pinot noir wines reporting on the chemical composition and sensory profiles under various conditions, including wines from California [7], Oregon [9, 18, 19, 70], British Columbia [14, 58, 109] and Burgundy [8, 61]. However, no studies have been conducted to characterize Pinot noir in a comprehensive manner that includes chemical and aroma recombination studies. This complete strategy utilizing both a chemical and sensorial approach is expected to provide a better understanding of the distinct aromas of Pinot noir wines from the Central Otago region of NZ. This in turn can provide information for viticulturists and winemakers to advance their practices based upon effects and manipulation of key target odorants, as well as supporting marketing of the typicality of the region's winemaking style.

4.2 Materials and methods

4.2.1 Wine samples

Two commercial Central Otago Pinot noir wines from Mt Difficulty Winery were chosen across two consecutive vintages, 2009 and 2010. Mt. Difficulty is an iconic winery and one of the largest producers of Pinot noir wines in the Central Otago region of NZ. Mt. Difficulty "Estate" Pinot noir is a blended wine made from various clones of Pinot noir grapes from different vineyards in the region. The "Premium" Mt. Difficulty Long Gully Single Vineyard Pinot noir wine is made from clone six Pinot noir grapes from a single vineyard. These wines were chosen as the Estate blend represents wine made from grapes that are grown across a large percentage (~40%) of the region with various soil types, microclimates and vine age while the Premium wine represented a more

traditional Bannockburn wine style of which grapes from a mature vineyard with more typical soils for this sub-region were used. Both wines were commercially made from *Vitis vinifera* cv. Pinot noir grapes grown at Bannockburn, Central Otago, NZ. The grapes were harvested at 24-26° Brix before being crushed, destemmed and pumped into a tank for pre-fermentation cold soak for seven days at 8-10 °C. The juice was then warmed to 18-20 °C and allowed to start primary fermentation spontaneously with wild yeasts. The must was left to ferment on skins for 17-20 days before being racked off and pumped into French oak barrels. The wine was then kept at 18-20 °C while allowing malo-lactic fermentation to occur spontaneously. Upon completion of malo-lactic fermentation, both wines were stored in barrels at 10-12 °C. The blended Estate wine was stored for 12 months and the Single Vineyard wine was stored for 16 months before bottling.

4.2.2 Reagents

All the chemicals used were of analytical grade. Sodium hydroxide, sodium acetate, diethyl ether, sodium sulfate, sodium dihydrogen phosphate dihydrate, hexane (96%), methanol and hydrochloric acid were from Scharlau (Sentmenat, Spain). *L*-cystein, ethyl acetate, hydrochloric acid, sodium acetate, *p*-hydroxymercuribenzoate, Dowex Resin 1x2 Cl^--form and *n*-alkane series were from Sigma-Aldrich (St. Louis, MO). Tris was from AppliChem (Darmstadt, Germany), dichloromethane from Merck (Darmstadt, Germany), and absolute ethanol from ECP Ltd. (Auckland, NZ).

Linalool (97%), geraniol (99%), *β*-citronellol (95%), *α*-terpineol (99%), phenylethyl alcohol (99%), hexanoic acid (98%), octanoic acid (99%), decanoic acid (99%), butyric acid (99%), isobutyric acid (99%), isovaleric acid (99%), 3-mercapto-hexanol (98%), ethyl butanoate (99%), ethyl lactate (95%), hexyl acetate (99%), acetovanillone (98%) and phenylethyl acetate (99%) were from Acros Organics. Nerol (97%), *α*-ionone (90%), *β*-ionone (96%), *cis*-3-hexen-1-ol (98%), methionol (99%), ethyl isobutyrate (99%), ethyl decanoate (99%), ethyl octanoate (99%), ethyl cinnamates (99%), ethyl hydrocinnamate (99%), ethyl anthranilate (99%), methyl anthranilate (99%), diethyl malate (97%), 4-ethylphenol (99%), ethyl isovalerate (99%), 1-

hexanol (98%), ethyl hexanoate (98%), diethyl succinate (99%), isobutyl acetate (99%), vanillin (98%), 2-furaldehyde (99%), guaiacol (100%), *γ*-nonalactone (97%), eugenol (99%), furfuryl alcohol (99%), 5-methyl furfural (99%), *trans*/*cis*-whiskey lactone (98%), 4-vinylguaiacol (98%), and benzaldehyde (99%) were purchased from Sigma-Aldrich. *Trans*-3-hexen-1-ol (97%), 4-decanol (98%) and 3-octanol (99%) were obtained from Lancaster (Ward Mill, MA). Isoamyl alcohol (98%) was from Pancreac Quimica (Barcelona, Spain). Benzyl alcohol (99%) was from Riedel-de Haën (Seelze, Germany). Isobutanol (99%) was from Scharlau. Isoamyl acetate (98%) was a product of Univar (Redmond, WA). 1-octen-3-1 (97%) and 4-vinylphenol (10% soln. in propylene glycol) came from Alfa Aesar (Ward Hill, MA). 3-mercapto-hexanol acetate (98%) was from Oxford Chemicals (Hartlepool, UK).

4.2.3 Isolation of volatile compounds for gas chromatography-mass spectroscopy (GC-MS) analyses

The extraction of the varietal thiol 3-mercapto-hexanol was carried out by applying the method described by Benkwitz *et al*. [41] for all wine samples in duplicate using 50 mL samples spiked with 4-methoxy-2-methyl-2-mercaptobutane and deuterated analogues of 3-mercapto-hexanol (3-mercapto(1-$^{2}H_{2}$)hexanol) and 3-mercaptohexyl acetate (3-mercapto(1-$^{2}H_{2}$) hexyl acetate) as internal standards.

A liquid-liquid extraction with ether:hexane (1/1, v/v) was undertaken in triplicate for the quantification of all other volatile compounds including monoterpenes, C_{13}-norisoprenoids, C_{6}-alcohols, higher alcohols, fatty acids, esters, acetate esters, lactones, aldehydes and volatile phenols according to the method developed by Kotseridis *et al.* [167]. This method, originally designed for extracting methoxypyrazines, was modified to accommodate the quantifications of other compounds in this study by extracting 50 mL of wine spiked with 3-octanol (1830 mg/L) and 4-decanol (123.7 mg/L) as internal standards, followed by concentrating the organic phase under a nitrogen flow to 100 µL. Immediate injection was carried out on an Agilent 6890N GC coupled with an Agilent MS 5973 (Santa Clara, CA). A pulsed splitless injection with a pulse and

purge time at 1.00 minute, purge flow of 12.0 mL/min, and total flow of 16.6 mL/min was delivered with helium as gas carrier onto a 19091N-136 HP-INNOWax capillary column (0.252 mm x 60 m x 0.25 μm; J&W Scientific, Folsom, CA). Analysis of C_6-alcohols, higher alcohols, fatty acids, esters (including ethyl and acetate esters), and volatile phenols was carried out at an initial oven temperature of 40°C held for 10 minutes, then raised to 170°C at 3°C /min, followed by an increase to 240°C at 70°C /min and held for 10 minutes before dropping down to 40°C at 60°C /min, resulting in a total run time of 70.7 minutes. Analysis of monoterpenes, C_{13}-norisoprenoids, lactones, aldehydes, and cinnamic esters was conducted at an initial oven temperature of 40°C held for 5 minutes, then raised to 200°C at 2°C /min, followed by an increase to 240°C at 80°C /min held for 5 minutes and finally brought back to 40°C at 80°C /min, resulting in a total run time of 100 minutes. The temperature of the interface line was set to 250°C. The ion source, operating in electron impact mode at 70 eV, was held at 250°C. The quadrupole temperature was set at 150°C. The GC-MS was operated in selective ion monitoring (SIM) mode for both methods (Table 4.1). A calibration curve was created for each volatile compound using the standard addition method. A model wine sample was extracted together with increasing concentrations of the compounds to be quantified using the same protocols and GC-MS parameters.

Linear retention indices were calculated using an *n*-alkane series. Identification of the constituents was obtained by the MS spectra, aroma descriptors, comparing retention indices of a standard or with commercial libraries (NIST 02, Kovats, Flavornet) and/or the literature. To confirm the retention indices, the pure standards were injected onto two capillary columns; a HP-INNOWax capillary column (60 m x 0.250 mm ID, 0.25 μm film thickness) and a DB-5 capillary column (30 m x 0.32 mm ID, 1 μm film thickness) (J&W Scientific).

4.2.4 Isolation of volatile compounds for gas chromatography-olfactometry analysis (GC-O)

The gas chromatographic analysis was carried out using an Agilent 6890N gas chromatograph equipped with a 7683B automatic liquid sampler, a G2613A auto-sampler, and a flame ionization detector (FID) (Santa Clara, CA). The inlet temperature was held at 230°C. 4 μL of the sample was injected in splitless mode, and delivered onto a HP-INNOWax capillary column (60 m x 0.250 mm ID, 0.25 μm film thickness), or a DB-5 capillary column (30 m x 0.32 mm ID, 1 μm film thickness) (J&W Scientific) using helium as carrier gas (133.6 kPa) at a constant flow rate of 1 mL/min. The method and the oven temperatures for the HP-INNOWax and DB-5 capillary columns was the same as given in the previous section. An Agilent GRAPHPACK 3D/2 precision-engineered crosspiece ensured the simultaneous detection of volatiles at the FID and the Gerstel Olfactory Detection Port (ODP 2) (Mülheim an der Ruhr, Germany) via a restriction capillary column (Gerstel, OD 0.36 mm, ID 0.15 mm). The temperature of the ODP transfer line was set to 230°C and the FID was operated at 250°C. A combination of H_2 (40 mL/min) and air (450 mL/min) was used as a flame and N_2 was the makeup gas at 20 mL/ min. GC-Oanalyses were performed by at least three panelists. If an odourwas detected, the panellistsnoted the retention time and an aroma descriptor.

4.2.5 Aroma extract dilution analysis (AEDA)

The wine samples were extracted using LiChrolut EN resin cartridges 500 mg in a Micro Kuderna-Danish concentrator (Sigma-Aldrich) and eluted with 5 mL of dichloromethane, based upon a previously developed method [191]. The extracts were stepwise diluted to obtain dilutions of 1:3, 1:9, 1:81… 1:6561 of the original solutions. The original extract and each dilution were assessed by three panellistsvia GC-Oon the HP-INNOWax capillary column. Each odorant was allotted a flavourdilution factor (FD) which corresponds to the maximum dilution at which at least one of the judges could perceive that odorant. The highest FD factor was used for each odorant amongst the three panellists.

4.2.6 Deodorisation of wines

The wine samples were deodorized adapting an established method [192]. LiChrolut EN resin (1 g) was packed into a cartridge and conditioned with 20 mL of dichloromethane and allowed to dry by letting air pass through under a vacuum. The resin was then washed with 10 mL of methanol followed by 20 mL of a 10% ethanol solution and again allowed to dry under a vacuum. The resin was transferred into a 1 L Schott bottle containing 750 mL of a Pinot noir wine sample. The headspace was purged with argon before sealing. The sample was stirred for 12 hours at 700 rpm. The resin was allowed to settle for one hour before being decanted.

4.2.7 Reconstitution and omission tests

Aroma models for the Pinot noir wines from the 2010 vintage were prepared using the deodorized wine matrix by adding selected compounds from stock solutions (made up in absolute ethanol), back to the original concentrations. The complete model containing all of the selected compounds was considered as the control. Additionally, five other models were prepared in the same manner but with the omission of a major aroma family, namely ethyl esters, C_{13}-norisoprenoids, monoterpenes, acetate esters, and selected oak-derived compounds. Table 4.2 shows the selected compounds for the reconstitution of the complete models and the compounds selected to represent each major aroma family for the omission models.

4.2.8 Sensory analysis

The trained panel comprised of one male and eight females, ranging from 35 to 57 years of age. Two preliminary lists of aroma descriptors were established (Table 4.3); one list comprised of aroma family-related descriptors, while the second list was more exhaustive and included a wide variety of specific aroma nuances. The preliminary lists were refined with the panel during the training phase. On the basis of the main differences perceived between the wines under investigation, the panellistsadapted the lists by removing some descriptors while adding others through group discussion and consensus. Panellistswere trained on an applicability rating

method, using a 10-point category scale [193]. Triplicate evaluations were conducted in sensory booths with red lighting and positive airflow to prevent bias from color or non-product-related odors and the sample presentation was monadic. 20 mL wine samples were served in standard XL5 wine glasses coded with 3-digit numbers and covered with watch glass lids. All wine samples were presented following a randomized balanced block design to minimize order and carry-over effects [121]. Six wines, the control and five omissions, were individually evaluated in triplicate with the panellistsallocated two minutes for each sample, followed by a two minute break for the assessment of the aroma families, while an extended time of three minutes per sample was given for the specific aroma nuances. A 15 minute break was made available between each set of six wines. This assessment model was carried out for both the Estate and Premium wines over a total of nine sessions. On a treatment-by-treatment basis, data for each descriptor and each of the three replicate evaluations by the panel were summarized by calculating a percentage of applicability. This index developed by Dravnieks [193] is based on both the frequency with which the descriptor was used to describe a particular treatment in a particular assessment replicate as well as the total score obtained by that descriptor out of the maximum total score attainable.

4.2.9 Statistical analysis

Microsoft Excel 2010 software was used for basic data analysis. For the sensory evaluations, the percentage of applicability data for the Estate and Premium sets of wines were analysed separately. On a descriptor-by-descriptor basis, percentages of applicability were averaged across the three assessment replicates. Differences between the control and the treatments were performed using Student's t-tests with the *p*-value set at 0.05 for each descriptor. All statistical analyses were carried out using SPSS 19 (IBM, Chicago, IL).

4.3 Results and discussion

4.3.1 GC-O and AEDA

As shown in Table 4.4, the AEDA resulted in the detection of at least 42 aromas, of which 41 were identified by MS spectra, a comparison of retention indices of standards, aroma descriptors and/or retention indices from the literature. Based on the AEDA values, esters were one of the major aroma families found to be potentially important to the Central Otago Pinot noir aroma showing high FD factors (≥81). Considering that the main constituents of wine are alcohols and acids, esters are formed easily and their development and contribution to red wine aroma is well known. The esters of isoacids including ethyl isobutyrate, ethyl butanoate, methyl butanoate, ethyl isovalerate, and ethyl-2-methyl butanoate displayed very high FD values. The fruity esters of fatty acids, ethyl octanoate presented high FD factors across both wines, however, ethyl hexanoate, displayed low FD values. Esters were also found to be of importance for Oregon Pinot noir wines, with ethyl butanoate, ethyl isobutyrate, ethyl hexanoate and ethyl decanoate showing the highest FD values in neutral fractions when AEDA was applied [18]. Despite the suggestion that esters of fatty acids in general are unimportant for distinguishing between wines [194], due to their fruity contribution, it is possible that the ratios of these compounds are distinctive for Central Otago Pinot noir. The cinnamic acid esters, ethyl hydrocinnamate and ethyl cinnamate, which derive from the shikimic acid pathway and contribute to floral, spicy, and honey characters of red wines, displayed high FD factors. The Estate wines exhibited particularly high values with FD factors of 729 to 6561 and Premium wines with a FD factor of 243. These potent esters were identified in Burgundy wines and were reported to be important in contributing fruity and cinnamon aroma nuances, despite their low concentrations [60, 61].

C_{13}-norisoprenoids are products of carotenoid breakdown and the hydrolysis of glycoconjugates (released during crushing). These grape-derived compounds, mainly *β*-ionone and *β*-damascenone, are present in most red wines, contributing distinct aromas of dark berries, violets, tea, and floral, and in this study showed moderate to very high FD scores. *β*-ionone scored

somewhat higher FD values (729, 243) for the Premium wine over the moderate FD factor of 81 for the Estate wine. *β*-damascenone had an extremely high FD factor of 6561 for the Premium and FD value of 2187 for the Estate wine. In the Oregon study, *β*-damascenone received only moderate FD values, while *β*-ionone was not mentioned [18]. It has been shown that concentrations of both *β*-damascenone and *β*-ionone can vary between vintages [27, 195], thus making it difficult to determine their true importance, since both are associated with different odourdescriptors depending on their concentration. Pineau *et al.* [25] suggested that the role of *β*-damascenone is one of an enhancer of red fruit aromas in red wines as opposed to an impact odorant, as has been proposed in previous studies [110, 149]. However, this powerful compound has since been reported as being a possible key odorant in Rosé wines [190] and Dornfelder [189].

Volatile fatty acids (C_6 to C_{10}) possess rancid, cheesy, sweaty odors and occur typically at detectable concentrations in wines spoiled by microbes, although their occurrence at or just below threshold can contribute to overall wine complexity. Isovaleric acid displayed high FD values for both wines. Isobutyric acid reached moderately high values for the Premium wine and butyric reached high values for the Estate wine. These potent fatty acids were regarded as important odorants in Oregon Pinot noir wines [18].

Other groups showing potential importance were higher alcohols and their acetates. Isoamyl alcohol, giving pungent cheese, over-ripened banana odors and isoamyl acetate had moderate to high FD scores of 729 and 81, respectively, for most samples, Phenyl ethyl alcohol, imparting rose odors, had extremely high FD values of 19,683 for the Estate wines and 729 for the Premium wines. Conversely, phenyl ethyl acetate (rose) seemed to be of lesser importance with FD factors of 9 to 27. This same trend was observed for Oregon Pinot noir wines with higher alcohols displaying the highest FD values of all the compounds and low FD factors for the acetate esters [18].

Volatile phenols are formed from phenolic acids and yeast esterase activity. These aromatic compounds can also be further transformed during the storage and aging of wine. The volatile

phenols, guaiacol, eugenol, and ethyl phenol which impart medicinal, clove and spicy aromas to the wines, showed signs of potential importance in Pinot noir aroma with high FD values. Both wines had very high scores for guaiacol (FD≥729) and eugenol (FD≥243) and moderate to high scores for ethyl phenol (FD≥81).

Lactones are another group of aroma compounds that presented high AEDA values. Lactones in wines are typically thought to be derived from oak storage. Both *cis* and *trans* whiskey lactone, imparting vanilla and coconut aromas, had high FD factors of 243 and 729 for the Estate and 729 and 2187 for the Premium wines, respectively. FD values of 81 were given for both wines for *γ*-nonalactone, which lends baked fruit and peach notes to red wine, although the perception threshold was not reached. Sotolon (maple syrup) and wine lactone (spice, baked bread) also showed potential importance with FD values ≥81 for all samples.

Individually, the Estate wines scored highest with ethyl isobutyrate (strawberry) and phenylethyl alcohol (rose) followed by isovaleric acid (cheese), ethyl cinnamate (honey, cinnamon), ethyl isovalerate (fruit, cherry) and *β*-damascenone (tea, floral, honey). The Premium wines scored highest with *β*-damascenone and guaiacol (medicinal, smoky) followed by *trans*-whiskey lactone (lactone, vanilla), 1-octen-3-o1 (mould, earthy), ethyl phenol (spice, cinnamon) and eugenol (spicy, clove). Because nearly all of the identified aroma compounds were found in both wines, the different aroma profiles are most likely caused by quantitative differences in particular odorants due to various reasons including ripeness and time in barrel. The Estate wines were blends consisting of several wine batches made from juices that ranged in ripeness from 24 to 26° Brix and harvested from vines that differed in clone and age from several vineyards having different soil types and aspects. The Premium wines, on the other hand, were from a single vineyard with one clone which was harvested at 26° Brix in a single event. These two distinct wine styles are common for the region and were chosen with the aim that collectively the results were more representative for the characterisationof Central Otago Pinot noir.

4.3.2 Chemical composition and OAVs

Approximately 51 volatile compounds commonly found in most red oaked wines were quantified via GC-MS for both Pinot noir wines from the 2009 and 2010 vintages. Odouractivity values (OAV) were obtained by dividing the quantitative data by the respective perception thresholds. As shown in Table 4.5, at least 22 of the odorants were present at concentrations above their corresponding perception thresholds in both Estate and Premium wines. According to the OAVs, *β*-damascenone, presented with the highest value, however, this value is most likely overstated as this figure is only reached when considering the perception threshold in the 10% w/w aqueous ethanol solution [10] as opposed to the red wine medium [25]. Ethyl octanoate, ethyl hexanoate, isovaleric acid and 3-mercapto-hexanol revealed high OAVs in both wines styles. Ethyl isovalerate and butyric acid had slightly higher OAVs in the Estate wines while whiskey lactone presented higher in the Premium wines which corresponded with the AEDA results. The cinnamic esters, volatile phenols, methionol, and hexanoic acid displayed lower OAVs although noteworthy when considering their potential contribution to the matrix and hence to the overall aroma.

4.3.3 Aroma reconstitution experiments

Aroma reconstitution experiments were conducted as a way to validate the quantitative data. The complete model was confirmed through a degree of difference test [196] using a 10-point rating scale. The scale was anchored with numbers (0 to 9) and words (match, somewhat different, different, very different and completely different). “Match”, reflecting the absence of difference between the overall aroma of a given sample and that of the authentic wine, corresponded to 0 on the scale, while “completely different” corresponded to 9. Scores between these two extremes reflected various degrees of differences. Ratings from 1 to 3, 4 to 5, and 6 to 8 corresponded to a wine perceived as being “somewhat different”, “different”, and “very different”, respectively, from the target in overall aroma. The sensory panel found an insignificant difference ($p > 0.05$) between the complete models’ aroma and the authentic wines’ aroma, and as a result both models were considered to be a suitable control. Using the data obtained from

the AEDA and OAVs experiments, different aroma models were prepared using the complete model matrix except with the absence of a major aroma family. Bench tests were performed by the laboratory staff and sensory team and the aroma models with the greatest differences were selected for the omission tests to be evaluated by the trained panel. The aroma families with their representative compounds can be found in Table 4.2.

When the complete models were compared to the wines with an omitted compound family, in both the Estate and Premium wines, some trends could be observed. Red fruit, black fruit and dried fruit, fruit jam, spice (vanilla, black pepper, black tea), woody (toast, smoky, leather) and mineral (flinty) received the highest applicability scores amongst the aroma family and specific aroma nuances for the complete models of both Estate and Premium wines. However, the omissions of one of the aroma families at a time had different effects when compared to the respective complete model.

Significance amongst both aroma family and specific descriptors was observed when comparing the Estate complete model to the omission models (Table 4.6). The omission of the monoterpenes did not show any significant differences in the aroma profile. The exclusion of ethyl esters revealed a significant increase in the mineral (flinty, chalky) descriptor and a slight increase in the woody (leather, toast) aroma notes. Surprisingly, there was only a small decrease in red fruit and black fruit characters. A decrease in red fruit and black fruit, significant for the black currant nuance, was observed when acetate esters were omitted. Nonetheless, a considerable increase in jammy notes was seen. This treatment also had a marked effect on the spice notes with a significant increase in aniseed and a slight increase in chocolate and cinnamon. Decreases in spice (black pepper, liquorice, vanilla), mineral (chalky), wood (caramel), floral (geranium) and fruit notes (dark berry, peach, raspberry) were seen with the exclusion of the C_{13}-norisoprenoids. Unexpectedly, the elimination of both acetate esters and C_{13}-norisoprenoids increased the fruit jam characters. The omission of oak-derived compounds had the expected effect of decreases in spice and woody notes (leather, toast) and increases in some floral and fruit notes.

The Premium omission models in general revealed a decrease in tannic and mineral notes. Table 6 shows the significant differences observed across the tested models. Unlike the Estate, the monoterpenes exclusion had significant effects on spice, floral, wood, and fruit jam notes. The exclusion of ethyl esters had little consequence with only an increase in wood (toast, caramel) and spice (clove, tobacco) notes. Similar to the Estate model, jammy notes were increased with the removal of acetate esters, although no changes were observed with red fruit and black fruit characters in this model. A decrease in floral, ash and leather was also determined. The omission model having the greatest impact on the aroma profile for the Premium wine was that of the C_{13}-norisoprenoids, which was congruent with the AEDA and OAV findings. A significant increase in animal notes was observed and there were considerable decreases in black fruit, spice (chocolate, tobacco), dried fruit, and fruit jam nuances. This result was expected as typically C_{13}-norisoprenoids are thought to enhance the fruity notes of ethyl cinnamate and ethyl octanoate in red wines indirectly [25]. The removal of oak derivatives did not have a profound effect on the woody or spicy notes in this model, with the exception of a decrease in leather. Given that the authentic wine matured eight months longer in oak barrels, a possible explanation is most likely due to the failure to remove an oak-derived compound through the deodorization process. Post-deodorization GC-MS analysis revealed that traces of some oak compounds remained. An increase in fruity and floral notes, such as plum, red berry, jam fruit, and rose, was observed as expected.

Overall, there were no profound changes and few consistent differences in the aroma profiles across the omission models when compared to the complete models for both the Estate and Premium wines. While there were clear differences in the profiles of each treatment, all the models, nonetheless, were perceived as typical of Pinot noir wines by the panelists. The interactions between volatile compounds and their effects on aromas are very complex and are not fully understood. The present results support the hypothesis that slight differences in the ratios of volatile compounds in a particular variety of wine can greatly change the perceived aroma. Other studies have shown the importance of binding interactions between the volatile

and non-volatile compounds [197-200], and how this depends on the composition of the non-volatile matrix [201]. Because the omission of compound families did not bring about large changes, despite some of them having high FD factors and OAVs, the suggestion can be made that Central Otago Pinot noir wines have an aromatic complexity that depends on many volatile compounds instead of a few key odorants. It has been suggested that wines with such complexity, having high concentrations of ethanol, ethyl esters, higher alcohols, volatile phenols, fatty acids and C_{13}-norisoprenoids, can form an aromatic buffer that can only be overpowered by the presence of an aroma with exceedingly different properties [153]. More detailed studies should be carried out to examine Pinot noir aromas further.

4.4 Conclusions

AEDA was applied to two distinct Central Otago Pinot noir wine styles over two consecutive vintages. The aroma compounds and/or compound families of importance varied across the wines, while a number of compounds were in common. The compounds with the highest FDs for the Estate wines were fruity esters and phenylethyl alcohol, while for the Premium wines the norisoprenoids and volatile phenols originating from oak had the highest FDs. Some of these differences may be explained by the Estate's blended composition comprised from various wine batches originating from a range of ripeness levels and diverse vineyards, and the Premium wine's extended time in oak barrels. As most compounds identified were found in both wines, it is possible that the major aromatic differences are related to variances in compound ratios. The elucidation of 51 aromatic compounds from two Central Otago Pinot noir wines over two consecutive vintages revealed *β*-damascenone, ethyl octanoate, ethyl hexanoate, ethyl isovalerate, isovaleric acid, and 3-mercapto-hexanol to be the most important odorants when considering odouractivity values. The aroma reconstitution experiments, which considered both chemical and sensorial properties, revealed no overwhelming differences when compound families were omitted. Overall, these results suggest that Central Otago Pinot noir wines do not

depend on a few key odorants for their aromatic complexity, but instead on the interactions of many aromatic compounds.

Abbreviations used

GC-O, Gas Chromatography-Olfactometry; GC-MS, Gas Chromatography-Mass Spectroscopy; OAV, odouractivity values; AEDA, aroma extract dilution analysis; FD, flavourdilution; FID, flame ionization detector; ODP, olfactory detection port; SIM, selective ion monitoring

Table 4.1 Linearity, quantification data and ions chosen for SIM mode for the compounds analysed in this study

Compound	Correlation coefficient (r^2)	Calibration range (µg/L)	Ions	Recovery in calibration range (%)	Average relative standard deviation (%)
C_{13}-norisoprenoids					
α-ionone	0.996	0.08 – 15.5	121,136,192	91 – 109	3.1
β-ionone	0.998	0.09 – 17.6	69,121,190	92 – 106	3.4
β-damascenone	0.998	0.08 – 15.5	171,178,192	98 – 107	5.7
monoterpenes					
linalool	0.998	0.15 – 29.7	71,93,121	93 – 127	7.8
nerol	0.996	0.29 – 57.3	69,41,93,121	94 – 108	5.6
geraniol	0.997	0.32 – 64.2	69,41,93,123	93 – 108	2.2
β-citronellol	0.997	0.20 – 40.3	41,69,82,123	97 – 110	3.4
α-terpineol	0.996	0.29 – 58.7	71,93,111	92 – 106	7.4
C_6-alcohols					
1-hexanol	0.997	124 – 24884	56,43,69	93 – 104	9.4
cis-3-hexen-1-ol	0.992	8.7 – 1747	41,67,82	91 – 102	3.8
trans-3-hexen-1-ol	0.996	4.18 – 835	67,41,82	90 – 106	
alcohols					
isoamyl alcohol	0.992	3338 – 200275	55,42,70	90 – 112	7.2
isobutanol	0.998	1888 – 113288	43,41,74	92 – 116	8.4
benzyl alcohol	0.999	33 – 2002	79,108,107	96 – 108	8.9
methionol	0.991	77 – 4596	106,61,58	91 – 111	6.9
phenylethyl alcohol	0.998	1920 – 115217	91,92,122	98 – 106	3.9
furfuryl alcohol		23 – 1364	98,81,97	98 – 114	8.6
fatty acids and isoacids					
hexanoic acid	0.997	560 - 11200	60,73,87	96 – 104	6.8
octanoic acid	0.999	460 – 36630	60,73,101	94 – 111	3.8
decanoic acid	0.998	50 - 1114	60,73,129	91 – 101	8.6
butyric acid	0.994	434 - 47190	60,73	95 – 105	7.7
isobutyric acid	0.999	148 – 11813	43,73,88	98 – 114	8.3

isovaleric acid	0.998	233 - 18666	60,87	97 – 110	6.7
varietal thiols					
3-mercapto-hexan-1-ol	0.998	0.49 – 11.2	134,100	92 – 106	5.4
3-mercaptohexyl acetate	0.999	0.061 – 1.75	116,101	96 – 104	–
ethyl esters of isoacids					
ethyl isobutyrate	0.991	5.5 – 401	43,71,116,88	95 – 104	9.2
ethyl isovalerate	0.991	5.2 – 99.1	88,85,115	97 – 105	7.8
ethyl esters of fatty acids					
ethyl butyrate	0.993	9.2 – 735	74,88,101	95 – 110	7.7
ethyl hexanoate	0.992	21 – 1708	88,99,101	96 – 105	5.4
ethyl decanoate	0.991	7.6 – 608	88,101,155	96 – 108	6.7
ethyl octanoate	0.993	32 – 2572	88,101,127	94 – 105	7.6
cinnamic esters					
ethyl cinnamate	0.990	0.14 – 27.9	131,103,176	92 – 107	7.1
ethyl hydrocinnamate	0.992	0.12 – 24.9	104,91,107	95 – 104	3.5
miscellaneous esters					
ethyl lactate	0.999	814 – 65132	45,43,75	98 – 112	2.6
ethyl anthranilate	0.992	0.14 – 28.9	119,165,92	96 – 109	6.7
methyl anthranilate	0.998	0.15 – 29.1	119,151,92	96 – 119	7.8
diethyl malate	0.999	9.4 – 1856	71,117,89	97 – 102	7.9
diethyl succinate	0.999	212 – 16979	101,129,73	97 – 114	8.6
acetate esters					
isobutyl acetate	0.991	7.8 – 627.1	43,56,73	93 – 101	8.2
isoamyl acetate	0.993	80 – 6434	70,55,87	92 – 100	5.5
hexyl acetate	0.995	6.8 – 553	56,61,84	94 – 101	4.7
phenylethyl acetate	0.992	6.2 – 499	104,43,91	94 – 103	6.2
volatile phenols					
4-ethylphenol	0.999	105 – 1055	107,122,77	99 – 108	6.9
4-vinylguaiacol	0.999	5.7 – 345	135,150,107	93 – 106	6.5
guaiacol	0.991	0.34 – 20.9	109,124,81	97 – 113	7.4
eugenol	0.998	2.04 – 123	164,149	96 – 106	4.1
lactones					

cis/trans whiskey lactone	0.995	9.2 – 536	99,71,87	97 – 110	8.3
γ-nonalactone	0.995	4.6 – 276	85,99	97 – 114	8.6
acetovanillone	0.995	96 – 5740	151,166,123	96 – 107	6.6
aldehydes					
2-furaldehyde	0.995	18 – 1086	96,95	98 – 115	7.9
5-methyl furfural	0.991	4.2 – 249	110,109	95 – 110	6.4
vanillin	0.998	13 – 701	152,151	95 – 119	9.0
benzaldehyde	0.999	1.04 – 62.4	77,106,105	99 – 114	7.9

Table 4.2 Compounds selected for the composition of the recombinant models

Compounds selected for the complete and omission models		
monoterpenes	**alcohols and isoacids**	**acetate esters**
linalool	isoamyl alcohol	isoamyl acetate
nerol	isobutanol	phenylethyl acetate
geraniol	benzyl alcohol	
citronellol	phenylethyl alcohol	**varietal volatile thiols**[a]
α-terpineol		3-mercapto-hexan-1-ol
C_{13}-norisoprenoids	**oak derivatives**	**ethyl esters**
β-ionone	furfuryl alcohol	ethyl isobutyrate
β-damascenone	4-ethylphenol	ethyl isovalerate
	guaiacol	ethyl butyrate
fatty acids and isoacids[a]	eugenol	ethyl hexanoate
hexanoic acid	(E/Z) whiskey lactone	ethyl decanoate
octanoic acid	γ-nonalactone	ethyl octanoate
decanoic acid	acetovanillone	ethyl cinnamate
butyric acid	2-furaldehyde	ethyl hydrocinnamate
isobutyric acid	5-methyl furfural	
isovaleric acid	vanillin	

[a] included in the complete and omission mode

Table 4.3 Sensory descriptors used in trained panel evaluations

Overall perception	**Specific definition**
Soft	Mellow, gentle, no burn
Sharp	Tingle in the nose, acidity, crisp
Weak/Bland	Watery, flat
Powerful	Intense, concentrated, strong
Light/Delicate	Light, fresh aromas
Heavy/Rich	Full, heavy aromas, opulent
Discordant	Unbalanced, disassociated
Balanced	No overpowering aroma families
Complex	Multi-layered wine (many different aroma families)
Hot	Alcohol burn
Pungent	Acrid, sulfuric
Aroma family	**Specific aroma nuances**
Orchard fruit	Pear, apple, quince, apricot, peach, nectarine, rock melon
Citrus fruit	Lemon, orange, grapefruit
Red fruit	Strawberry, raspberry, redcurrant, red cherry
Black fruit	Black cherry, blackcurrant, blackberry, blueberry, Plum, boysenberry
Jammy/lolly	Fruit jam, cooked fruit, fruity lolly
Dried fruit	Date, fig, prune, raisins, Muscat, fermented fruit
Floral	Jasmine, lilac, violet, chamomile, rose, honeysuckle, geranium, bergamot
Spice	Vanilla, clove, nutmeg, cinnamon, ginger, chocolate, aniseed/fennel, liquorice, black pepper, curry, chili
Nuts	Almond, walnut, hazelnut
Woody	Caramel, toasted bread, coffee, fresh wood, burnt wood, smoky, tobacco
Green/vegetal	Artichoke, asparagus, cauliflower, green beans, olives, bay leaf, thyme, hay, herbaceous/cut grass
Animal	Musk, cat urine, wet wool/wet dog
Forest floor	Mushroom, humus, earthy
Lactic	Sour milk, cream, butter
Mineral	Flinty, ash, chalky
Tannic	Leather, black tea

Table 4.4 Compounds detected by aroma extract dilution analysis in conjunction with their corresponding retention indices (RI) on two columns, and flavourdilution (FD) factors for the 2009 and 2010 Estate and Premium wines obtained on the INNOWax column

Compound	RI-INNOWax	RI-DB-5	Identity	Descriptor	FD factors			
					2009 Estate	2010 Estate	2009 Premium	2010 Premium
Ethyl isobutyrate	980	756	MS,ST,A	Strawberry, fruit	19,683	6561	81	81
2,3-butanedione	989	584	MS,AR,L	Caramel, toffee	3	81	27	27
Methyl butanoate	1011	733	MS,AR	Ether, solvent, sweet	81	81	729	243
Ethyl butanoate	1034	800	MS,ST,A	Fruit	243	243	81	81
Ethyl isovalerate	1049	826	MS,ST,A	Fruit, cherry	2187	2187	249	729
Ethyl 2-methyl butanoate	1068	862	MS,ST,A	Fruit, resin	243	243	81	81
Isoamyl acetate	1088	833	MS,ST,A	Fruit	27	81	81	81
Isoamyl alcohol	1208	802	MS,ST,A	Cheese, over ripe banana	243	729	729	729
Isoamyl isobutyrate	1223	822	A,L	Baked fruit	3	3	9	9
Ethyl hexanoate	1230	1021	MS,ST,A	Fruit	9	27	9	9
Hexyl acetate	1269	1048	MS,ST,A	Lolly, fruit	3	3	3	3
1-octen-3-ol	1302	904	MS,ST,A	Mould, earthy	27	27	2187	2187
2-heptanol	1334	854	MS,ST,A	Coconut milk, sweet	27	9	9	9
1-hexanol	1370	861	MS,ST,A	Cut grass	3	0	3	3
Trans-3-hexen-1-ol	1380	883	MS,ST,A	Green grass	27	0	81	3
Cis-3-hexanol	1390	866	MS,ST,A	Green	27	27	27	27
Unknown	1428	1330		Solvent, fuel	9	9	9	9
Ethyl octanoate	1437	1214	MS,ST,A	Baked fruit	81	9	81	81
Methional	1457	876	MS,ST,A	Vegetable, potato	3	3	9	9
Isobutyric acid	1589	782	MS,ST,A	Cheese	3	3	81	81
Ethyl decanoate	1626	1402	MS,ST,A	Fruit	3	9	3	3
Butyric acid	1646	813	MS,ST,A	Cheese	81	81	3	27
Isovaleric acid	1687	868	MS,ST,A	Cheese	6561	2187	243	81
A-terpineol	1698	1204	MS,ST,A	Sweet, floral	9	9	3	3
Methionol	1711	1070	MS,ST,A	Vegetables, potato	9	9	9	9
β-citronellol	1751	1233	MS,ST,A	Dusty, floral	9	9	9	9
β-phenylethyl acetate	1785	1271	MS,ST,A	Rose	9	27	9	9
β-damascenone	1815	1395	MS,ST,A	Tea, floral, honey	2187	2187	6561	6561
Geraniol	1844	1276	MS,ST,A	Floral	27	27	27	27
Guaiacol	1856	1080	MS,ST,A	Medicinal, smoky	729	729	6561	6561
Ethyl hydrocinnamate	1883	1361	MS,ST,A	Honey, floral	729	729	243	243
Phenylethyl alcohol	1918	1109	MS,ST,A	Roses	19,683	19,683	729	729

β-ionone	1920	1493	MS,ST,A	Dark berries, violet	81	81	729	729
(*E*)-whiskey lactone	1956	1603	MS,ST,A	Lactone, vanilla	243	243	729	729
(*Z*)-whiskey lactone	1963	1612	MS,ST,A	Coconut	729	729	2187	2187
γ-nonalactone	2027	1343	MS,ST,A	Baked fruit, peach	81	81	81	81
Ethyl cinnamate	2096	1472	MS,ST,A	Honey, cinnamon, vanilla	2187	6561	243	243
Eugenol	2152	1344	MS,ST,A	Spicy, clove ,wood	243	729	729	2187
Ethyl phenol	2171	1172	MS,ST,A	Spice ,cinnamon ,floral	81	81	729	2187
Sotolon	2192	1111	MS,ST,A	Toast, maple syrup	81	243	81	243
Wine lactone	2198	1444	A,L	Spice, baked bread	81	81	81	81
Isoeugenol	2239	1891	MS,ST,A	Aniseed ,spice	27	27	27	27

* MS = mass spectra, ST = standard compound, A = aroma descriptor, L = retention indices from the literature

Table 4.5 Concentration (µg/L) and odouractivity values (OAV) of aroma compounds in the Estate and Premium wines for 2009 and 2010 vintages. OAVs are reported as the mean of the 2009 and 2010 concentrations

Compound	Estate 2009	Estate 2010	Premium 2009	Premium 2010	Perception Threshold (µg/L)	OAV Estate	OAV Premium
C_{13}-norisoprenoids							
α-ionone	0.34 ± 0.01	0.28 ± 0.01	0.61 ± 0.03	0.55 ± 0.02	2.6[10]	0.1	0.2
β-ionone	0.29 ± 0.01	0.31 ± 0.02	0.41 ± 0.01	0.42 ± 0.01	0.09[10]	3.3	4.6
β-damascenone	4.13 ± 0.33	4.02 ± 0.21	5.25 ± 0.42	5.44 ± 0.11	0.05 [10], 7[25]	94, 0.6	95, 0.8
Monoterpenes							
Linalool	2.25 ± 0.25	5.37 ± 0.41	3.84 ± 0.32	4.6 ± 0.28	25[10]	0.2	0.2
Nerol	5.24 ± 0.34	5.98 ± 0.44	4.84 ± 0.12	3.79 ± 0.21	400[41]	<0.1	<0.1
Geraniol	14.7 ± 0.3	16.2 ± 0.2	12.4 ± 0.4	13.6 ± 0.4	20[10]	0.8	0.7
β-citronellol	9.9 ± 0.6	11.1 ± 0.4	6.9 ± 0.1	9.3 ± 0.2	100[10]	0.1	0.1
α-terpineol	2.52 ± 0.12	3.28 ± 0.27	6.55 ± 0.53	6.68 ± 0.49	250[10]	<0.1	<0.1
C_6-alcohols							
1-hexanol	1272 ± 112	904 ± 89	809 ± 68	950 ± 102	8000[10]	0.1	0.1
Cis-3-hexen-1-ol	43 ± 3	34 ± 4	22 ± 1	25 ± 3	400[10]	0.1	<0.1
Trans-3-hexen-1-ol	35 ± 6	27 ± 4	20 ± 3	18 ± 2	1000[41]	<0.1	<0.1
Alcohols							
Isoamyl alcohol	104295± 8211	150538 ± 10421	125485 ± 9102	144426 ± 9921	30000[66]	4.2	4.5
Isobutanol	12277 ± 1008	14655 ± 1256	11072 ± 802	10436 ± 979	40000[66]	0.3	0.3
Benzyl alcohol	1100 ± 89	1374 ± 75	1589 ± 157	1799 ± 203			
Methionol	901 ± 83	1626 ± 110	498 ± 31	1970 ± 123	1000[66]	1.3	1.2
Phenylethyl alcohol	89212 ± 3256	134980 ± 4589	68719 ± 2227	77774 ± 4522	14000[28]	8.0	5.2
Furfuryl alcohol	1523 ± 81	2006 ± 89	4128 ± 411	4084 ± 426	15000[85]	0.1	0.3
Fatty acids and isoacids							
Hexanoic acid	1217 ± 65	854 ± 55	1142 ± 79	712 ± 66	420[28]	2.5	2.2
Octanoic acid	936 ± 23	911 ± 42	1038 ± 40	1302 ± 53	500[28]	1.8	2.3
Decanoic acid	71 ± 6	135 ± 10	66 ± 8	87 ± 7	1000[28]	0.1	0.1
Butyric acid	1756 ± 105	1845 ± 127	1026 ± 112	1142 ± 101	173[66]	10.4	6.3
Isobutyric acid	389 ± 28	421 ± 41	895 ± 72	758 ± 64	2300[66]	0.2	0.4
Isovaleric acid	665 ± 56	563 ± 37	489 ± 15	275 ± 25	33.4[66]	18	11
Varietal thiols							
3-mercapto-hexan-1-ol	1.31 ± 0.10	0.917 ± 0.04	1.53 ± 0.06	1.41 ± 0.08	0.06[41]	19	25

3-mercaptohexyl acetate	nd	nd	nd	nd	0.004[41]	<0.0	<0.0
Ethyl esters of isoacids							
Ethyl isobutyrate	54 ± 6	45 ± 2	29 ± 3	25 ± 3	15[10]	3.3	1.8
Ethyl isovalerate	49 ± 3	54 ± 4	23 ± 2	27 ± 3	3[10]	17	8.3
Ethyl esters of fatty acids							
Ethyl butyrate	153 ± 10	125 ± 11	89 ± 9	75 ± 4	20[10]	7.0	4.1
Ethyl hexanoate	338 ± 24	372 ± 21	312 ± 17	342 ± 12	14[10]	25	23
Ethyl decanoate	194 ± 12	207 ± 10	164 ± 15	174 ± 13	200[10]	1.0	0.8
Ethyl octanoate	384 ± 21	374 ± 33	329 ± 26	318 ± 28	5[10]	76	65
Cinnamic esters							
Ethyl cinnamate	4.1 ± 0.2	2.3 ± 0.2	3.2 ± 0.1	1.6 ± 0.3	1.1[10]	2.9	2.2
Ethyl hydrocinnamate	2.31 ± 0.08	1.54 ± 0.04	1.22 ± 0.03	1.11 ± 0.07	1.6[10]	1.2	0.7
Miscellaneous esters							
Ethyl lactate	141206 ± 3645	134921 ± 3339	169549 ± 4223	191724 ± 5223	154000[10]	0.9	1.2
Ethyl anthranilate	1.52 ± 0.12	1.3 ± 0.09	0.74 ± 0.04	0.89 ± 0.05			
Methyl anthranilate	0.29 ± 0.01	0.22 ± 0.03	0.20 ± 0.02	0.18 ± 0.01	3.0[60]	0.1	0.1
Diethyl malate	109 ± 9	148 ± 12	69 ± 5	79 ± 6	10000[41]	<0.1	<0.1
Diethyl succinate	11279 ± 1002	17112 ± 1279	12493 ± 1123	10869 ± 1056	200000[10]	0.1	0.1
Acetate esters							
Isobutyl acetate	27 ± 2	32 ± 3	42 ± 4	58 ± 4	1600[10]	<0.1	<0.1
Isoamyl acetate	249 ± 11	233 ± 10	254 ± 16	189 ± 14	30[10]	8.0	7.4
Hexyl acetate	14.2 ± 0.7	10.6 ± 0.8	12.5 ± 0.4	18.6 ± 0.7	700[41]	<0.1	<0.1
Phenylethyl acetate	13.2 ± 0.6	18.1 ± 1.2	11.6 ± 1.2	13.3 ± 0.9	250[10]	0.1	<0.1
Volatile phenols							
4-ethylphenol	454 ± 33	421 ± 28	727 ± 41	685 ± 57	600[85]	0.7	1.2
4-vinylguaiacol	6.3 ± 0.3	7.6 ± 0.7	13.2 ± 1.1	11.3 ± 0.4	1100[85]	<0.1	<0.1
Guaiacol	3.1 ± 0.4	4.4 ± 0.7	10.3 ± 0.6	9.2 ± 0.3	9.5[85]	0.4	1.0
Eugenol	18.2 ± 0.8	16.9 ± 0.2	25.3 ± 0.6	21.8 ± 0.8	6[85]	2.8	3.8
Lactones							
(*E/Z*)-whiskey lactone	351 ± 35	418 ± 36	752 ± 53	604 ± 54	67[85]	5.7	10
γ-nonalactone	18 ± 2	16 ± 1	29 ± 2	30 ± 3	30[85]	0.6	1.0
Acetovanillone	2089 ± 135	2658 ± 104	2999 ± 233	2815 ± 227			
Aldehydes							
2-furaldehyde	265 ± 18	200 ± 16	32 ± 3	20 ± 4	14100[85]	<0.1	<0.1
5-methyl furfural	12.3 ± 0.8	10.5 ± 0.2	18.1 ± 1.4	15.6 ± 1.2	16000[85]	<0.1	<0.1

Vanillin	21 ± 4	31 ± 2	44 ± 5	59 ± 3	65[85]	0.4	0.8
Benzaldehyde	15.4 ± 1.2	14.6 ± 1.4	10.2 ± 0.8	18.6 ± 1.3	2000[10]	<0.1	<0.1

Table 4.6 Omission tests for Estate and Premium complete models

	Effect on aroma	
complete models versus complete models without:	**Estate**	**Premium**
Monoterpenes	ns	Increase in lactic***, chocolate*, fruit jam***, rose* Decrease in ash*
Ethyl esters	Increase in mineral*	Increase in toast/wood*
Acetate esters	Increase in aniseed* Decrease in black currant**	Increase in flinty* Decrease in ash**
C_{13}-norisoprenoids	Increase in caramel* Decrease in geranium*, liquorice*, peach***, raspberry*	Increase in animal*, chocolate* Decrease in mineral*
Oak derivatives	Increase in geranium**, red cherry** Decrease in leather*, toast/wood*	Increase in plum*

ns = not significant, *significant at $p < 0.05$, **significant at $p < 0.01$, ***significant at $p < 0.001$

Chapter 5

Effect of Cluster Thinning *Vitis vinifera* cv Pinot noir on Wine Volatile and Phenolic Composition and Quality

Tanya E. Rutan, Mandy Herbst-Johnstone, Paul A. Kilmartin*

[1]Wine Science Program, School of Chemical Sciences, University of Auckland, Private Bag 92019, Auckland, New Zealand

*Corresponding author (email: p.kilmartin@auckland.ac.nz)

For submission to Journal of Agricultural and Food Chemistry

Abstract

The effects on wine quality and volatile composition of two cluster thinning regimes on *Vitis vinifera* cv. Pinot noir in vineyards located in Central Otago, NZ were studied across three consecutive seasons. There were strong negative correlations observed with pH and bunch weights and strong positive associations with titratable acidity (TA) across all seasons. A strong relationship was observed across all three seasons between cluster thinning and the concentrations of the C_{13} norisoprenoids, monoterpenes, fatty acids, cinnamic esters, ethyl isobutyrate, ethyl hexanoate, β-phenylethyl alcohol and all polyphenols. Triangular sensory studies revealed that cluster thinning led to wines that were significantly different from the control wines. In paired comparison tests the sensory terms herbaceous and acidic consistently received the highest ranking in the controls. The thinning treatments generally received higher marks in the descriptors fruity, spice, sweet and body, but there were no consistent differences in the remaining attributes of floral, bitter and astringent. Cluster thinning had a measurable effect on timing of harvest, chemical composition, perceived aromatics, mouth-feel and overall quality, but there were no clear differences with regards to the intensities of thinning treatments.

Key words: cluster thinning, wine quality, Pinot noir, wine aroma, crop load, polyphenols, sensory

5.1 Introduction

Pinot noir is becoming an increasingly important red grape for NZ and is increasing in demand. According to the latest statistics from NZ Wine Growers, Pinot noir plantings, harvested tons, and exported litres have risen over the last few years [202]. Central Otago, located on the South Island of NZ, is one of the country's prominent regions renowned for its ability to grow high quality Pinot noir grapes. This inland mountainous region has a semi-continental climate with very low rainfall and its winegrowing sub-regions are comprised of mostly glacially derived soils with rich deposits of mica and schist. This unique climate provides ideal growing conditions for Pinot noir grapes where the region has over 1500 ha of plantings responsible for 80% of the area's production.

There are many grape growing factors that enter into the production of quality wines. There is a common belief that Pinot noir vines are sensitive to crop yield, therefore, high quality is often associated with low yielding vines. Cluster thinning is one way to control the crop load of a vine by simply removing whole clusters after the berries have set to achieve the desired yield. This method is widely used in the Central Otago region and is thought to provide more favourable conditions for the retained clusters by changing the ratio between leaf area and total fruit weight per plant. This increase in leaf area promotes berry development and maturation by providing a more beneficial situation for photosynthesis to occur [161].

Wine quality is determined by many factors including colour, aroma, mouth-feel and complexity of flavours. All of these components depend greatly on the maturity and ripeness of the grapes and a range of studies have investigated these relationships. Cluster thinning was shown to have positive effects on the timing of fruit maturity or accumulation of soluble solids in Riesling [203], Pinot noir [204], Trebbiano [205], Nebbiolo [206], Cabernet Sauvignon [207, 208], Syrah [162, 209], Tempranillo [162] and Merlot [210, 211]. Many of these studies [126, 162, 206, 208, 209, 211] along with an investigation of Shiraz [212], and of red wines in general [213], have also established a close

relationship between crop thinning and an increase in phenolic composition, which is responsible for colour and mouth-feel. Research has also revealed positive sensory differences in Riesling [214] and Syrah [209], while wines made from lower yielding vines displayed increased currant aromas in Pinot noir [126] and increased vegetative and herbaceous characters in Cabernet Sauvignon [215] and Chardonnay Musqué [168].

On the other hand, cluster thinning had little to no effect on fruit composition with Cabernet Sauvignon [207, 216] and Riesling [214] grapes and no differences in overall wine quality in Cabernet Sauvignon [171], Gewürztraminer [172] and Sangiovese [173]. Additionally, a decline in quality attributes was observed in Sauvignon blanc [163, 170] wines when crops were thinned.

The aromatic and phenolic makeup of a wine play a major role in the desirability and the perception of quality and is a primary factor when taking into account the typicality of a region's wine style. There have been few reports on the relationship between the aromatic and phenolic chemical composition of Pinot noir wines and cluster thinning. The objective of this study was to investigate the effects of three crop thinning regimes over three consecutive seasons on the chemical composition and quality of wines made from Pinot noir grapes. This research was performed in a vineyard in Central Otago, NZ where crop thinning is a common practice used to lower yields with the aim of increasing the quality and value of the wines produced.

5.2 Materials and methods

5.2.1 Vineyard site and experimental design

Seventeen year old Pinot noir vines, located in Central Otago, NZ, were subjected to crop load management treatments in 2009, 2010 and 2011. Vines were spaced at 2 x 3 meters (vine x row) in north-south oriented rows and trained to a three wire vertical shoot position system with five vines per panel.

The experimental design was created after careful assessment of the vineyard aspects of which there were various clones planted in north-south oriented rows (with multiple clones often on the same row) and bands of different soil textures with an east-west orientation. Each clone was planted in what was thought to be the most favourable soil type for growth, productivity and management making clones follow an east-west arrangement. This configuration was optimal for the grower; however, caused some difficulty with trial design. In an effort to capture each soil type and clone and enable ease of management the vineyard was divided into three north-south oriented segments where one section remained as a control with no thinning, which yielded a heavy crop. The other two sections were bunch thinned to two different yields, namely, moderate and low. All thinning was performed during the same week and prior to veraison.

Panels across each section were randomly selected at the beginning of the study for data collection including canopy assessment, bunch weights and pruning weights. The bunch thinning regime used to achieve a moderate yield included leaving two bunches on all strong, tall shoots and one on shorter shoots. Low yields were achieved with more intense thinning by leaving only one bunch on strong shoots, both tall and shorter shoots. All bunches in both treatments were removed if the shoot was weak and/or stunted.

5.2.2 Yield components

The average number of clusters per vine was calculated by counting all remaining clusters (post thinning) from each data panel and dividing by the total number of vines. Average cluster weights were quantified following hand harvesting the clusters from each data panel and weighing with a mobile platform scale (Wedderburn, Sydney, Australia). The total weight was then divided by the total number of vines. The yield for each area was calculated by multiplying the average number of clusters and the average cluster weight by the total number of vines in the complete trial area and then dividing by the total area.

The vines were pruned according to grower specifications and cane pruning weights were collected from each data panel across each area.

5.2.3 Canopy characterisation

Vines within each trial segment underwent the same viticultural practices with regards to irrigation, leaf plucking, pruning and spray regimes according to grower specifications. Point quadrat analysis [166] was used to characterize canopy density around veraison and after leaf removal in each year. To assess vine performance, the crop load, defined as the ratio of exposed leaf area to fruit, was determined using the Ravaz Index [165]. This index is defined as the ratio of exposed leaf area to fruit and is calculated by dividing total yield per vine by the pruning weight recorded during the winter following each season.

5.2.4 Juice composition

Following hand harvesting of the full trial area, the grapes were crushed and de-stemmed (Diemme Enologia SpA, Lugo (RA), Italy) and standard analyses including pH (Orion Star A211, Thermo Scientific, Waltham, MA, USA), temperature, titratable acidity and °Brix (Mettler Toledo Densito

30PX, Global Science, Greenbelt, MA, USA) were carried out according to methods typically used in wineries [164].

5.2.5 Winemaking

Wines were made in duplicate following hand harvesting of fruit from each treatment area and evenly dividing before processing. The grapes were harvested at 25-26° Brix before being crushed, de-stemmed and pumped into a stainless steel tank for pre-fermentation cold soak for seven days at 8-10° C. Keeping to the standard practices of the winery, the juice was warmed to 18-20° C and inoculated with 200 mg/kg of selected *Saccharomyces cerevisiae* yeast (EC1118 Lallemand Inc., Montreal, Canada). The must was left to ferment on skins for 17-20 days whereby the cap was manually punched down twice daily. Alcoholic fermentation was confirmed as completed (residual sugar < 1 g/L) using glucose/fructose enzymatic methods (Mega-zyme, Wicklaw, Ireland) according to manufacturer's instructions using a Genesys™ 10 Thermospectronic (Daly City, CA, USA). The wines were racked and samples were collected and sulphited at 35 mg/L to inhibit malolactic fermentation. The samples were kept at 4° C for three months before analysis.

5.2.6 Reagents

All the chemicals used were of analytical grade. Sodium hydroxide, sodium acetate, diethyl ether, sodium sulfate, sodium dihydrogen phosphate dihydrate, hexane (96%), absolute methanol (99%), acetic acid glacial (99%), acetonitrile (gradient grade) and hydrochloric acid were from Scharlau (Sentmenat, Spain). Ammonium sulfate was from Univar (Redmond, WA). Methyl cellulose and tartaric acid were from Sigma-Aldrich (St. Louis, MO). Dichloromethane was from Merck (Darmstadt, Germany). Potassium metabisulfite was from Redox Pty Ltd (Auckland, NZ) and absolute ethanol (99%) from ECP Ltd. (Auckland, NZ).

Commercial volatile compounds, used as external standards, included linalool (97%), geraniol (99%), *β*-citronellol (95%), *α*-terpineol (99%), phenylethyl alcohol (99%), hexanoic acid (98%), octanoic acid (99%), decanoic acid (99%),ethyl butanoate (99%), ethyl lactate (95%), and phenylethyl acetate (99%) from Acros Organics. Nerol (97%), *β*-ionone (96%), *cis*-3-hexen-1-ol (98%), methionol (99%), ethyl isobutyrate (99%), ethyl decanoate (99%), ethyl octanoate (99%), ethyl cinnamates (99%), ethyl hydrocinnamate (99%), ethyl anthranilate (99%), methyl anthranilate (99%), diethyl malate (97%), ethyl isovalerate (99%), 1-hexanol (98%), ethyl hexanoate (98%), diethyl succinate (99%), isobutyl acetate (99%), were purchased from Sigma-Aldrich. *Trans*-3-hexen-1-ol (97%), 4-decanol (98%) and 3-octanol (99%) were obtained from Lancaster (Ward Mill, MA). Isoamyl alcohol (98%) was from Pancreac Quimica (Barcelona, Spain). Isobutanol (99%) was from Scharlau. Isoamyl acetate (98%) was a product of Univar.

Commercial polyphenols, used as external standards, included gallic acid monohydrate (98%), vanillic acid (97%), syringic acid (95%), caffeic acid (98%), *p*-coumaric acid (98%), *trans*-resveratrol (99%), quercetin dihydrate (98%), quercetin-3-β-D-glucoside (90%), catechin hydrate (98%), epicatechin (90%), epigallocatechin (95%) and epicatechin-*o*-gallate (98%) were purchased from Sigma-Aldrich. Oenine chloride, used as an external standard for monomeric anthocyanins, was obtained from Extrasynthese (Genay Cedex, France).

5.2.7 Isolation of volatile compounds for GC-MS analyses

A liquid-liquid extraction with ether: hexane (1/1, v/v) was undertaken in triplicate for the quantification of volatile compounds including monoterpenes, C_{13}-norisoprenoids, C_6-alcohols, higher alcohols, fatty acids, esters and acetate esters according to an established method [167]. This method, originally designed for extracting methoxypyrazines, was modified to accommodate the quantifications of other compounds in this study. Volatile compounds were extracted from 50 mL of wine spiked with 3-octanol and 4-decanol [41] as internal standards three times, followed by

concentrating the organic phase under a nitrogen flow to 100 µL. Immediate injection was carried out on an Agilent 6890N GC coupled with an Agilent MS 5973 (Santa Clara, CA). A pulsed splitless injection with a pulse and purge time at 1.00 minute, purge flow of 12.0 mL/min, and total flow of 16.6 mL/min was delivered with helium as gas carrier onto a 19091N-136 HP-INNOWax capillary column (0.252mm x 60m x 0.25µm; J&W Scientific, Folsom, CA, USA). Analysis of C_6-alcohols, higher alcohols, fatty acids, esters (including ethyl and acetate esters) was carried out at an initial oven temperature of 40°C held for 10 minutes, then raised to 170°C at 3°C/min, followed by an increase to 240°C at 70°C/min and held for 10 minutes before dropping down to 40°C at 60°C/min, resulting in a total run time of 70.7 minutes. Analysis of monoterpenes, C_{13}-norisoprenoids and cinnamic esters was conducted at an initial oven temperature of 40°C held for 5 minutes, then raised to 200°C at 2°C/min, followed by an increase to 240°C at 80°C/min held for 5 minutes and finally brought back to 40°C at 80°C/min, resulting in a total run time of 100 minutes. The temperature of the interface line was set to 250°C. The ion source, operating in electron impact mode at 70 eV, was held at 250°C. The quadrupole temperature was set at 150°C. The GC-MS was operated in selective ion monitoring (SIM) mode for both methods.

Calibration curves were created for each volatile compound using the standard addition method and employing (Table 5.1) the same extraction protocols and GC-MS parameters, respectively.

5.2.8 Polyphenol analysis

The analysis of individual wine phenolics was conducted using Reverse-Phased HPLC, based on a method developed at the University of Auckland [176]. In the method, the use of a binary gradient elution system consisting of an acidified mobile phase (acetic acid) and an organic solvent (acetonitrile) provides an efficient separation of different wine phenolic compounds. In addition, monitoring wine phenolics at four different wavelengths of 280 nm, 320 nm, 365 nm, and 520 nm

also allows the simultaneous identification and quantification of various compounds in a single HPLC run.

Samples were injected into a Hewlett-Packard (Palo Alto, CA, USA) Agilent 1100 series instrument coupled to a Diode Array Detector (G1315B) a Column Heater (G1316A), an Auto-sampler (G1313A), a Quaternary Pump (G1311A) and a Degasser (G1379A). The column used was a Phenomenex Luna C18 (250 mm x 4.6 mm; pore size of 5 microns; Agilent). Milli-Q water (Barnstead; Thermo Scientific), acetonitrile, and a 5 % (v/v) acetic acid solution were used for the mobile phase. The column temperature was set at 25°C with a maximum column pressure of 259 bar and minimum pressure of 10 bar. The injection volume was set at 20 μL and a flow rate of 0.8ml/min. These parameters were constant for each sample with a run time of 150 minutes. Samples were filtered with a Sartorius Minisart (RC 15) 0.45 micron polytetrafluoroethylene syringe filters (Agilent) into Grace Ram 12 x 32 mm amber vials (Agilent). After degassing with argon, vials were sealed with Sun 9mm silicon septas and screw caps (Agilent) for direct injection.

The quantification of polyphenols was carried out using external standards to construct calibration curves employing the same extraction protocols and RP-HPLC parameters, respectively. Due to poor solubility of polyphenol compounds in water, all individual stock solutions of commercial standards, except quercetin, were prepared in absolute ethanol. The stock solution of quercetin was prepared in methanol due to its poor solubility in ethanol. Serial dilutions of stock solutions in model wine (5 g/L tartaric acid, 12 % v/v aqueous ethanol, pH adjusted to 3.6) were then performed to obtain different polyphenol concentrations, chosen to be in the range of these compounds usually found in red wines (Table 5.1).

5.2.8.1 Tannin analysis

The tannin content was quantified in triplicate with 0.25 mL of sample using the Methyl Cellulose Precipitation (MCP) tannin assay [177]. Quantification is based on subtracting the absorbance values

at 280 nm (A^{280}) of solutions both with and without tannin precipitation, and converting to epicatechin equivalents. The calibration curve was constructed using eight concentrations of (-)-epicatechin ranging from 5 to 250 mg/L (r^2= 0.99). Spectrophotometric absorbance values were obtained using a Genesys ™ 10 Thermospectronic and Eppendorf (Hamburg, Germany) disposable cuvettes (2 mm pathway). Centrifugation was performed using an Eppendorf Centrifuge 5804.

5.2.9 Sensory analysis

All of the wines underwent sensory analysis approximately three months after sampling, prior to malo-lactic fermentation and exposure to oak. Analysis was conducted by a group of 15 expert oenologists (7 men and 8 women, between 24 and 45 years of age) from Mt. Difficulty Winery and the Wine Science staff at the University of Auckland. 30 mL wine samples were presented in approved glasses [217] labelled with three-digit random codes and covered with glass Petri dishes according to a random arrangement. All samples were served at room temperature and were evaluated at individual stations. Panellists paused for five minutes between sample sets to limit adaptation effects and sensory fatigue.

Firstly, separate triangular tests were performed for each of the three experimental wines where each subject was presented with three random coded samples for each test. Subjects were informed that two of the samples were identical and that one was different. The subject tasted each product from left to right and selected the sample they perceived as different from the other two. Secondly, the samples underwent a paired comparison test where the subjects were asked to rank the wines in order of intensity of a particular sensory characteristic, namely, fruity, floral, herbaceous, spicy, acidic, sweet, bitter and astringent [120].

5.2.10 Statistical analysis

All chemical data are expressed as the arithmetic average ± the standard deviation. One factor analysis of variance (ANOVA) was carried out with SPSS software (SPSS Inc., Chicago, IL), and comparisons between samples were conducted using Tukey's HSD posthoc test. R coefficients were calculated using Pearson correlation coefficient. Significance for the triangular tests was determined using the method set out by Meilgaard *et al*. [120] The paired comparison tests were calculated using the statistics of Friedman [120].

5.3 Results and discussion

5.3.1 Canopy and fruit characterisation

No significant differences were observed with regards to the canopy density and the Ravaz Index, (which remained between 5 and 6) for each experimental condition for each season, demonstrating good vine balance (data not shown). There were no problems with flowering, fruit set or disease in any season. The data for the chemical composition of the grapes (Table 5.2) showed a strong negative correlation between bunch weights and cluster thinning with the treated vines always producing bunches with an average weight more than the control. Some significant differences ($p = <0.05$) were noted when comparing weights for the 2009 and 2010 season. This tendency could be a result of compensation in the treated vines where the fruit to carbohydrate ratio was greater.

5.3.2 Basic juice composition

As shown in Table 5.2, there were only slight differences in °Brix amongst the samples within each year, as the target °Brix were reached by all treatments with a moderate to strong correlation between treatments. However, the low yield grapes reached the target °Brix for harvesting five to

ten days earlier than the control grapes, and one to seven days earlier than the moderate yield grapes for each year. The moderate thinning treatment also reached target °Brix one to seven days earlier than the control across the three vintages. Differences in the pH were observed within each year, with a very strong negative correlation being observed. Significant differences were observed in titratable acidity in 2009 and 2011, and there was a strong positive correlation with the treatments every year. Both pH and titratable acidity are considered for the timing of harvest and when evaluating the quality of the grape juice. Maintaining a low pH (<3.8) is important for colour stability in red wines and has a beneficial antimicrobial effect, while acids give wine its freshness and modifies the perception of other tastes and mouth-feel sensations [21].

5.3.3 Quantification of aroma compounds

A total of 33 aroma compounds were quantified by means of GC-MS (Table 5.3-5.5) in each year. There were trends observed amongst several compound groups that suggested a correlation between their concentrations and cluster thinning treatments. The removal of crop, as seen in this study, typically allowed more light exposure to the fruit. The concentrations of numerous grape aroma compounds are influenced by increased exposure to sunlight, including free norisoprenoids as well as glycosylated norisoprenoids and monoterpenes. The two C_{13} norisoprenoids examined in this study are released during crushing of the grapes and have low aroma perception thresholds (Table 5.1). Both compounds were present in the wines at concentrations above their perception thresholds across all treatments in every year (Table 5.3-5.5), and significant differences were observed between the different treatments within each year. An association between the level of thinning and β-damascenone and *β*-ionone was also observed. As thinning levels increased, the concentrations of both of these compounds increased in all three seasons. This study and previous studies [27, 195] have shown that the concentrations of C_{13} norisoprenoids can vary between seasons. Furthermore, a slight variance in their concentration can result in exceedingly different

aromatic nuances and subsequent contribution in wines. β-damascenone, in particular, has been shown to be of potential importance in Pinot noir wines [218]. The variance in concentrations is thought to be caused by environmental conditions such as temperature, weather and sunlight, where the quality of light, rather than the intensity, plays a role in regulating the accumulation of norisoprenoids [160].

The monoterpenes quantified in this study were well below perception threshold, and for this reason it has been suggested in previous studies that monoterpenes may be of little importance in the overall aroma of Pinot noir wines [147, 218]. However, it is worth noting that in most cases, the control was significantly lower in concentrations of monoterpenes than both cluster thinning treatments and the relationship between the cluster thinning levels and the concentration of the monoterpenes was similar to the C_{13} norisprenoids in that as more fruit was removed, the concentrations increased across all three vintages. This finding was similar to a bunch shading study on Syrah grapes where shaded bunches had fewer total numbers of monoterpene precursors, most likely due to the increased light exposure in the treatment areas favouring the accumulation of glycosylated monoterpenes [219].

Another group of compounds affected by light are the cinnamic and anthranilic esters, which are thought to be derived from the shikimic pathway [220] and phenyl pyruvate [221]. While the biosynthesis of these volatile phenylpropanoids has not been studied extensively in grapes, there are studies that show how this group of compounds can contribute significantly to the distinct aromas of the Cabernet Sauvignon and Merlot musts [222] and Pinot Noir wines [61, 218]. In this study, the cinnamic esters exceeded their thresholds and their concentrations consistently increased when the level of thinning was increased. The anthranilic esters showed no trends and did not meet their respective perception thresholds; however, significant differences were noted between treatments in most cases. This may indicate that the additional light allowed with cluster thinning may only affect compounds derived from the aromatic amino acid, phenylalanine.

The medium chain fatty acids examined in this study are thought to be dependent on a number of conditions including grape and must composition [74, 75]. The concentrations were found to be just over perception threshold with the exception of decanoic acid. Fatty acids are thought to contribute to a wine's complexity when the concentrations are at or slightly under threshold [66]. Significant differences were seen between treatments with the more intense thinning treatment showing lower concentrations of fatty acids in all cases. Given that medium chain fatty acids have been shown to be correlated with stuck and sluggish ferments due to their inhibitory effects on *S. cerevisiae*, practices that keep these compounds at low to moderate concentrations, such as cluster thinning, would appear to produce positive outcomes.

There were varied results with esters quantified in this study. The monocarboxylic esters, typically the most aromatic in wine aroma, all showed significant differences between treatments across all three years and treatments, and their concentrations exceeded the respective perception thresholds with the exception of ethyl decanoate. Of the acetate esters, isoamyl acetate, thought to contribute or lift the intensity of the other fruity notes present [132], was the only acetate ester at a concentration above the perception threshold. The two treatments of crop thinning did not seem to have a consistent effect on the concentrations of esters in this study across each season with exception to ethyl isobutyrate and ethyl hexanoate, where concentrations of both were consistently lower as crop thinning levels increased. These esters are derived from fatty acids and have been shown to be important in the overall aroma of Pinot Noir [218]. This observation is most likely due to the concentration of fatty acid precursor concentrations in the juice, which, as mentioned before, are influenced by the amount of light during grape maturation, instead of the enzymatic biosynthesis [82] involved in the formation of most ethyl esters. The acetate esters had moderate to strong positive correlations in most cases. The concentrations of the dicarboxylic acid esters quantified in this study, namely ethyl lactate, diethyl malate and diethyl succinate, did not reach their respective perception thresholds and no trends were observed.

When considering the alcohols, the C_6 alcohols did not meet threshold in any of the samples; however, nearly all higher alcohols exceeded their respective perception thresholds and significant differences were observed in most seasons. There were no consistent trends observed across the treatments with the exception of 1-hexanol, where a strong positive correlation was shown and β-phenylethyl alcohol where a strong negative correlation was noted across all three vintages. β-phenylethyl alcohol has been shown to be of importance in the aroma of Pinot Noir [218], contributing rose-like aromas, and is a derivative of phenylalanine.

Overall, for the volatiles quantified in this study, there were trends noticed amongst the compounds that were affected by the amount of light the grapes were exposed to, namely C13 norisoprenoids and monoterpenes. Relationships were also revealed where cluster thinning may have resulted in changing the grape composition and its subsequent must, especially that of the aromatic amino acid, phenylalanine.

5.3.4 Phenolics composition

The phenolic and tannin composition of each wine sample was analysed and is displayed in Tables 5.6-5.8. Significant differences and very close associations with the levels of crop thinning and the concentrations of the phenolics were observed across all compounds in every year. As crop levels decreased with more intense thinning, the phenolic concentrations increased. This result is not unexpected and follows observations made with different varieties as stated earlier. The non-flavonoids, dependent on the derivatives from phenylalanine via the shikimic pathway, were increased by up to 25% with moderate bunch thinning, and by up to 57% with intense thinning, when compared to the control. While the non-flavonoids do not contribute sensory attributes directly to the wine, there are some aromatic compounds, namely the cinnamic esters, which depend on these compounds and play an important role in the overall aroma of Pinot noir wines. The flavonoids, also dependent on phenylalanine, displayed a 21% and 54% increase with moderate and intense thinning

treatments, respectively. The flavonoid polyphenols are found in the skins and seeds of grapes where they absorb UV radiation and provide protection for the fruit. The increased direct exposure to UV and blue radiation from the moderate and intense thinning treatments would for the most part explain the increased synthesis of these compounds. It has been reported that exposure to sunlight has a considerable effect on flavonol content in berries [223]. Given that flavonols, flavan-3-ols and anthocyanins provide co-pigmentation factors, bitterness and pigmentation, respectively, to red wines, the increase in these compounds would most likely have a direct effect on the quality of the wine. The total grape tannins reported in this study, as the samples were not exposed to oak, increased by up to 36% with thinning treatments. These condensed tannins can contribute both bitterness and astringency in red wines providing sensorial structure and mouth-feel. The retention of these tannins is also dependent on their polymerization with anthocyanins, which in turn increases the wine's aging ability and quality.

5.3.5 Sensory difference tests

Table 5.9 shows the results of the triangular sensory tests of the three pairs of wines for each year. The assessors successfully distinguished ($p < 0.05$) the control and both treatments each year with exception of the moderate crop thinning treatment in 2009. The assessors were only able to distinguish the moderate and intense crop thinning treatments in 2010. Table 5.10 displays the results of the paired comparison ranking tests for each season. This test provides the assessor with an opportunity to put the wines in order of intensity (from least to most) for a particular characteristic, with the maximum possible score being 45 in this situation (15 assessors and three wines). The Friedman test (F) was applied to the values and is considered significant if it is greater than or equal to the critical value corresponding to the chosen threshold. In this experiment, the threshold was 5, 1 and 0.1 %, with critical values of 5.99, 9.21 and 13.82, respectively. Significant differences were seen in every season with regards to the sensory terms herbaceous and acidic, with

the control wines receiving the highest ranking scores every time. The thinning treatments received higher marks in every case for the descriptors fruity, spice, sweet and body; however, significant differences were not observed every year. The observation of increased sweetness amongst the thinned treatments is interesting considering that all of the wine samples had no residual sugar. In this case the decreased acidity from lower TAs, and increased body from increased concentrations of phenolics, may have brought about the heightened perception of sweetness. The assessors could not determine any consistent differences when considering the remaining attributes of floral, bitter and astringent. These results indicate that cluster thinning has an effect on the perceived aromatics and mouth-feel of the resultant wines, when compared to no thinning. However, particular levels of thinning may only make a slight to no consistent difference.

5.4 Conclusions

In summary, vines exposed to intense cluster thinning reached the target °Brix for harvesting earlier than the moderately thinned treatment, and both treatments reached target earlier than the control. Significant differences were observed for the chemical composition of each treatment, with consistent increases in pH and bunch weights and decreases in TAs were observed when more fruit was removed from increased cluster thinning treatments. A close relationship was observed across all three seasons between cluster thinning and concentrations of the C_{13} norisoprenoids, monoterpenes, fatty acids, cinnamic esters, ethyl isobutyrate, ethyl hexanoate, β-phenylethyl alcohol and all phenolics. Significant differences were observed for the wine sensory properties, and wines made from thinning treatments were found to be less herbaceous and acidic than the control with no thinning. The wines made from thinning treatments were also perceived as being fruitier, spicy, sweeter and having more body when compared to the control wines; however, no consistent trends were observed between the two treatments. Therefore, it can be concluded that cluster thinning may

have an effect on ripening times and the non-volatile and volatile chemical composition of the subsequent wines. These effects may also lead to detectable sensory differences in the final product. It would seem that crop thinning, while a costly practice due to increased labour and yield reduction, is effective in influencing wine quality. However, a particularly intense level of thinning was not necessary to achieve the differences in the wines, as a moderate level of thinning also provided a marked enhancement to several attributes.

Table 5.1 Linearity, quantification data and perception thresholds for the volatile compounds and phenolics and phenolics analysed in this study

Compound	Correlation coefficient (r^2)	Calibration range (µg/L)	Recovery in calibration range (%)	Average relative std deviation (%)	Perception threshold (µg/L)
C_{13}-norisoprenoids					
β-ionone	0.998	0.09 – 17.6	92 – 106	3.4	0.09[10]
β-damascenone	0.998	0.08 – 15.5	98 – 107	5.7	0.05[10]
monoterpenes					
linalool	0.998	0.15 – 29.7	93 – 127	7.8	25[10]
nerol	0.996	0.29 – 57.3	94 – 108	5.6	400[41]
geraniol	0.997	0.32 – 64.2	93 – 108	2.2	20[10]
β-citronellol	0.997	0.20 – 40.3	97 – 110	3.4	100[10]
α-terpineol	0.996	0.29 – 58.7	92 – 106	7.4	250[10]
C_6-alcohols					
1-hexanol	0.997	124 – 24884	93 – 104	9.4	8000[10]
cis-3-hexen-1-ol	0.992	8.7 – 1747	91 – 102	3.8	400[10]
trans-3-hexen-1-ol	0.996	4.18 – 835	90 – 106		1000[41]
alcohols					
isoamyl alcohol	0.992	3338 – 200275	90 – 112	7.2	30000[10]
isobutanol	0.998	1888 – 113288	92 – 116	8.4	40000[10]
methionol	0.991	77 – 4596	91 – 111	6.9	1000[10]
phenylethyl alcohol	0.998	1920 – 115217	98 – 106	3.9	14000[10]
fatty acids					
hexanoic acid	0.997	560 – 11200	96 – 104	6.8	420[10]
octanoic acid	0.999	460 – 36630	94 – 111	3.8	500[10]
decanoic acid	0.998	50 – 1114	91 – 101	8.6	1000[10]
ethyl esters of isoacids					
ethyl isobutyrate	0.991	5.5 – 401	95 – 104	9.2	15[10]
ethyl isovalerate	0.991	5.2 – 99.1	97 – 105	7.8	3[10]
ethyl esters of fatty acids					
ethyl butyrate	0.993	9.2 – 735	95 – 110	7.7	20[10]
ethyl hexanoate	0.992	21 – 1708	96 – 105	5.4	14[10]
ethyl decanoate	0.991	7.6 – 608	96 – 108	6.7	200[10]
ethyl octanoate	0.993	32 – 2572	94 – 105	7.6	5[10]

cinnamic esters					
ethyl cinnamate	0.990	0.14 – 27.9	92 – 107	7.1	1.1[10]
ethyl hydrocinnamate	0.992	0.12 – 24.9	95 – 104	3.5	1.6[10]
miscellaneous esters					
ethyl lactate	0.999	814 – 65132	98 – 112	2.6	154000[10]
ethyl anthranilate	0.992	0.14 – 28.9	96 – 109	6.7	
methyl anthranilate	0.998	0.15 – 29.1	96 – 119	7.8	3[60]
diethyl malate	0.999	9.4 – 1856	97 – 102	7.9	10000[41]
diethyl succinate	0.999	212 – 16979	97 – 114	8.6	200000[41]
acetate esters					
isobutyl acetate	0.991	7.8 – 627.1	93 – 101	8.2	1600[10]
isoamyl acetate	0.993	80 – 6434	92 – 100	5.5	30[10]
phenylethyl acetate	0.992	6.2 – 499	94 – 103	6.2	250[10]
Non-flavonoids					
Benzoic acid					
gallic acid	0.992	1.5 – 200	97 – 119	2.1	
Hydroxycinnamic acids					
trans-caftaric acid	0.999	0.5 – 200	95 – 106	2.0	
cis-coutaric acid	0.992	0.2 – 100	95 – 110	1.5	
trans-coutaric acid	0.999	0.2 – 100	92 – 100	1.5	
caffeic acid	0.991	0.5 – 50	94 – 102	2.3	
Stilbenes					
trans-resveratrol	0.998	0.3 – 40	92 – 100	2.0	
Flavonoids					
Flavonols					
quercetin	0.993	0.6 – 85	92 – 100	3.0	
quercetin-3-glucoside	0.991	0.5 – 50	95 – 101	3.6	
Flavan-3-ols					
epicatechin	0.999	2.0 – 300	99 – 115	1.2	
catechin	0.999	3.0 – 350	99 – 120	1.9	
Anthocyanins					
delphinidin-3-glucoside	0.991	4.0 – 1000	95 – 104	2.5	
cyanidin-3-glucoside	0.996	4.0 – 1000	96 – 107	3.6	
petunidin-3-glucoside	0.996	4.0 – 1000	96 – 102	2.5	
peonidin-3-glucoside	0.994	4.0 – 1000	96 – 101	3.1	
malvidin-3-glucoside	0.992	4.0 - 1000	99 – 116	3.2	

Table 5.2 °Brix, pH, TA, bunch weight and harvest date for the grapes harvested in 2009 to 2011 with corresponding r coefficients

	°Brix	pH	TA (g/L)	Bunch weight (g/L)	Harvest date
2009					
Control - 7.4 t/ha	26.3 ± 0.3	3.19 ± 0.02 a	8.81 ± 0.11 a	110 ± 5 a	12th April 09
Moderate CT – 6.1 t/ha	26.2 ± 0.2	3.23 ± 0.02 b	8.42 ± 0.18 b	121 ± 3 b	10th April 09
Intense CT – 3.5 t/ha	26.5± 0.2	3.26 ± 0.02 b	8.18 ± 0.19 b	127 ± 3 b	2nd April 09
r coefficient	-0.78	-0.96	0.95	-0.94	
2010					
Control – 8.5 t/ha	25.6 ± 0.2	3.17 ± 0.02 a	9.31 ± 0.10	91 ± 4	22nd April
Treatment 1 – 6.7 t/ha	25.8 ± 0.4	3.21 ± 0.01 b	9.23 ± 0.15	95 ± 9	18th April
Treatment 2 – 3.2 t/ha	26.1± 0.3	3.27 ± 0.02 c	9.18 ± 0.17	105 ± 6	17th April
r coefficient	-0.99	-0.99	0.95	-0.99	
2011					
Control – 9.3 t/ha	25.4 ± 0.3	3.23 ± 0.03 a	8.94 ± 0.19 a	138 ± 6 a	5th April
Treatment 1 – 7.7 t/ha	25.1 ± 0.3	3.32 ± 0.03 b	7.68 ± 0.13 b	149 ± 4 b	6th April
Treatment 2 - 4.4 t/ha	25.6± 0.5	3.39 ± 0.01 c	7.38 ± 0.12 c	152 ± 5 b	29th March
r coefficient	-0.56	-0.96	0.86	-0.87	

r coefficient calculated using Pearson correlation coefficient

Table 5.3 Volatile composition of wines made from Pinot noir grapes at different crop yields, achieved through bunch thinning for the 2009 vintage

2009	Control 7.4 t/ha	Moderate CT 6.1 t/ha	Intense CT 3.5 t/ha	r coefficient
C_{13} norisoprenoids				
β-ionone	0.23 ± 0.01 a	0.26 ± 0.01 a	0.41 ± 0.02 b	-0.98
β-damascenone	3.1 ± 0.6 a	2.9 ± 0.8 a	4.3 ± 0.2 b	-0.97
monoterpenes				
linalool	2.2 ± 0.1 a	4.1 ± 0.1 b	4.6 ± 0.4 b	-0.87
nerol	4.9 ± 0.4 a	6.1 ± 0.2 b	6.4 ± 0.2 b	-0.86
geraniol	11 ± 4 a	12 ± 4 a	16 ± 6 b	-0.99
citronellol	5.4 ± 0.3 a	7.9 ± 0.5 b	8.2 ± 0.2 b	-0.81
α-terpineol	2.7 ± 0.1 a	2.8 ± 0.2 ab	3.3 ± 0.4 b	-0.98
C_6 alcohols				
1-hexanol	2175 ± 294 a	1606 ± 84 b	1360 ± 63 b	0.81
cis-3-hexen-1-ol	19 ± 2 a	53 ± 3 b	37 ± 1 c	-0.36
trans-3-hexen-1-ol	37 ± 5 a	67 ± 5 b	51 ± 6 c	-0.29
alcohols				
isoamyl alcohol	71072 ± 3757 a	82205 ± 4652 b	77865 ± 4783 ab	-0.44
isobutanol	15140 ± 182 a	10209 ± 198 b	10823 ± 102 b	0.67
methionol	1339 ± 96 a	1140 ± 51 b	1221 ± 76 ab	0.43
β-phenyl ethanol	112575 ± 2303 a	125767 ± 3383 b	158473 ± 2024 c	-0.99
fatty acids				
hexanoic acid	1024 ± 23 a	900 ± 34 b	667 ± 25 c	0.99
octanoic acid	1014 ± 18 a	931 ± 40 b	674 ± 36 c	0.99
decanoic acid	662 ± 17 a	502 ± 30 b	374 ± 23 c	0.97
ethyl esters of isoacids				
ethyl isobutyrate	28 ± 3 a	38 ± 5 b	25 ± 2 a	0.40
ethyl isovalerate	56 ± 7 a	48 ± 6 a	30 ± 4 b	0.99
ethyl esters of fatty acids				
ethyl butyrate	139 ± 12	152 ± 15	154 ± 13	-0.83
ethyl hexanoate	435 ± 34 a	282 ± 16 b	236 ± 18 b	0.88
ethyl decanoate	205 ± 10	172 ± 16	189 ± 16	0.31
ethyl octanoate	326 ± 19 a	269 ± 19 b	283 ± 10 b	0.58
cinnamic esters				
ethyl cinnamate	2.11 ± 0.05 a	2.08 ± 0.06 a	3.79 ± 0.02 b	-0.94
ethyl hydrocinnamate	1.39 ± 0.05 a	1.35 ± 0.03 a	2.66 ± 0.03 b	-0.94
miscellaneous esters				
ethyl lactate	75246 ± 2103 a	99012 ± 2165 b	85873 ± 2593 c	-0.26
ethyl anthranilate	1.31 ± 0.11 a	1.24 ± 0.06 a	1.51 ± 0.04 b	-0.83
methyl anthranilate	0.41 ± 0.04 a	0.78 ± 0.03 b	0.52 ± 0.03 a	-0.10
diethylmalate	155 ± 25	166 ± 26	144 ± 38	0.65
diethyl succinate	1177 ± 44 a	1048 ± 20 b	1157 ± 25 a	-0.05
acetates				
isobutyl acetate	122 ± 15 a	91 ± 18 ab	66 ± 12 b	0.97
isoamyl acetate	279 ± 23 a	306 ± 16 a	204 ± 22 b	0.82
phenylethyl acetate	19 ± 6 a	21 ± 4 a	36 ± 5 b	-0.97

Table 5.4 Volatile composition of wines made from Pinot noir grapes at different crop yields, achieved through bunch thinning for the 2010 vintage

2010	Control 8.5 t/ha	Moderate CT 6.7 t/ha	Intense CT 3.2 t/ha	r coefficient
C_{13} norisoprenoids				
β-ionone	0.61 ± 0.03 a	0.71 ± 0.04 b	0.89 ± 0.05 c	-0.99
β-damascenone	2.7 ± 0.3 a	3.6 ± 0.4 b	4.5 ± 0.7 c	-0.98
monoterpenes				
linalool	2.3 ± 0.2 a	5.1 ± 0.1 b	6.1 ± 0.4 c	-0.90
nerol	4.1 ± 0.1 a	5.9 ± 0.3 b	6.0 ± 0.1 b	-0.79
geraniol	12 ± 3 a	15 ± 4 b	16 ± 2 b	-0.89
citronellol	11 ± 2 a	16 ± 2 ab	19 ± 1 b	-0.94
α-terpineol	2.2 ± 0.3 a	2.5 ± 0.2 ab	3.1 ± 0.3 b	-0.99
C_6 alcohols				
1-hexanol	1942 ± 83 a	1480 ± 79 b	1223 ± 74 c	0.94
cis-3-hexen-1-ol	36 ± 5 a	48 ± 5 b	27 ± 2 a	0.58
trans-3-hexen-1-ol	62 ± 2 a	52 ± 2 b	41 ± 3 c	0.98
alcohols				
isoamyl alcohol	264934 ± 1999	257014 ± 9276	281154 ± 8675	-0.78
isobutanol	17224 ± 619 a	15342 ± 1178 a	11751 ± 1001 b	0.77
methionol	1226 ± 31 a	1672 ± 47 b	1357 ± 64 a	-0.10
β-phenyl ethanol	75281 ± 3173 a	83617 ± 1271 b	87178 ± 3529 b	-0.92
fatty acids				
hexanoic acid	973 ± 20 a	989 ± 34 a	756 ± 27 b	0.92
octanoic acid	766 ± 26 a	821 ± 40 b	590 ± 11 c	0.84
decanoic acid	416 ± 25 a	364 ± 27 b	298 ± 13 c	0.99
ethyl esters of isoacids				
ethyl isobutyrate	52 ± 2 a	46 ± 4 a	36 ± 3 b	0.99
ethyl isovalerate	43 ± 5 a	56 ± 3 b	50 ± 4 ab	-0.37
ethyl esters of fatty acids				
ethyl butyrate	161 ± 15 a	189 ± 11 b	153 ± 17 a	0.38
ethyl hexanoate	451 ± 22 a	499 ± 12 a	290 ± 10 b	0.84
ethyl decanoate	249 ± 9 a	273 ± 14 a	153 ± 10 b	0.86
ethyl octanoate	336 ± 17 a	372 ± 19 a	253 ± 16 b	0.80
cinnamic esters				
ethyl cinnamate	2.6 ± 0.3 a	2.9 ± 0.1 b	3.3 ± 0.2 c	-0.99
ethyl hydrocinnamate	1.50 ± 0.03 a	2.09 ± 0.09 b	2.49 ± 0.13 c	-0.95
miscellaneous esters				
ethyl lactate	84218 ± 2546 a	91772 ± 1655 b	81899 ± 2078 a	0.40
ethyl anthranilate	1.65 ± 0.05	1.46 ± 0.06	1.50 ± 0.09	0.62
methyl anthranilate	0.33 ± 0.04 a	0.27 ± 0.03 a	0.47 ± 0.03 b	-0.80
diethylmalate	153 ± 16 a	170 ± 19 b	125 ± 11 c	0.75
diethyl succinate	1311 ± 40	1665 ± 32	1240 ± 46	0.33
acetates				
isobutyl acetate	77 ± 4 a	87 ± 4 a	60 ± 7 b	0.75
isoamyl acetate	267 ± 13 a	194 ± 12 b	206 ± 18 c	0.65
phenylethyl acetate	20 ± 2 a	23 ± 3 b	39 ± 1 b	-0.98

Table 5.5 Volatile composition of wines made from Pinot noir grapes at different crop yields, achieved through bunch thinning for the 2011 vintage

2011	Control 9.3 t/ha	Moderate CT 7.7 t/ha	Intense CT 4.4 t/ha	r coefficient
C_{13} norisoprenoids				
β-ionone	0.44 ± 0.04 a	0.59 ± 0.04 b	0.79 ± 0.02 c	-0.99
β-damascenone	1.6 ± 0.1 a	1.9 ± 0.3 b	3.0 ± 0.2 c	-0.99
monoterpenes				
linalool	2.2 ± 0.2 a	4.7 ± 0.1 b	5.0 ± 0.5 b	-0.81
nerol	4.9 ± 0.2 a	6.8 ± 0.6 b	7.7 ± 0.5 c	-0.92
geraniol	12 ± 1	13 ± 2	16 ± 2	-0.99
citronellol	13 ± 1 a	15 ± 2 ab	16 ± 4 b	-0.92
α-terpineol	1.8 ± 0.1 a	2.1 ± 0.2 b	2.4 ± 0.2 b	-0.98
C_6 alcohols				
1-hexanol	3142 ± 166 a	2311 ± 54 b	1892 ± 126 c	0.93
cis-3-hexen-1-ol	44 ± 3 a	82 ± 11 b	48 ± 4 a	0.10
trans-3-hexen-1-ol	105 ± 16 a	102 ± 13 a	72 ± 6 b	0.97
alcohols				
isoamyl alcohol	185380 ± 5514 a	186651 ± 7809 a	168237 ± 1898 b	0.92
isobutanol	21911 ± 1479	25769 ± 1057	26477 ± 814	-0.83
methionol	987 ± 27 a	1421 ± 19 b	727 ± 38 c	0.55
β-phenyl ethanol	54134 ± 2333 a	72054 ± 2190 b	87491 ± 1019 c	-0.97
fatty acids				
hexanoic acid	753 ± 33 a	504 ± 18 b	510 ± 12 b	0.73
octanoic acid	632 ± 40 a	570 ± 15 b	384 ± 20 c	0.99
decanoic acid	302 ± 25 a	236 ± 15 b	184 ± 14 c	0.96
ethyl esters of isoacids				
ethyl isobutyrate	76 ± 4 a	72 ± 3 a	59 ± 3 b	0.99
ethyl isovalerate	65 ± 3 a	60 ± 2 b	55 ± 2 c	0.98
ethyl esters of fatty acids				
ethyl butyrate	224 ± 10 a	181 ± 16 b	216 ± 17 a	-0.02
ethyl hexanoate	489 ± 24 a	485 ± 13 a	342 ± 22 b	0.95
ethyl decanoate	218 ± 19 a	181 ± 6 b	269 ± 11 c	-0.73
ethyl octanoate	480 ± 46 a	383 ± 11 b	622 ± 40 c	-0.73
cinnamic esters				
ethyl cinnamate	4.0 ± 0.2 a	4.5 ± 0.1 a	4.9 ± 0.3 b	-0.96
ethyl hydrocinnamate	1.7 ± 0.3 a	1.9 ± 0.1 a	2.6 ± 0.3 b	-0.99
miscellaneous esters				
ethyl lactate	11103 ± 1247 a	14914 ± 1156 b	11453 ± 660 a	0.11
ethyl anthranilate	1.40 ± 0.06 a	1.52 ± 0.04 b	1.39 ± 0.05 ab	0.26
methyl anthranilate	0.37 ± 0.05 a	0.26 ± 0.03 b	0.50 ± 0.03 c	0.70
diethylmalate	242 ± 16 a	259 ± 16 b	214 ± 17 c	0.76
diethyl succinate	1509 ± 26 a	1829 ± 30 b	1394 ± 35 c	0.44
acetates				
isobutyl acetate	84 ± 14	93 ± 13	80 ± 11	0.48
isoamyl acetate	93 ± 13 a	132 ± 14 b	62 ± 6 c	0.61
phenylethyl acetate	22 ± 1	26 ± 2	28 ± 4	-0.93

Table 5.6 Phenolic composition (mg/L) and tannins (mg/L epicatechin equivalents) of wines made from Pinot noir grapes at different crop yields, achieved through bunch thinning for the 2009 vintage

2009	Control 7.4 t/ha	Moderate cluster thin 6.1 t/ha	Intense cluster thin 3.5 t/ha	r coefficient
Non-flavonoids				
Benzoic acid				
gallic acid	19 ± 1 a	25 ± 1 b	28 ± 2 b	-0.93
Hydroxycinnamic acids				
trans-caftaric acid	18 ± 3 a	20 ± 4 a	30 ± 2 b	-0.99
cis-coutaric acid	0.78 ± 0.04 a	0.96 ± 0.02 b	1.24 ± 0.06 c	-0.99
trans-coutaric acid	2.9 ± 0.4 a	3.9 ± 0.7 b	4.5 ± 0.6 c	-0.99
caffeic acid	1.7 ± 0.2 a	1.8 ± 0.5 a	3.6 ± 0.2 b	-0.97
Stilbenes				
trans-resveratrol	0.8 ± 0.3 a	1.2 ± 0.1 b	1.3 ± 0.3 b	-0.87
Flavonoids				
Flavonols				
quercetin	3.3 ± 0.3 a	3.5 ± 0.4 b	5.7 ± 0.8 b	-0.96
quercetin-3-glucoside	6.5 ± 0.3 a	8.7 ± 0.6 b	8.9 ± 0.3 b	-0.78
Flavan-3-ols				
epicatechin	174 ± 5 a	183 ± 8 b	245 ± 22 c	-0.97
catechin	197 ± 13 a	246 ± 19 b	344 ± 31 c	-0.98
Anthocyanins				
delphinidin-3-glucoside	9.5 ± 0.8 a	9.8 ± 0.4 a	13.3 ± 0.6 b	-0.96
cyanidin-3-glucoside	3.2 ± 0.3 a	3.6 ± 0.2 a	4.2 ± 0.4 b	-0.99
petunidin-3-glucoside	19 ± 2 a	22 ± 3 b	26 ± 4 c	-0.99
peonidin-3-glucoside	21 ± 4 a	25 ± 3 b	37 ± 5 c	-0.99
malvidin-3-glucoside	147 ± 23 a	186 ± 14 b	206 ± 26 c	-0.93
Tannins				
total tannins	378 ± 81 a	431 ± 72 a	550 ± 99 b	-0.99

Table 5.7 Phenolic composition and tannins (mg/L epicatechin equivalents) of wines made from Pinot noir grapes at different crop yields, achieved through bunch thinning for the 2010 vintage

2010	Control 8.5 t/ha	Moderate cluster thin 6.7 t/ha	Intense cluster thin 3.2 t/ha	r coefficient
Non-flavonoids				
Benzoic acid				
gallic acid	33 ± 4 a	36 ± 5 a	46 ± 3 b	-0.98
Hydroxycinnamic acids				
trans-caftaric acid	32 ± 2 a	35 ± 3 a	41 ± 3 b	-0.99
cis-coutaric acid	1.2 ± 0.4 a	1.3 ± 0.4 a	1.7 ± 0.3 b	-0.99
trans-coutaric acid	5.6 ± 0.4 a	7.7 ± 0.4 b	9.1 ± 0.3 c	-0.95
caffeic acid	1.6 ± 0.4 a	1.7 ± 0.6 a	2.2 ± 0.6 b	-0.97
Stilbenes				
trans-resveratrol	0.6 ± 0.1 a	0.8 ± 0.2 b	1.2 ± 0.4 c	-0.99
Flavonoids				
Flavonols				
quercetin	3.6 ± 0.7 a	4.8 ± 0.2 b	6.2 ± 0.6 c	-0.99
quercetin-3-glucoside	10 ± 5 a	11 ± 4 ab	13 ± 4 b	-0.99
Flavan-3-ols				
epicatechin	147 ± 14 a	183 ± 12 b	196 ± 24 c	-0.90
catechin	200 ± 15 a	255 ± 27 b	288 ± 28 c	-0.95
Anthocyanins				
delphinidin-3-glucoside	10 ± 4 a	14 ± 6 b	17 ± 6 c	-0.95
cyanidin-3-glucoside	3.3 ± 0.6 a	3.4 ± 0.2 b	4.4 ± 0.2 b	-0.95
petunidin-3-glucoside	21 ± 4 a	24 ± 7 b	37 ± 6 c	
peonidin-3-glucoside	25 ± 5 a	27 ± 2 a	42 ± 3 b	-0.98
malvidin-3-glucoside	192 ± 18 a	215 ± 25 b	243 ± 27 c	-0.99
Tannins				
total tannins	338 ± 70 a	442 ± 91 b	572 ± 115 c	-0.99

Table 5.8 Phenolic composition and tannins (mg/L epicatechin equivalents) of wines made from Pinot noir grapes at different crop yields, achieved through bunch thinning for the 2011 vintage

2011	Control 9.3 t/ha	Moderate cluster thin 7.7 t/ha	Intense cluster thin 4.4 t/ha	r coefficient
Non-flavonoids				
Benzoic acid				
gallic acid	33 ± 2 a	39 ± 6 b	45 ± 5 c	-0.98
Hydroxycinnamic acids				
trans-caftaric acid	20 ± 6 a	37 ± 4 b	46 ± 2 c	-0.93
cis-coutaric acid	1.5 ± 0.2 a	1.6 ± 0.3 a	2.1 ± 0.4 b	-0.99
trans-coutaric acid	4.9 ± 0.3 a	7.2 ± 0.4 b	11.8 ± 0.7 c	-0.99
caffeic acid	1.4 ± 0.2 a	1.6 ± 0.3 a	2.5 ± 0.4 b	-0.99
Stilbenes				
trans-resveratrol	0.7 ± 0.2 a	1.5 ± 0.2 b	1.9 ± 0.4 c	-0.93
Flavonoids				
Flavonols				
quercetin	4.1 ± 0.5 a	5.8 ± 0.2 b	8.2 ± 0.7 c	-0.99
quercetin-3-glucoside	8.1 ± 0.2 a	10.9 ± 1.2 b	11.9 ± 1.4 c	-0.89
Flavan-3-ols				
epicatechin	119 ± 15 a	140 ± 24 b	172 ± 25 c	-0.99
catechin	266 ± 36 a	279 ± 33 b	352 ± 29 c	-0.98
Anthocyanins				
delphinidin-3-glucoside	8 ± 1 a	12 ± 3 b	15 ± 1 b	-0.94
cyanidin-3-glucoside	3.1 ± 0.5 a	4.2 ± 0.4 ab	5.1 ± 0.9 b	-0.94
petunidin-3-glucoside	16 ± 1 a	20 ± 3 b	26 ± 7 c	-0.99
peonidin-3-glucoside	29 ± 3 a	36 ± 4 b	47 ± 2 c	-0.99
malvidin-3-glucoside	157 ± 11 a	205 ± 20 b	270 ± 29 c	-0.99
Tannins				
total tannins	281 ± 72 a	408 ± 88 b	491 ± 99 b	-0.95

Table 5.9 Results from triangle test for wines made from the 2009 to 2011 harvests. The top number indicates number of tasters with a positive identification

	Positive identification		
	2009	**2010**	**2011**
control vs moderate cluster thin	5/15	6/15*	7/15*
control vs intense cluster thin	7/15**	7/15**	8/15***
moderate cluster thin vs intense cluster thin	4/15	6/15*	5/15

* significance $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$

Table 5.10 Results of paired comparison test for wines made from the 2009 to 2011 harvests. C = control, MCT = moderate cluster thin, ICT = intensive cluster thin and F = Freidman score. Significance noted using critical values and is indicated as * $\propto \leq 0.05$, ** $\propto \leq 0.01$

	2009				2010				2011			
	C	MCT	ICT	F	C	MCT	ICT	F	C	MCT	ICT	F
Herbaceous	35 ± 4	31 ± 2	25 ± 1	6.62*	38 ± 4	29 ± 3	24 ± 2	9.04*	38 ± 2	23 ± 2	29 ± 3	7.96*
Fruity	24 ± 5	34 ± 3	31 ± 2	5.56	24 ± 3	31 ± 5	35 ± 4	6.36*	22 ± 2	33 ± 3	35 ± 3	7.47*
Floral	31 ± 5	28 ± 4	31 ± 3	2.26	31 ± 7	32 ± 1	27 ± 6	4.13	28 ± 5	31 ± 5	31 ± 1	2.44
Spice	22 ± 3	32 ± 2	36 ± 5	8.67*	27 ± 1	34 ± 5	29 ± 1	3.60	23 ± 4	30 ± 3	37 ± 1	7.78*
Acidic	38 ± 2	25 ± 1	27 ± 3	7.07*	39 ± 3	27 ± 2	24 ± 2	8.58*	38 ± 3	29 ± 3	23 ± 2	8.76*
Sweet	26 ± 6	30 ± 6	34 ± 5	6.18*	27 ± 5	33 ± 4	30 ± 4	3.47	25 ± 4	31 ± 7	34 ± 8	8.31*
Bitter	29 ± 6	27 ± 2	34 ± 4	3.96	29 ± 3	32 ± 3	28 ± 3	1.56	31 ± 6	28 ± 5	32 ± 5	3.42
Astringent	28 ± 2	30 ± 4	31 ± 2	1.42	31 ± 4	27 ± 2	32 ± 6	3.24	26 ± 1	33 ± 2	31 ± 3	2.44
Body	21 ± 5	33 ± 3	36 ± 3	10.8**	27 ± 5	33 ± 3	30 ± 3	2.98	25 ± 2	29 ± 6	36 ± 6	7.69*

* significance $p \leq 0.05$, ** $p \leq 0.01$

Chapter 6

Conclusions and Future Work

Pinot noir is a difficult grape variety to cultivate and is greatly influenced by the local climate, topography of the land and soil type. More so than other red wines, the flavours, aromas and texture of Pinot noir wines are thought to be directly related to the location of where the grapes were grown and to the practices of the winemaker. The marketing success of place of origin labeling, while dependent upon the product, has been successful for the wine industry, and is often used to differentiate wines in foreign markets, act as a marker of quality and influence consumer purchasing decisions.

Pinot noir wines, despite their origin, are quite capricious and can encompass a range of flavours often depending on vintage. However, these wines are often generalised in the description of their flavours and textures, and it is often presumed that wines from the same country or region will have similar sensory properties and therefore similar volatile and non-volatile compositions. There have been numerous studies conducted on the aromas of Pinot noir wines as discussed in Chapter 2. However, while revealing substantial information about the chemical components of Pinot noir wines from many of the major Pinot noir growing regions, most of these studies considered only a few wines from one region leaving a gap of information of how the chemical components of Pinot noir wines from different regions compare using the same methodology of extraction and quantification.

6.1 Chemical Survey of Pinot noir Wines from Different Countries

The present research is the first time that a large number of Pinot noir wines from several countries were analysed using the same methods over two consecutive vintages. While this data gave a better understanding of the typical makeup of Pinot noir wines, it also showed that these wines can vary differently, not only between, but within regions. Therefore, the oversimplification when characterising these wines as a varietal or associating particular attributes belonging solely to a specific region should be avoided, or at least carefully considered.

The data collected in this study, as discussed in Chapter 3, showed that most wine samples exceeded the perception thresholds for aroma compounds belonging to several families, including C_{13} norisoprenoids, higher alcohols, esters of isoacids and fatty acids and cinnamic esters. This finding gave reason to consider the importance of these aroma compounds to the overall varietal aromas of Pinot noir wines regardless of origin. There was an observation of ethyl phenols and tannins being consistently higher in the French Pinot noir wines; however, this could be more related to winemaking or viticultural practices, as much as to the grape source.

Another consideration should be given to vintage variation. It is commonly accepted worldwide that the unique conditions of climate vary from year to year and has a major influence on fruit composition. This study showed that this occurred when evaluating the aroma compounds found in various Pinot noir wines across two vintages using Principal Component Analysis. The NZ and Australian Pinot noir wines grouped closely, while the French wines were slightly more variable in the first year, while in the second year the French and NZ wines grouped more closely while the wines from the USA were more variable. It is worth noting that the phenomenon of global climate change may intensify these variations from one growing season to the next and winegrowers must begin to have some insight on how this may affect their region's wine style and typicality.

Some compounds were not available to our lab during this part of the study but were obtained later. There have also been advances in extraction methods that would be beneficial for quantifying trace compounds that were undetectable with previous methods. Hence, further chemical analyses of additional Pinot noir wines from a range of regions using these advanced methods of extraction, and perhaps focus on quantifying specific potentially important compounds, would be beneficial in future research. The inclusion of non-volatile constituents would also be of useful, as it is now accepted that these components have binding interactions within the wine matrix, as discussed in Chapter 4. These wines should undergo sensory analyses in parallel with the chemical data to ensure that any similarities or differences have a perceivable effect on the final product.

6.2 Characterising the Key Aroma Compounds in Central Otago Pinot noir wines

Like many other red wines, with exception to Syrah, there have been no genuine impact compounds identified in Pinot noir thus far, only suggestions as to possible key odorants. Moio and Etievant (1995) suggested that ethyl anthranilate, methyl anthranilate, ethyl cinnamate and 2,3-diydrocinnamte were important odorants in Burgundy Pinot noir. Later, using GC-O, Fang and Qian (2005), suggested that ethyl butanoate, ethyl isobutyrate, ethyl hexanoate, and ethyl decanoate were potential key odorants for Oregon Pinot noir as these volatiles held the highest FD values in neutral fractions when AEDA was applied. However, there were no sensory studies to determine or confirm the role or importance of any of these prospective odorants in the Burgundy study and no sensory reconstitution studies to verify their contribution in the Oregon study. Before this research was undertaken, no studies had been conducted to characterise Pinot noir in a comprehensive manner that included both chemical and sensory evaluations using aroma recombination studies (Chapter 4).

This holistic chemical and sensorial approach, paired with the tactic of analysing two diverse Pinot noir wines that were made with grapes from various vineyards and reflected different winemaking styles, time in barrel and price points, provided for a more complete and thorough examination for revealing key aromatic compounds in Central Otago Pinot noir. The composition results showed that aroma compounds and/or compound families of importance varied across the wines, while a number of compounds were in common. These differences were thought to be caused by viticultural management, timing of harvest, degree of ripeness, winemaking practices and barrel selection and storage time, which all play a role in the region's typicality and perceived wine style. While some grape varieties have particularly high concentrations of compounds, such as 3MH in Sauvignon blanc, that can prevail over these influences, Central Otago Pinot noir does not. The aroma reconstitution experiments revealed no overwhelming differences when compound families were omitted, and suggested that these wines do not depend on a few key odorants for their aromatic complexity, but rather on the interactions of many aromatic compounds. Further GC-O studies could possibly reveal unidentified compounds which may have a genuine impact on the overall aroma.

The method used for the deodorisation of the Pinot noir wines was very successful and the use of the original Pinot noir wine matrix for the recombinant evaluations was novel for this study. Future research should focus on the compounds determined in this study to be of greater importance to the overall aroma of Pinot noir wines through additional aroma reconstitution analysis. Manipulation of the ratios of these compounds and/or additions tests, where compounds or compound families are added above original concentrations to the model, could reveal further interactions in the matrix that may affect the overall aroma in a profound way. Future work could include the analysis of more Pinot noir wines than this study as the extensive time consuming AEDA analysis could be bypassed.

6.3 Effects of Cluster Thinning on the Aromatic and Phenolic Compounds of Pinot noir Wines

Understanding how these important volatiles can be altered in the grape composition and ultimately in the wine is essential. It is widely accepted that viticultural practices can alter the grapes' constituents and hence play a key role in overall wine quality. A common practice that is thought to increase quality is to reduce yield through cluster thinning. This research is the first of its kind to conduct field trials where cluster thinning was performed at a rate that represented the common practices of the Central Otago region. It was also conducted at a level where the wines could be made at a commercial scale. Working on this larger scale presented its own difficulties, but was important, given a previous MSc project discussed in Chapter 1, which revealed that micro and/or macro scale ferments are not consistent in the concentrations of compounds compared to ferments undertaken on a commercial scale. The current study was also the first to show what effects cluster thinning may have on volatile compounds in Pinot noir.

With much diligence, the field trials in the vineyard and in the winery were successful across all three seasons. Many of the results were not unexpected, as discussed in Chapter 5, as some of the same trends have been observed in other varieties. The vines exposed to intense cluster thinning reached the target °Brix for harvesting earlier than the moderately thinned treatment, and both treatments reached target earlier than the control. Consistent increases in pH, bunch weights and phenolics, and decreases in TAs, were observed when more fruit was removed as cluster thinning treatments increased. What was of more interest were the significant differences when comparing the chemical composition of each treatment and the close relationship observed across all three seasons between cluster thinning and the concentrations of C_{13} norisoprenoids, monoterpenes, fatty acids, cinnamic esters, ethyl isobutyrate, ethyl hexanoate, β-phenylethyl alcohol. As discussed in Chapter 4, the compounds listed above, with exception to monoterpenes, were considered important compounds

in the overall aroma when considering flavourdilution and odour activity values in Central Otago Pinot noir wines.

Significant differences were observed for the wine sensory properties, and wines made from thinning treatments were found to be less herbaceous and acidic than the control with no thinning. The wines made from thinning treatments were also perceived as being fruitier, spicy, sweeter and having more body when compared to the control wines. However, no consistent trends were observed between the two treatments. Overall, it was concluded that cluster thinning can affect the sensory properties of Pinot nor wines and most likely in a positive way. It would seem that crop thinning, while a costly practice due to increased labor and yield reduction, is effective in influencing wine quality. However, a particularly intense level of thinning was not necessary to achieve the differences in the wines, as a moderate level of thinning also provided a marked enhancement to several attributes. Mt. Difficulty found the results very satisfactory and have since changed their thinning regimes across their vineyards. According to their viticulturist and winemaker, this change has significantly reduced their labor costs while increasing yield to heighten production without any loss of quality.

Future studies evaluating the effects of different timings of crop thinning (ie. at flowering, before veraison, after veraison), as well as the effects on additional non-volatile compounds would enhance the knowledge gained from this study. Further sensory studies of Pinot noir wines made from differing crop levels using descriptive analysis would be beneficial in forecasting and planning for varying wine styles resulting from vintage differences, viticultural management and/or unexpected climate events.

6.4 Summary

In summary, as consumers become more educated about wine, their expectations and standards of quality will increase. Consumers today are becoming more interested and aware of the origin of their food and beverages. Ultimately, it is the wine industry's responsibility to increase their knowledge

on how to make the best product and deliver this to the consumer in a way that benefits them directly. Associating a particular region with consistent high quality wine is one way to achieve this objective. This research is a starting point and provides a foundation for the winegrowers of Central Otago to continue in their duty to reach this aspiration.

References

1. Maharaj, S., *That temperamental grape - research scale winemaking of New Zealand Pinot noir* in *Chemistry-Wine Science*. 2008, University of Auckland: Auckland.
2. Rutan, T., *Chemical and Sensory Profiles of Research Wines from New Zealand Pinot noir Regions*, in *Chemical Sciences*. 2009, University of Auckland.
3. Newman, J.D. and J. Chappell, Isoprenoid biosynthesis in plants: carbon partitioning within the cytoplasmic pathway. Crit Rev Biochem Mol Biol, 1999. **34**(2): p. 95-106.
4. Rohmer, M., The discovery of of a mevalonate-independent pathwayfor isoprenoid biosynthesis in bacteria, algae and higher plants. Nat. Prod. Rep., 1999. **16**: p. 565-574.
5. Maicas, S. and J.J. Mateo, Hydrolysis of terpenyl glycosides in grape juice and other fruit juices: a review. Applied Microbiology and Biotechnology, 2005. **67**(3): p. 322-335.
6. Rocha, S.M., E. Coelho, J. Zrostlíková, I. Delgadillo and M.A. Coimbra, Comprehensive two-dimensional gas chromatography with time-of-flight mass spectrometry of monoterpenoids as a powerful tool for grape origin traceability. Journal of Chromatography A, 2007. **1161**(1–2): p. 292-299.
7. Brander, C.F., R.E. Kepner and A.D. Webb, Identification of some volatile compounds of wine of Vitis vinifera cultivar Pinot noir. Am. J. Enol. Vitic., 1980. **31**: p. 69.
8. Schreier, P., F. Drawert and K.O. Abraham, Identification and determination of volatile constituents in Burgundy Pinot noir wines. Lebensm.-Wiss. Technol., 1980. **13**: p. 318.
9. Miranda-Lopez, R., L.M. Libbey, B.T. Watson and M.R. McDaniel, Identification of additional odor-active compounds in Pinot noir wines. Am. J. Enol. Vitic., 1992. **43**: p. 90.
10. Escudero, A., E. Campo, L. Farina, J. Cacho and V. Ferreira, Analytical characterisationof the aroma of five premium red wines. Insights into the role of odourfamilies and the concept of fruitiness of wines. J. Agric. Food Chem., 2007. **55**(11): p. 4501-4510.
11. Ribéreau-Gayon, P., D. Dubourdieu and B. Donèche, *Handbook of enology*. 2nd ed. 2006, Chichester, West Sussex, England ; Hoboken, NJ: John Wiley. v.
12. Pigella, R., A. Bosso, R. Di Stefano, L. Corino and G. Malerba, Pinot noir varietal characterisationby polyphenols and aroma precursors study. Rivista di Viticoltura e di Enologia, 1998. **51**(1): p. 45-62.
13. Bonino, M., R. Schellino, C. Rizzi, R. Aigotti, C. Delfini and C. Baiocchi, Aroma compounds of an Italian wine (Ruché) by HS-SPME analysis coupled with GC-ITMS. Food Chem., 2003. **80**(1): p. 125-133.
14. Girard, B., D. Yuksel, M.A. Cliff, P. Delaquis and A.G. Reynolds, Vinification effects on the sensory, colour and GC profiles of Pinot noir wines from British Columbia. Food Res. Int., 2001. **34**(6): p. 483-499.
15. Tomasino, E., R. Harrison, J. Breitmeyer, R. Sedcole, R. Sherlock and A. Frost, Aroma composition of 2-year-old New Zealand Pinot Noir wine and its relationship to sensory characteristics using canonical correlation analysis and addition/omission tests. Aust. J. Grape Wine R., 2015. **21**(3): p. 376-388.
16. Song, J., R.E. Smart, R.G. Dambergs, A.M. Sparrow, R.B. Wells, H. Wang and M.C. Qian, Pinot Noir wine composition from different vine vigour zones classified by remote imaging technology. Food Chem., 2014. **153**: p. 52-59.
17. Feng, H., F. Yuan, P.A. Skinkis and M.C. Qian, Influence of cluster zone leaf removal on Pinot noir grape chemical and volatile composition. Food Chem., 2015. **173**: p. 414-423.
18. Fang, Y. and M. Qian, Aroma compounds in Oregon Pinot Noir wine determined by aroma extract dilution analysis (AEDA). Flavour Fragr. J., 2005. **20**: p. 22.
19. Fang, Y. and M.C. Qian, Quantification of selected aroma-active compounds in Pinot noir wines from different grape maturities. J. Agric. Food Chem., 2006. **54**(22): p. 8567-8573.
20. Hernández, L.F., J.C. Espinosa, M. Fernández-González and A. Briones, [beta]-Glucosidase activity in a Saccharomyces cerevisiae wine strain. Int. J. Food Microbiol., 2003. **80**(2): p. 171-176.

21. Jackson, R.S., *Wine science : principles, practice, perception*. 2nd ed. 2000, San Diego: Academic Press. xv, 648.
22. Moreno, J.J., F. Cerpa-Calderón, S.D. Cohen, Y. Fang, M. Qian and J.A. Kennedy, Effect of postharvest dehydration on the composition of pinot noir grapes (Vitis vinifera L.) and wine. Food Chem., 2008. **109**(4): p. 755-762.
23. Hall, H., *Impact of yeast present during pre-fermentation cold maceration on Pinot noir wine aroma*, in *Enology*. 2012, Oregon State University.
24. Yuan, F. and M.C. Qian, Development of C13-norisoprenoids, carotenoids and other volatile compounds in Vitis vinifera L. Cv. Pinot noir grapes. Food Chem., 2015. **192**: p. 633-641.
25. Pineau, B., J.C. Barbe, C. Van Leeuwen and D. Dubourdieu, Which impact for beta-damascenone on red wines aroma? J. Agric. Food. Chem., 2007. **55**(10): p. 4103-4108.
26. Fischer, U., *Mass balance of aroma compounds during the dealcoholization of wine: Correlation of chemical and sensory data*. 1995, University of Hanover: Hanover.
27. Kotseridis, Y., R.L. Baumes, A. Bertrand and G.K. Skouroumounis, Quantitative determination of [beta]-ionone in red wines and grapes of Bordeaux using a stable isotope dilution assay. J. Chromatogr. A, 1999b. **848**(1-2): p. 317-325.
28. Ferreira, V., R. Lopez and J.F. Cacho, Quantitative determination of the odorants of young red wines from different grape varieties. J. Sci. Food Agric., 2000. **80**: p. 1659.
29. Tominaga, T., C. Peyrot des Gachons and D. Dubourdieu, A new type of flavourprecursors in *Vitis vinifera* L. cv. Sauvignon blanc: s-cysteine conjugates. J Agric Food Chem., 1998. **46**: p. 5215-5219.
30. Tominaga, T., I. Masneuf and D. Dubourdieu, A S-cysteine conjugate, precursor of aroma of white Sauvignon. Journal International des Sciences de la Vigne et du Vin 1995. **29**(4): p. 227-232.
31. Schneider, R., F. Charrier, A. Razungles and R. Baumes, Evidence for an alternative biogenetic pathway leading to 3-mercaptohexanol and 4-mercapto-4-methylpentan-2-one in wines. Anal. Chim. Acta., 2006. **563**(1-2): p. 58-64.
32. Peyrot Des Gachons, C., T. Tominaga and D. Dubourdieu, Sulfur aroma present in S-glutathione conjugate form: Identification of S-3-(Hexan-1-ol)-glutathione in must from *Vitis vinifera* L. cv. Sauvignon blanc. J. Agric. Food Chem., 2002. **50**: p. 4076-4079.
33. Harsh, M., F. Benkwitz, A. Frost, B. Colonna-Ceccaldi, R.C. Gardner and J. Salmon, New precursor of 3-mercaptohexan-1-ol in grape juice: thiol forming potential and kinetics during early stages of must fermentation. J. Agric. Food Chem., 2013. **61**: p. 3703-3713.
34. Subileau, M., R.m. Schneider, J.-M. Salmon and E. Degryse, New Insights on 3-Mercaptohexanol (3MH) Biogenesis in Sauvignon Blanc Wines: Cys-3MH and (E)-Hexen-2-al Are Not the Major Precursors. J. Agr. Food Chem., 2008. **56**(19): p. 9230-9235.
35. Swiegers, J. and I. Pretorius, Modulation of volatile sulfur compounds by wine yeast. Applied Microbiology and Biotechnology, 2007. **74**(5): p. 954-960.
36. Roland, A., R. Schneider, F. Charrier, F. Cavelier, M. Rossignol and A. Razungles, Distribution of varietal thiol precursors in the skin and the pulp of Melon B. and Sauvignon Blanc grapes. Food Chem., 2011. **125**(1): p. 139-144.
37. Capone, D.L. and D.W. Jeffery, Effects of Transporting and Processing Sauvignon blanc Grapes on 3-Mercaptohexan-1-ol Precursor Concentrations. J. Agr. Food Chem., 2011. **59**(9): p. 4659-4667.
38. Darriet, P., T. Tominaga, E. Demole and D. Dubourdieu, Evidence of the presence of a 4-mercapto-4-methylpentan-2-one precursor in Vitis vinifera Sauvignon blanc grape variety C.R. Acad. Sci. Series III - VIE, 1993. **316**(11): p. 1332.1335.
39. Tominaga, T.D., P.; Dubourdieu, D., Identification of 3-mercaptohexyl acetate in Sauvignon wine, a powerful aromatic compound exhibiting box-tree odor. Vitis, 1996. **35**(4): p. 207-210.
40. Tominaga, T., A. Furrer, R. Henry and D. Dubourdieu, Identification of new volatile thiols in the aroma of Vitis vinifera L. var. Sauvignon blanc wines. Flavour Frag. J., 1998. **13**(3): p. 159-162.

41. Benkwitz, F., T. Tominaga, P.A. Kilmartin, C. Lund, M. Wohlers and L. Nicolau, Identifying the chemical composition related to the distinct aroma characteristics of New Zealand Sauvignon blanc wines. Am. J. Enol. Vitic., 2012. **63**(1): p. 62-72.
42. Bouchilloux, P., P. Darriet, R. Henry, V. Lavigne-Cruege and D. Dubourdieu, Identification of volatile and powerful odorous thiols in Bordeaux red wine varieties. J. Agr. Food Chem., 1998. **46**(8): p. 3095-3099.
43. Tominaga, T., R. Baltenweck-Guyot, C.P. Gachons and D. Dubourdieu, Contribution of Volatile Thiols to the Aromas of White Wines Made From Several Vitis vinifera Grape Varieties. Am. J. Enol. Vitic., 2000. **51**(2): p. 178-181.
44. Benkwitz, F., L. Nicolau, C. Lund, M. Beresford, M. Wohlers and P.A. Kilmartin, Evaluation of Key Odorants in Sauvignon Blanc Wines Using Three Different Methodologies. J. Agr. Food Chem., 2012. **60**(25): p. 6293-6302.
45. Blanchard, L., P. Darriet and D. Dubourdieu, Reactivity of 3-Mercaptohexanol in Red Wine: Impact of Oxygen, Phenolic Fractions, and Sulfur Dioxide. Am. J. Enol. Vitic., 2004. **55**(2): p. 115-120.
46. Murat, M.L., T. Tominaga and D. Dubourdieu, Impact of some components on Bordeaux roses and clairets aromaMise en évidence de composés clefs dans l'arôme des vins rosés et clairets de Bordeaux International Journal of Vine and Wine Sciences, 2001. **35**: p. 99-105.
47. Ferreira, V., N. Ortin, A. Escudero, R. Lopez and J. Cacho, Chemical characterisationof the aroma of Grenache rosé wines: aroma extract dilution analysis, quantitative determination, and sensory reconstitution studies. J Agric Food Chem., 2002. **50**: p. 4048-4054.
48. Franson, P., Sulfur compounds in winemaking. A report from the Vinquiry seminar on the role of sulfur compounds in wine. Vineyard and Winery Management; USA, 2004. **30**(2): p. 43-47.
49. Benkwitz, F. and L. Nicolau, Characterising the aroma of New Zealand Sauvignon blanc. Australia and New Zealand Grapegrower and Winemaker, 2006. **509**: p. 46-49.
50. Crouzet, J., M. Nicolas, I. Molina and J. Valentin, *Enzymes occurlng In the formation of sixcarbon aldehydes and alcohols in grapes*, in *Progesss in Flavour Research*, J. Adda, Editor. 1984, Elsevier Science: Amsterdam. p. 401-408.
51. Joslin, W.S. and C.S. Ough, Cause and Fate of Certain C6 Compounds Formed Enzymatically in Macerated Grape Leaves During Harvest and Wine Fermentation. Am. J. Enol. Vitic., 1978. **29**(1): p. 11-17.
52. Ramey, D., A. Bertrand, C.S. Ough, V.L. Singleton and E. Sanders, Effects of Skin Contact Temperature on Chardonnay Must and Wine Composition. Am. J. Enol. Vitic., 1986. **37**(2): p. 99-106.
53. Killian, E. and C.S. Ough, Fermentation Esters -- Formation and Retention as Affected by Fermentation Temperature. Am. J. Enol. Vitic., 1979. **30**(4): p. 301-305.
54. Meunier, J.M. and E.W. Bott, Behaviour of different volatile constituents of Burgundy wines during malolactic fermentation. Chemie, Mikrobiologie, Technologie der Lebensmittel, 1979(6): p. 92-95.
55. Kwan, W.-O. and B.R. Kowalski, Pattern recognition analysis of gas chromatographic data. Geographic classification of wines of Vitis vinifera cv Pinot Noir from France and the United States. J. Agr. Food Chem., 1980. **28**(2): p. 356-359.
56. Kwan, W.-O. and B.R. Kowalski, Correlation of objective chemical measurements and subjective sensory evaluations. wines of vitis vinifera variety 'pinot noir' from france and the united states. Anal. Chim. Acta., 1980. **122**(2): p. 215-222.
57. Massoutier, C., H. Alexandre, M. Feuillat and C. Charpentier, Isolation and characterisationof cryotolerant Saccharomyces strains Vitis, 1998. **37**(1): p. 55-59.
58. Girard, B., T.G. Kopp, A.G. Reynolds and M. Cliff, Influence of vinification treatments on aroma constituents and sensory descriptors of Pinot noir wines. Am. J. Enol. Vitic., 1997. **48**(2): p. 198-206.

59. Fischer, U., M. Strasser and K. Gutzler, Impact of fermentation technology on the phenolic and volatile composition of German red wines. International Journal of Food Science & Technology, 2000. **35**(1): p. 81-94.
60. Aubry, V., P.X. Etievant, C. Ginies and R. Henry, Quantitative determination of potent flavourcompounds in Burgundy Pinot noir wines using a stable isotope dilution assay. J. Agric. Food Chem., 1997. **45**: p. 2120.
61. Moio, L. and P.X. Etievant, Ethyl anthranilate, ethyl cinnamate, 2,3-dihydrocinnamate, and methyl anthranilate: four important odorants identified in Pinot noir wines of Burgundy. Am. J. Enol. Vitic., 1995. **46**: p. 392.
62. Holley, R., B. Stoyla and D. Holley, The identification of some volatile constituents of concord grape juice Journal of Food Science, 1955. **20**(4): p. 326-331.
63. Stevens, K.L., W.H. McFadden and R. Teranishi, Volatiles from Grapes I. Some Volatiles from Concord Essence. Journal of Food Science, 1965. **30**(6): p. 1006-1007.
64. Swiegers, J.H., E.J. Bartowsky, P.A. Henschke and I.S. Pretorius, Yeast and bacterial modulation of wine aroma and flavour. Aust. J. Grape Wine R., 2005. **11**(2): p. 139-173.
65. Bell, S.-J. and P.A. Henschke, Implications of nitrogen nutrition for grapes, fermentation and wine. Aust. J. Grape Wine R., 2005. **11**(3): p. 242-295.
66. Francis, I.L. and J.L. Newton, Determining wine aroma from compositional data. Aus.t J. Grape Wine R., 2005. **11**(2): p. 114-126.
67. Rossouw, D., T. Næs and F.F. Bauer, Linking gene regulation and the exo-metabolome: A comparative transcriptomics approach to identify genes that impact on the production of volatile aroma compounds in yeast. BMC Genomics, 2008. **9**(1): p. 1-10.
68. Rossouw, D., R. Olivares-Hernandes, J. Nielsen and F.F. Bauer, Comparative Transcriptomic Approach To Investigate Differences in Wine Yeast Physiology and Metabolism during Fermentation. Appl. Environ. Microbiol., 2009. **75**(20): p. 6600-6612.
69. Maigre, D., Behaviour of Pinot noir with permanent grass cover and influence of nitrogen fertilization. 2. Analytic and sensorial results. Revue suisse de viticulture, arboriculture, horticulture, 2002. **34**(4): p. 239-244.
70. Miranda-Lopez, R., L.M. Libbey, B.T. Watson and M.R. McDaniel, Odouranalysis of Pinot noir wines from grapes of different maturities by a gas chromatography-olfactometry technique (Osme). J. Food Sci., 1992. **57**: p. 985.
71. Fischer, U., *Wine Aroma*, in *Flavours and Fragrances*. 2007. p. 241-267.
72. Berry, D.R. and D.C. Watson, *Production of organoleptic compounds*, in *Yeast Biotechnology*, D.R. Berry, I. Russell, and G.G. Stewart, Editors. 1987, Allen and Unwin: London. p. 345-368.
73. Romano, A., M.C. Perello, A. Lonvaud-Funel, G. Sicard and G. de Revel, Sensory and analytical re-evaluation of "Brett character". Food Chem., 2009. **114**(1): p. 15-19.
74. Edwards, C.G., R.B. Beelman, C.E. Bartley and A.L. McConnell, Production of decanoic acid and other volatile compounds and the growth of yeast and malolactic bacteria during vinification. Am. J. Eno.l Viticult., 1990. **41**(1): p. 48-56.
75. Bardi, L., C. Cocito and M. Marzona, Saccharomyces cerevisiae cell fatty acid composition and release during fermentation without aeration and in absence of exogenous lipids. Int. J. Food Microbiol., 1999. **47**(1): p. 133-140.
76. Bisson, L.F., Stuck and Sluggish Fermentations. Am. J. Enol. Viticult., 1999. **50**(1): p. 107-119.
77. McDaniel, M.R., R. Miranda-Lopez, B.T. Watson, N.J. Micheals and L.M. Libbey, *Pinot noir aroma: a sensory/gas chromatographic approach*, in *Flavours and Off-flavours*, G. Charalambous, Editor. 1990, Elsevier Science: Amsterdam. p. 23-35.
78. Blackmore, S.J., *Composition of volatiles of pinot noir wine as affected by differing levels of DAP supplementation*, in *Oenology*. 2014, Lincoln University.
79. Mason, A.B. and J.-P. Dufour, Alcohol acetyltransferases and the significance of ester synthesis in yeast. Yeast, 2000. **16**(14): p. 1287-1298.
80. Daudt, C.E. and C.S. Ough, Variations in Some Volatile Acetate Esters Formed during Grape Juice Fermentation. Effects of Fermentation Temperature, SO2, Yeast Strain, and Grape Variety. Am. J. Enol. Vitic., 1973. **24**(3): p. 130-135.

81. Saerens, S.M.G., F.R. Delvaux, K.J. Verstrepen and J.M. Thevelein, Production and biological function of volatile esters in Saccharomyces cerevisiae. Microbiol. Biotech., 2010. **3**(2): p. 165-177.
82. Saerens, S.M.G., F. Delvaux, K.J. Verstrepen, P. Van Dijck, J.M. Thevelein and F.R. Delvaux, Parameters Affecting Ethyl Ester Production by Saccharomyces cerevisiae during Fermentation. Appl. Environ. Microbiol., 2008. **74**(2): p. 454-461.
83. Vestrepen, K.J., S.D. Laere, B.M. Vanderhaegen, G. Derdelinckx, J.P. Dufour, I.S. Pretorius, J. Winderickx, J.M. Thevelein and F.R. Delvaux, Expression levels of the yeast alcohol acetyltransferase genes ATF1, Lg-ATF1, and ATF2 control the formation of a broad range of volatile esters. Appl Environ Microbiol, 2003. **69**.
84. Peinado, R.A., J. Moreno, J.E. Bueno, J.A. Moreno and J.C. Mauricio, Comparative study of aromatic compounds in two young white wines subjected to pre-fermentative cryomaceration. Food Chem., 2004. **84**(4): p. 585-590.
85. Chatonnet, P., C. Viala and D. Dubourdieu, Influence of Polyphenolic Components of Red Wines on the Microbial Synthesis of Volatile Phenols. Am. J. Enol. Vitic., 1997. **48**: p. 443.
86. Kennison, K.R., M.R. Gibberd, A.P. Pollnitz and K.L. Wilkinson, Smoke-Derived Taint in Wine: The Release of Smoke-Derived Volatile Phenols during Fermentation of Merlot Juice following Grapevine Exposure to Smoke. J. Agr. Food Chem., 2008. **56**(16): p. 7379-7383.
87. Spillman, P.J., M.A. Sefton and R. Gawel, The contribution of volatile compounds derived during oak barrel maturation to the aroma of a Chardonnay and Cabernet Sauvignon wine. Aust. J. Grape Wine R., 2004. **10**(3): p. 227-235.
88. Chatonnet, P., The aromatic compounds of oakwood liberated into the wine. Origin and processing of oak used in cooperage. Influence of toasting process in cooperage. J. Int. Sci. Vigne Vin, 1992: p. 81-91.
89. Chatonnet, P., D. Dubourdieu and J.B. Lavigne, Synthesis of volatile phenols by Saccharomyces cerevisiae in wines. J. Sci. Food Agr., 1993. **62**(2): p. 191-202.
90. Fugelsang, K.C. and B.W. Zoecklein, Population Dynamics and Effects of Brettanomyces bruxellensis Strains on Pinot noir (Vitis vinifera L .) Wines. Am. J. Enol. Vitic., 2003. **54**(4): p. 294-300.
91. Chira, K. and P.-L. Teissedre, Extraction of oak volatiles and ellagitannins compounds and sensory profile of wine aged with French winewoods subjected to different toasting methods: Behaviour during storage. Food Chem., 2013. **140**(1–2): p. 168-177.
92. Abbott, N., J.-L. Puech, C. Bayonove and R. Baumes, Determination of the Aroma Threshold of the cis and trans Racemic Forms of β-Methyl-γ-Octalactone by Gas Chromatography-Sniffing Analysis. Am. J. Enol. Vitic., 1995. **46**(3): p. 292-294.
93. Reazin, G.H., Chemical Mechanisms of Whiskey Maturation. Am. J. Enol. Viticult., 1981. **32**(4): p. 283-289.
94. Waterhouse, A.L. and J.P. Towey, Oak Lactone Isomer Ratio Distinguishes between Wine Fermented in American and French Oak Barrels. J. Agr. Food Chem., 1994. **42**(9): p. 1971-1974.
95. Alañón, M.E., L. Castro-Vázquez, M.C. Díaz-Maroto, M.H. Gordon and M.S. Pérez-Coello, A study of the antioxidant capacity of oak wood used in wine ageing and the correlation with polyphenol composition. Food Chem., 2011. **128**(4): p. 997-1002.
96. Towey, J.P. and A.L. Waterhouse, The Extraction of Volatile Compounds From French and American Oak Barrels in Chardonnay During Three Successive Vintages. Am. J. Enol. Viticult., 1996. **47**(2): p. 163-172.
97. Guillén, M.a.D. and M.a.J. Manzanos, Study of the volatile composition of an aqueous oak smoke preparation. Food Chem., 2002. **79**(3): p. 283-292.
98. Prida, A. and P. Chatonnet, Impact of Oak-Derived Compounds on the Olfactory Perception of Barrel-Aged Wines. Am. J. Enol. Viticult., 2010. **61**(3): p. 408-413.
99. Chatonnet, P., I. Cutzach, M. Pons and D. Dubourdieu, Monitoring Toasting Intensity of Barrels by Chromatographic Analysis of Volatile Compounds from Toasted Oak Wood. J. Agr. Food Chem., 1999. **47**(10): p. 4310-4318.

100. Sauvageot, F. and F. Feuillat, The Influence of Oak Wood (Quercus robur L., Q. petraea Liebl.) on the Flavourof Burgundy Pinot noir. An Examination of Variation Among Individual Trees. Am. J. Enol. Viticult., 1999. **50**(4): p. 447-455.
101. Rapp, A. and H. Mandery, Wine aroma. Cellular and Molecular Life Sciences (CMLS), 1986. **42**(8): p. 873-884.
102. Stone, H. and J.L. Sidel, *Sensory Evaluation Practices*. Third ed. Food Science and Technology International Series, ed. S. Taylor. 2004, Amsterdam: Elsevier Academic Press.
103. Watson, M., Sensory Characteristics of Food. Nutrition & Food Science, 1992. **92**(4): p. 4-6.
104. Stone, H., J.L. Sidel, A. Woolsey, S. Oliver and R.C. Singleton, Sensory evaluation by quantitative descriptive analysis. Food Technology, 1974. **28**(11): p. 24-34.
105. Murray, J.M., C.M. Delahunty and I.A. Baxter, Descriptive sensory analysis: past, present and future. Food Res. Int., 2001. **34**(6): p. 461-471.
106. Lawless, H.T. and H. Heymann, *Sensory evaluation of food: principals and practice*. 1998, New York: Chapman and Hall.
107. Lesschaeve, I. and S. Issanchou, Could selection tests detect the future performance of descriptive panellists? Food Qual. Prefer., 1996. **7**(3-4): p. 177-183.
108. Piggott, J.R., S.J. Simpson and S. Williams, Sensory analysis. International Journal of Food Science & Technology, 1998. **33**(1): p. 7-12.
109. Cliff, M.A. and M.C. Dever, Sensory and compositional profiles of British Columbia Chardonnay and Pinot noir wines. Food Res. Int., 1996. **29**(3-4): p. 317-323.
110. Kotseridis, Y. and R. Baumes, Identification of impact odorants in Bordeaux red grape juice, in the commercial yeast used for its fermentation, and in the produced wine. J. Agric. Food Chem., 2000. **48**(2): p. 400-406.
111. Falqué, E., A.C. Ferreira, T. Hogg and P. Guedes-Pinho, Determination of aromatic descriptors of Touriga Nacional wines by sensory descriptive analysis. Flavour Frag. J., 2004. **19**(4): p. 298-302.
112. Guinard, J.-X. and M. Cliff, Descriptive Analysis of Pinot noir Wines from Carneros, Napa, and Sonoma. Am. J. Enol. Vitic., 1987. **38**(3): p. 211-215.
113. Vilanova, M., S. Zamuz, J. Taradaguila and A. Masa, Descriptive analysis of wines from Vitis vinifera cv. Albariño. Journal of the Science of Food and Agriculture, 2008(88): p. 819-823.
114. Munoz, A.M. and G.V. Civille, Universal, product and attribute specific scaling and the development of common lexicons in descriptive analysis. J. Sens. Stud., 1998. **13**(1): p. 57-75.
115. Noble, A.C., R.A. Arnold, J. Buechsenstein, E.J. Leach, J.O. Schmidt and P.M. Stern, Modification of a Standardized System of Wine Aroma Terminology. Am. J. Enol. Vitic., 1987. **38**(2): p. 143-146.
116. Rainey, B., Importance of reference standards in training panelists. J. Sens. Stud., 1986. **1**(2): p. 149-154.
117. Mcdaniel, M., L.A. Henderson, B.T. Watson Jr and D. Heatherbell, Sensory panel training and screening for descriptive analysis of the aroma of pinot noir fermented by several strains of malolactic bacteria. J. Sens. Stud., 1987. **2**(3): p. 149-167.
118. Parr, W.V., J.A. Green, K.G. White and R.R. Sherlock, The distinctive flavour of New Zealand Sauvignon blanc: Sensory characterisation by wine professionals. Food Qual. Prefer., 2007. **18**(6): p. 849-861.
119. Parr, W.V., D. Valentin, J.A. Green and C. Dacremont, Evaluation of French and New Zealand Sauvignon wines by experienced French wine assessors. Food Qual. Prefer., 2010. **21**(1): p. 56-64.
120. Meilgaard, M., G.V. Civille and B.T. Carr, *Sensory evaluation techniques*. 4th ed. Overall difference tests: does a sensory differnce exist between samples. 2007, Boca Raton: CRC. 448.
121. Lawless, H.T. and H. Heymann, *Sensory Evaluation of Food. Principles and Practices*. Second ed. 2010, New York: Chapman & Hall. 850 p.
122. Ross, C.F. and K. Weller, Effect of serving temperature on the sensory attributes of red and white wines. J. Sens. Stud., 2008. **23**(3): p. 398-416.

123. Tourbier, I.A. and R.L. Doty, Sniff Magnitude Test: Relationship to OdourIdentification, Detection, and Memory Tests in a Clinic Population. Chem. Senses, 2007. **32**(6): p. 515-523.
124. Seubert, J., A.F. Rea, J. Loughead and U. Habel, Mood Induction with Olfactory Stimuli Reveals Differential Affective Responses in Males and Females. Chem. Senses, 2009. **34**(1): p. 77-84.
125. Oberg, C., M. Larrson and L. Backman, Differential sex effects in olfactory functioning: The role of verbal processing. J Int Neuropsychol Soc, 2002. **8**: p. 691-698.
126. Reynolds, A.G., S. Yerle, B. Watson, S.F. Price and D.A. Wardle, Fruit environment and crop level effects on Pinot noir. III. Composition and descriptive analysis of Oregon and British Columbia wines. Am. J. Enol. Vitic., 1996. **47**(3): p. 329-339.
127. Reynolds, A.G., M. Cliff, D.A. Wardle and M. King, Evaluation of winegrapes in British columbis: chardonnay and pinot noir clones. Horttechnology, 2004. **14**(4): p. 594-602
128. Sauvageot, F. and P. Vivier, Effects of Malolactic Fermentation on Sensory Properties of Four Burgundy Wines. Am. J. Enol. Vitic., 1997. **48**(2): p. 187-192.
129. Ledderhof, D., A.G. Reynolds, L. Manin and R. Brown, Influence of water status on sensory profiles of Ontario Pinot noir wines. LWT - Food Sci. Techno., 2014. **57**(1): p. 65-82.
130. Takush, D.G. and J.P. Osborne, Impact of yeast on the aroma and flavour of Oregon Pinot Noir wine. Aust. J. Grape Wine R., 2012. **18**(2): p. 131-137.
131. Ferreira, V. and J. Cacho, *Identification of Impact Odorants of Wines*, in *Wine Chemistry and Biochemistry*, M. Moreno-Arribas and M.C. Polo, Editors. 2009, Springer New York. p. 393-415.
132. Ferreira, V., *Volatile aroma compounds and wine sensory attributes.*, in *In Managing Wine Quality*, A.G. Reynolds, Editor. 2010, Woodhead Publishing Ltd.: Cambridge, England. p. pp. 3-28.
133. Delahunty, C., Gas chromatography-olfactory J. Sep. Sci., 2006. **29**: p. 2107-2125.
134. Pollien, P., A. Ott, F. Montigon, M. Baumgartner, R. Muñoz-Box and A. Chaintreau, Hyphenated Headspace-Gas Chromatography-Sniffing Technique: Screening of Impact Odorants and Quantitative Aromagram Comparisons. J. Agr. Food Chem., 1997. **45**(7): p. 2630-2637.
135. van Ruth, S.M. and C.H. O'Connor, Evaluation of three gas chromatography-olfactometry methods: comparison of odour intensity-concentration relationships of eight volatile compounds with sensory headspace data. Food Chem., 2001. **74**(3): p. 341-347.
136. van Ruth, S.M., Methods for gas chromatography-olfactometry: a review. Biomol. Eng., 2001. **17**(4–5): p. 121-128.
137. Debonneville, C., B. Orsier, I. Flament and A. Chaintreau, Improved Hardware and Software for Quick Gas Chromatography−Olfactometry Using CHARM and GC-"SNIF" Analysis. Anal. Chem., 2002. **74**(10): p. 2345-2351.
138. Le Guen, S., C. Prost and M. Demaimay, Characterisationof odorant compounds of mussels (Mytilus edulis) according to their origin using gas chromatography–olfactometry and gas chromatography–mass spectrometry. Journal of Chromatography A, 2000. **896**(1–2): p. 361-371.
139. Acree, T.E., J. Barnard and D.G. Cunningham, A procedure for the sensory analysis of gas chromatographic effluents. Food Chem., 1984. **14**(4): p. 273-286.
140. Ullrich, F. and W. Grosch, Identification of the most intense volatile flavour compounds formed during autoxidation of linoleic acid. Z Lebensm Unters Forch, 1987. **184**(4): p. 277-282.
141. Grosch, W., Determination of potent odourants in foods by aroma extract dilution analysis (AEDA) and calculation of odour activity values (OAVs). Flavour Frag. J., 1994. **9**(4): p. 147-158.
142. Grosch, W., Detection of potent odorants in foods by aroma extract dilution analysis. Trends Food Sci. Technol., 1993. **4**(3): p. 68-73.

143. Abbott, N., P. Etievant, S. Issanchou and D. Langlois, Critical evaluation of two commonly used techniques for the treatment of data from extract dilution sniffing analysis. J. Agr. Food Chem., 1993. **41**(10): p. 1698-1703.
144. Etiévant, P.X., G. Callement, D. Langlois, S. Issanchou and N. Coquibus, OdourIntensity Evaluation in Gas Chromatography−Olfactometry by Finger Span Method. J. Agr. Food Chem., 1999. **47**(4): p. 1673-1680.
145. Peťka, J., V. Ferreira, M.A. González-Viñas and J. Cacho, Sensory and Chemical Characterisationof the Aroma of a White Wine Made with Devín Grapes. J. Agr. Food Chem., 2006. **54**(3): p. 909-915.
146. Plutowska, B. and W. Wardencki, Headspace solid-phase microextraction and gas chromatography–olfactometry analysis of raw spirits of different organoleptic quality. Flavour Frag. J., 2009. **24**(4): p. 177-185.
147. Robinson, A.L., P.K. Boss, P.S. Solomon, R.D. Trengove, H. Heymann and S.E. Ebeler, Origins of grape and wine aroma. Part 1. Chemical components and viticultural impacts. Am. J. Enol. Vitic., 2014. **65**(1): p. 1-24.
148. Molyneux, R.J. and P. Schieberle, Compound Identification: A Journal of Agricultural and Food Chemistry Perspective. J. Agr. Food Chem., 2007. **55**(12): p. 4625-4629.
149. Aznar, M., R. Lopez, J.F. Cacho and V. Ferreira, Identification and quantification of impact odorants of aged red wines from Rioja. GC-olfactometry, quantitative GC-MS, and odourevaluation of HPLC fractions. J. Agric. Food Chem., 2001. **49**: p. 2924.
150. Ferreira, V., M. Aznar, R. Lopez and J. Cacho, Quantitative gas chromatography-olfactory carried out at different dilutions of an extract. Key differences in the odourprofiles of four high-quality Spanish aged red wines. J. Agric. Food Chem., 2001a. **49**: p. 4818-4824.
151. Ferreira, V., R. Lopez and M. Aznar, *Olfactometry abd aroma extract dilution analysis of wines.*, in *In analysis of taste and aroma*, J. Jackson, Editor. 2001b, Springer-Verlag: Berlin. p. 89-122.
152. Risticevic, S., Y. Chen, L. Kudlejova, R. Vatinno, B. Baltensperger, J.R. Stuff, D. Hein and J. Pawliszyn, Protocol for the development of automated high-throughput SPME-GC methods for the analysis of volatile and semivolatile constituents in wine samples. Nat. Protocols, 2010. **5**(1): p. 162-176.
153. Escudero, A., B. Gogorza, M.A. Melus, N. Ortin, J. Cacho and V. Ferreira, Characterisationof the aroma of a wine from Maccabeo. Key role played by compounds with low odouractivity values. J. Agric. Food Chem., 2004. **52**(11): p. 3516-3524.
154. Grosch, W., Evaluation of the Key Odorants of Foods by Dilution Experiments, Aroma Models and Omission. Chem. Senses, 2001. **26**(5): p. 533-545.
155. Audouin, V., F. Bonnet, Z.M. Vickers and G.A. Reineccius. *Limitations in the use of odouractivity values to determine important odorants in foods*. in *ACS Symposium Series*. 2001. Washington, DC; American Chemical Society; 1999.
156. Guth, H., Identification of character impact odorants of different white wine varieties. J. Agric. Food Chem., 1997. **45**: p. 3022.
157. Hopfer, H., S.E. Ebeler and H. Heymann, How Blending Affects the Sensory and Chemical Properties of Red Wine. Am. J. Enol. Viticult., 2012.
158. Dal Santo, S., T.G. B., S. Zemoni, M. Fasoli, L. Farina, A. Anesi and F. Guzzo, The plasticity of the grapevine berry transcription. Genome Biol., 2013. **14**: p. R54.
159. Mira de Orduña, R., Climate change associated effects on grape and wine quality and production. Food Res. Int., 2010. **43**(7): p. 1844-1855.
160. Schultz, H., Climate change and viticulture: A European perspective on climatology, carbon dioxide and UV-B effects. Aust. J. Grape Wine R., 2000. **6**(1): p. 2-12.
161. Kliewer, W.M. and N.K. Dokoozlian, Leaf area/crop weight ratios of grapevines: Influence on fruit composition and wine quality. Am. J. Enol. Vitic., 2005. **56**(2): p. 170-181.
162. Gil-Munoz, R.R., J.L. Vila-Lopez, Fernandez-Fernandez and A. Martinez-Cutillas, Effects of cluster thinnig on anthocyanin extractability and chromatic parameters of Syrah and Tempranillo grapes and wines. J. Int. Sci. Vigne Vin, 2009. **43**: p. 45-53.

163. Gal, Y., A. Naor and B. Bravdo, Effect of shoot density, crop level and crop load on fruit and wine quality of Sauvignon Blanc grapes. Acta. Hortic., 1996. **427**: p. 151-159.
164. Iland, P., *Chemical analysis of grapes and wine : techniques and concepts*. 2004, Campbelltown, SA, Australia: Patrick Iland Wine Promotions PTY LTD. iv, 110.
165. Ravaz, M.L., L'effeullage de la vigne. Ann. l'Ecole Nat. Agric. Montpellier, 1911. **11**: p. 216-244.
166. Smart, R.E. and M. Robinson, *Sunlight into wine: A handbook for winegrape canopy management.* 1991, Adelaide, AU: Winetitles.
167. Kotseridis, Y., R.L. Baumes, A. Bertrand and G.K. Skouroumounis, Quantitative determination of 2-methoxy-3-isobutylpyrazine in red wines and grapes of Bordeaux using a stable isotope dilution assay. J. Chromatogr. A, 1999a. **841**(2): p. 229-237.
168. Reynolds, A.G., J. Schlosser, D. Sorokowsky, R. Roberts, J. Willwerth and C. De Savigny, Magnitude of viticultural and enological effects. II. Relative impacts of cluster thinning and yeast strain on composition and sensory attributes of Chardonnay Musqué. Am. J. Enol. Vitic., 2007. **58**(1): p. 25-41.
169. Chapman, D.M., J.H. Thorngate, M.A. Matthews, J.X. Guinard and S.E. Ebeler, Yield effects on 2-methoxy-3-isobutylpyrazine concentration in Cabernet Sauvignon using a solid phase microextraction gas chromatography/mass spectrometry method. J. Agric. Food Chem., 2004. **52**: p. 5431.
170. Naor, A., Y. Gal and B. Bravdo, Shoot and cluster thinning influence vegetative growth, fruit yield, and wine quality of Sauvignon blanc grapevines. J Am. Soc. Hortic. Sci., 2002. **127**: p. 628-634.
171. Ough, C.S. and R. Nagaoka, Effect of cluster thinning and vineyard yields on grape and wine composition and wine quality of Cabernet Sauvignon. Am. J. Enol. Vitic., 1984. **35**(1): p. 30-34.
172. Reynolds, A.G. and D.A. Wardle, Impact of various canopy manipulation techniques on growth, yield, fruit composition, and wine quality of Gewürztraminer. Am. J. Enol. Vitic., 1989. **40**(2): p. 121-129.
173. Filippetti, I., S. Ramazzotto, M. Centinari, B. Bucchetti and C. Intrieri, Effects of cluster thinning on grape composition: preliminary experiences on Sangiovese grapevines. Acta. Hortic., 2007. **754**: p. 227-234.
174. Ahmed, Z.U., J.P. Johnson, X. Yang, C.K. Fatt, H.S. Teng and L.C. Boon, Does country of origin matter for low-involvement products? Int. Market. Rev., 2004. **21**(1): p. 102-120.
175. Louw, C., D. La Grange, I.S. Pretorius and P. van Rensburg, The effect of polysaccharide-degrading wine yeast transformants on the efficiency of wine processing and wine flavour. J. Biotechnol., 2006. **125**(4): p. 447-461.
176. Zou, H., P. Kilmartin, M. Inglis and A. Frost, Extraction of phenolic compounds during vinification of Pinot Noir wine examined by HPLC and cyclic voltammetry. Aust. J. Grape Wine R., 2002. **8**(3): p. 163-174.
177. Sarneckis, C.J., R.G. Dambergs, P. Jones, M. Mercurio, M.J. Herderich and P.A. Smith, Quantification of condensed tannins by precipitation with methyl cellulose: development and validation of an optimised tool for grape and wine analysis. Aust. J. Grape Wine R., 2006. **12**(1): p. 39-49.
178. Kotseridis, Y., R. Baumes and G.K. Skouroumounis, Synthesis of labelled [2H4][beta]-damascenone, [2H2]2-methoxy-3- isobutylpyrazine, [2H3][alpha]-ionone, and [2H3][beta]-ionone, for quantification in grapes, juices and wines. J. Chromatogr. A, 1998. **824**(1): p. 71-78.
179. Feuillat, M., C. Charpentier and C. Massoutier, Enological importance of cryotolerant *Saccharomyces cerevisiae* yeast strains. Revue des Oenologues 1997. **24**(85): p. 18-21.
180. Curtin, C., B. Dambergs, B. Bramley, G. Cowey, M. Holdstock, A. Coulter, E. Kennedy, B. Travis, L. Francis and P. Godden, *Perception of "Brett" in Australian and New Zealand Pinot noir wines*, in *Australian Wine Industry Technical Conference*. 2007: Melbourne.

181. Guedes de Pinho, P., E. Falqué , M. Castro, H. Oliveira e Silva, M. B. and A.C. Silva Ferreira, Further Insights into the Floral Character of Touriga Nacional Wines. Journal of Food Science, 2007. **72**(6): p. S396-S401.
182. Cortell, J.M., M. Halbleib, A.V. Gallagher, T.L. Righetti and J.A. Kennedy, Influence of vine vigor on grape (Vitis vinifera L. cv. Pinot Noir) and wine proanthocyanidins. J. Agric. Food Chem, 2005. **53**: p. 5798-5808.
183. Kennedy, J.A., M.A. Matthews and A.L. Waterhouse, Effect of Maturity and Vine Water Status on Grape Skin and Wine Flavonoids. Am. J. Enol. Vitic., 2002. **53**(4): p. 268-274.
184. Sacchi, K.L., L.F. Bisson and D.O. Adams, A Review of the Effect of Winemaking Techniques on Phenolic Extraction in Red Wines. Am. J. Enol. Vitic., 2005. **56**(3): p. 197-206.
185. Harbertson, J.F., R.E. Hodgins, L.N. Thurston, L.J. Schaffer, M.S. Reid, J.L. Landon, C.F. Ross and D.O. Adams, Variability of Tannin Concentration in Red Wines. Am. J. Enol. Vitic., 2008. **59**(2): p. 210-214.
186. Imre, S.P., J.L. Mauk, S. Bell and J. Dougherty, Correlations among ground penetrating radar, electromagnetic induction and vine trunk circumference data: towards quantifying terroir in New Zealand Pinot Noir vineyards. Progrès Agricole et Viticole 2009. **126**(1): p. 8-11.
187. Tomasino, E., R. Harrison, R. Sedcole and A. Frost, Regional differentiation of New Zealand Pinot noir wine by wine professionals using canonical variate analysis. Am. J. Enol. Vitic., 2013. **64**(3): p. 357-363.
188. Gómez García-Carpintero, E., E. Sánchez-Palomo, M.A. Gómez Gallego and M.A. González-Viñas, Characterisationof impact odorants and sensory profile of Bobal red wines from Spain's La Mancha region. Flavour Frag. J., 2012. **27**(1): p. 60-68.
189. Frank, S., N. Wollmann, P. Schieberle and T. Hofmann, Reconstitution of the flavoursignature of Dornfelder red wine on the basis of the natural concentrations of its key aroma and taste compounds. J. Agric. Food Chem., 2011. **59**(16): p. 8866-8874.
190. Masson, G. and R. Schneider, Key compounds of Provence Rose wine flavor. Am. J. Enol. Vitic., 2009. **60**(1): p. 116-122.
191. Cullere, L., A. Escudero, J. Cacho and V. Ferreira, Gas chromatography olfactometry and chemical quantitative study of the aroma of six premium quality Spanish aged red wines. J. Agric. Food Chem. , 2004. **52**(6): p. 1653-1660.
192. San-Juan, F., V. Ferreira, J. Cacho and A. Escudero, Quality and aromatic sensory descriptors (mainly fresh and dry fruit character) of Spanish red wines can be predicted from their aroma-active chemical composition. J. Agric. Food Chem. , 2011. **59**(14): p. 7916-7924.
193. Dravnieks, A., *Atlas of odorcharacter profiles*. 1985, Philadelphia, PA: American Society for Testing and Materials.
194. Mansfield, A., J. Schirle-Keller and G.A. Reineccius, Identification of odor-iImpact compounds in red table wines produced from Frontenac grapes. Am. J. Enol. Vitic., 2011. **62**(2): p. 169-176.
195. Kotseridis, Y., R.L. Baumes and G.K. Skouroumounis, Quantitative determination of free and hydrolytically liberated [beta]-damascenone in red grapes and wines using a stable isotope dilution assay. J. Chromatogr. A, 1999c. **849**(1): p. 245-254.
196. Young, T.A., S. Pecore, N. Stoer, F. Hulting, N. Holschuh and F. Case, Incorporating test and control product variability in degree of difference tests. Food Qual. Prefer., 2008. **19**(8): p. 734-736.
197. Aronson, J. and S.E. Ebeler, Effect of polyphenol compounds on the headspace volatility of flavors. Am. J. Enol. Vitic., 2004. **55**(1): p. 13-21.
198. Dufour, C. and C.L. Bayonove, Interactions between wine polyphenols and aroma substances. An insight at the molecular level. J. Agric. Food Chem., 1999. **47**(2): p. 678-684.
199. Dufour, C. and C.L. Bayonove, Influence of wine structurally different polysaccharides on the volatility of aroma substances in a model system. J. Agric. Food Chem., 1999. **47**(2): p. 671-677.
200. Ferreira, V., I. Jarauta, R. Lopez and J. Cacho, Quantitative determination of sotolon, maltol and free furaneol in wine by solid-phase extraction and gas chromatography ion-trap mass spectrometry. J. Chromato A, 2003. **1010**(1): p. 95-103.

201. Saenz-Navajas, M., E. Campo, L. Cullere, P. Fernandez-Zurbano, D. Valentin and V. Ferreira, Effects of the nonvolatile matrix on the aroma perception of wine. J. Agric. Food Chem. , 2010. **58**(9): p. 5574-5585.
202. New Zealand Winegrowers Annual Report http://www.nzwine.com/info-centre/statistics/ 2015.
203. Reynolds, A.G., C.G. Edwards, D.A. Wardle, D.R. Webster and M. Dever, Shoot density affects Riesling grapevines I: vine performance. J Am. Soc. Hortic. Sci., 1994a. **119**: p. 874-880.
204. Reynolds, A.G., S.F. Price, D.A. Wardle and B.T. Watson, Fruit environment and crop level effects on Pinot noir. I. Vine performance and fruit composition in British Columbia. Am. J. Enol. Vitic., 1994b. **45**(4): p. 452-459.
205. Arfelli, G., B. Zironi, B. Marangoni, A. Amati and M. Castellari, The effects of cluster thinning on some ripening parameters and wine quality. Acta. Hortic., 1996. **427**: p. 379-385.
206. Guidoni, S., P. Allara and A. Schubert, Effect of cluster thinning on berry skin anthocyanin composition of *Vitis vinifera* cv. Nebbiolo. Am. J. Enol. Vitic., 2002. **53**(3): p. 224-226.
207. Nuzzo, V. and M.A. Matthews, Response of fruit growth and ripening to crop level in dry-farmed Cabernet Sauvignon on four rootstocks. Am. J. Enol. Vitic., 2006. **57**(3): p. 314-324.
208. Petrie, P.R. and P.R. Clingeleffer, Crop thinning (hand versus mechanical), grape maturity and anthocyanin concentration: outcomes from irrigated Cabernet Sauvignon (*Vitis vinifera* L.) in a warm climate. Aust. J. Grape Wine R., 2006. **12**(1): p. 21-29.
209. Gil, M., M. Esteruelas, E. González, N. Kontoudakis, J. Jiménez, F. Fort, J.M. Canals, I. Hermosín-Gutiérrez and F. Zamora, Effect of two different treatments for reducing grape yield in *Vitis vinifera* cv Syrah on wine composition and quality: berry thinning versus cluster thinning. J. Agric. Food Chem., 2013. **61**(20): p. 4968-4978.
210. Bowen, P., C. Bogdanoff, K. Usher, B. Estergaard and M. Watson, Effects of irrigation and crop load on leaf gas exchange and fruit composition in red winegrapes grown on a loamy sand. Am. J. Enol. Vitic., 2011. **62**(1): p. 9-22.
211. King, P.D., D.J. McClellan and R.E. Smart, Effect of severity of leaf and crop removal on grape and wine composition of Merlot vines in Hawke's Bay vineyards. Am. J. Enol. Vitic., 2012. **63**(4): p. 500-507.
212. Wolf, T.K. and M.K. Cook, Seasonal deacclimation patterns of three grape cultivars at constant, warm temperature. Am. J. Enol. Vitic., 1992. **43**(2): p. 171-179.
213. Avizcuri-Inac, J., A. Gonzalo-Diago, J. Sanz-Asensio, M. Martinez-Soria, M. Lopez-Alonso, M. Dizy-Soto, J. Echa¡varri-Granado, L. Vaquero-Ferna¡ndez and P. Ferna¡ndez-Zurbano, Effect of Cluster Thinning and Prohexadione Calcium Applications on Phenolic Composition and Sensory Properties of Red Wines. J. Agr. Food Chem., 2013. **61**(5): p. 1124-1137.
214. Preszler, T., T.M. Schmit, V. H. and J. E., Cluster thinning reduces the economic sustainability of Riesling production. Am. J. Enol. Vitic., 2013.
215. Chapman, D.M., M.A. Matthews and J.X. Guinard, Sensory attributes of Cabernet Sauvignon wines made from vines with different crop yields. Am. J. Enol. Vitic., 2004. **55**(4): p. 325-334.
216. Keller, M., L.J. Mills, R.L. Wample and S.E. Spayd, Cluster thinning effects on three deficit-irrigated *Vitis vinifera* cultivars. Am. J. Enol. Vitic., 2005. **56**(2): p. 91-103.
217. ISO, *Sensory analysis— identification and selection of descriptors for establishing a sensory profile by a multidimensional approach*, in *The International Organization for Standardization*. 1994: Geneva, Switzerland.
218. Rutan, T., M. Herbst-Johnstone, B. Pineau and P.A. Kilmartin, Characterisationof the Aroma of Central Otago Pinot noir Wines Using Sensory Reconstitution Studies. Am. J. Enol. Vitic., 2014.
219. Bureau, S.M., R.L. Baumes and A.J. Razungles, Effects of vine or bunch shading on the glycosylated flavourprecursors in grapes of Vitis vinifera L. Cv. Syrah. J. Agr. Food Chem., 2000. **48**(4): p. 1290-1297.
220. Dudareva, N., E. Pichersky and J. Gershenzon, Biochemistry of plant volatiles. Plant Physiol., 2004. **135**(4): p. 1893-1902.

221. Orlova, I., A. Marshall-ColÃ³n, J. Schnepp, B. Wood, M. Varbanova, E. Fridman, J.J. Blakeslee, W.A. Peer, A.S. Murphy and D. Rhodes, Reduction of benzenoid synthesis in petunia flowers reveals multiple pathways to benzoic acid and enhancement in auxin transport. The Plant Cell, 2006. **18**(12): p. 3458-3475.
222. Francis, I.L., S. Kassara, A.C. Noble and P.J. Williams, *The contribution of glycoside precursors to Cabernet Sauvignon and Merlot aroma: Sensory and compositional studies.*, in *Chemistry of Wine Flavor*, A.L. Waterhouse and S.E. Ebeler, Editors. 1998, Am Chemical Society: Washington DC. p. 13-30.
223. Adams, O.D., Phenolics and ripening in grape berries. Am. J. Enol. Viticult., 2006. **57**(3): p. 249-256.