The compositional basis of the aroma of Riesling and unwooded Chardonnay wine

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

January 2005

TABLE OF CONTENTS

ABSTRACT

The aim of this research project was to explore, in detail, the relationship between volatile composition and wine aroma for two white wine varieties, namely Riesling and unwooded Chardonnay, so that the most influential volatile aroma compounds to the aroma of these two varieties could be identified. Twenty Australian commercial wines of each variety were analysed by quantitative sensory descriptive analysis and targeted for the chemical analysis of more than 45 volatile compounds. The compositional and sensory data sets were related using multivariate methods (e.g. PCA and PLS), and aroma volatiles were identified that related to the specific sensory properties of each variety. Most of the Riesling and several of the unwooded Chardonnay sensory properties were well predicted by the compositional data and several compounds were identified as important to the aroma of each variety. The unwooded Chardonnay wines were higher in concentration of various fermentation-derived compounds than were the Riesling wines, and these volatiles played an important role in the sensory properties of this variety. The Riesling wines were higher in concentration of grape-derived compounds including the monoterpenes, norisoprenoids, and dimethyl sulfide. These compounds, and also many of the fermentation-derived compounds, were identified as important contributors to the aroma of the Riesling wines. The results from this study have greatly advanced our understanding of the complex interactions between volatile compounds and the role that they play in the specific aroma nuances of white wines.

The prediction of sensory properties of the Riesling and unwooded Chardonnay wines was investigated using rapid instrumental techniques, namely mass spectrometry based electronic nose (MS Enose) and visible and near infrared (VIS-NIR) spectroscopy. A combination of MS Enose and VIS-NIR gave the best predictive results compared to either method alone. Promising results were achieved for many of the sensory properties indicating that this technique shows good potential for application.

The so-called ‘wine lactone’ (3a,4,5,7a-tetrahydro-3,6-dimethylbenzofuran-2(3H)-one) is known to be an important white wine odorant. The formation of wine lactone was investigated from two potential precursors, namely (E)-2,6-dimethyl-6-hydroxyocta-2,7-dienoic acid and the glucose ester of this acid, in model wine at room temperature and 45°C. The hydrolytic results show that the rate of formation of wine lactone is too slow for either the acid or the glucose ester to be major precursors to wine lactone in young white wine. Therefore, different precursors are most likely responsible for the formation of wine lactone in young white wine.

DEDICATION

I dedicate this thesis to my Lord God, who made my heart, mind and soul, and who is and forever will be, my only audience.

> *Whatever you do, work at it with all your heart, as working for the Lord, not for men, since you know you will receive an inheritance from the Lord as a reward. It is the Lord you are serving.* Colossians 3:23

DECLARATION

This work contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference is made.

I consent to this copy of my thesis, when deposited in the University Library, being made available for photocopying and loan.

Signed _____ Date _____

ACKNOWLEDGEMENTS

I thank the Grape and Wine Research and Development Corporation and the Cooperative Research Centre for Viticulture for providing funding that allowed me the privilege of pursuing a PhD and for the opportunity to attend and present my research at conferences in Australia and overseas.

I am very grateful to the many wine producers and wine makers across Australia who provided generous support of this research through the contribution of wine and through openly providing detailed information about the wines included in this study.

I thank my supervisors Drs Leigh Francis, Mark Sefton and Markus Herderich for the opportunity to work under their supervision and for their valued guidance, support and advice thoughout my PhD. I also thank Dr Daniel Cozzolino, who has been an integral part of the success of this project, for his mentorship and friendship. I also thank Professors Peter Høj, Sakkie Pretorius and the staff and students at The Australian Wine Research Institute for their on-going support and encouragement throughout my time at the Institute.

In particular, I acknowledge Tracey Siebert, Dr Alan Pollnitz, Dr Kate Howell, Dr Markus Herderich and Dr Mark Sefton for their participation in the development of the analytical method for fermentation-derived compounds and Dr George Skouroumounis for his advice on the synthetic component of this project. I also acknowledge those who were involved with developing the various analytical methods that were used by the author, including Tracey Siebert, Dr Alan Pollnitz, Dimitra Capone, Katryna van Leeuwen, Dr Gordon Elsey and Geoff Cowey. I thank Dr Aggie Cox (nee Janusz) for analysing the study wines for TPB.

For their assistance and expertise with the sensory analysis of the study wines I thank Dr Leigh Francis and Kate Lattey, and also the staff and students of the AWRI for their involvement as panellists.

I thank Dr Daniel Cozzolino for his expert advice on multivariate data analysis and for his direction over the rapid instrumental component of this project. I acknowledge Dr. Weis Cynkar for her assistance with the electronic nose work. I also thank Dr Vicente Ferreira for his comments and advice for the project, particularly in the area of multivariate analysis and the grouping of chemical variables.

For the wine lactone work, I thank Dr Gordon Elsey, Anders Hakansson, Kevin Pardon, and Dr George Skouroumounis who provided expert advice and assistance with the synthetic part of this study. Dr Gordon Elsey made significant contributions by synthesising bulk

deuterium labelled and unlabelled wine lactone that was used for analytical method development and Kevin Pardon assisted by separating wine lactone isomers **1a** and **1c** by chromatography. I also thank Dimitra Capone who provided expert advice and assistance with analytical method development, Katryna van Leeuwen who assisted in the preparation of samples for the hydrolytic study and Dr Mark Sefton who provided outstanding supervision and direction for this project.

I thank all those who provided comments and feedback on my thesis, including my supervisors, Dr Daniel Cozzolino, Dr Alan Pollnitz, my father Daryll Pain and my husband Graeme Smyth. I also thank the wonderful Ingrid Oates and the team from the JFM Library for providing me fast and easy access to all of the journal articles and books that I needed to conduct my research.

I thank my friends, in particular my fellow postgraduate students and my church family (NEVC), for their friendship, support and encouragement in the good times and the tough times throughout my PhD studies.

I especially thank my parents, Daryll and Hazel Pain, for their unconditional love and support, and for giving me so many opportunities in life to pursue my dreams. I also gratefully thank my husband, Graeme John Smyth who continues to love me, and believe in me, in everything I do.

PUBLICATIONS AND PRESENTATIONS

The following publications were derived from this research:

Refereed Papers:

Cozzolino, D.; Smyth, H.E.; Lattey, K.A.; Cynkar, W.U.; Janik, L.; Dambergs, R.G.; Francis, I.L.; and Gishen, M., *Relationship between sensory analysis and near infrared spectroscopy in Australian Riesling and Chardonnay wines.* Analytica Chimica Acta, 2005. In press.

Siebert, T.E.; Smyth, H.E.; Capone, D.L.; Neuwöhner, C.; Pardon, K.; Skouroumounis, G.; Herderich, M.J.; Sefton, M.A.; and Pollnitz, A.P., *Stable Isotope Dilution Analysis of wine fermentation products by HS-SPME-GC-MS.* Analytical and Bioanalytical Chemistry, 2005. 381: 937-947.

Cozzolino, D.; Smyth, H.; and Gishen, M., *Feasibility study on the use of visible and near infrared spectroscopy together with chemometrics to discriminate between commercial white wines of different varietal origins.* Journal of Agricultural and Food Chemistry, 2003. 51: 7703-7708.

Papers and posters presented at conferences:

Cozzolino, D.; Smyth, H.E.; Lattey, K.A.; Cynkar, W.U.; Janik, L.; Dambergs, R.G.; Francis, I.L.; and Gishen, M., *Relationship between sensory analysis and near infrared spectroscopy in Australian Riesling and Chardonnay wines.* Near Infrared Spectroscopy: Proceedings of the 12th International conference, Auckland, NZ Vic, 10 - 15th April 2005. Paper Accepted.

Smyth, H.E.; Cozzolino, D.; Herderich, M.; Sefton, M.A.; Francis, I.L., *Relating volatile composition to wine aroma: identification of key aroma compounds in Australian white wines.* Proceedings of the 12th Australian Wine Industry Technical Conference, Melbourne Vic, 25 - 29th July 2004. Paper presented.

Cozzolino, D.; Smyth, H.E.; Dambergs, R.G.; Gishen, M., *Multivariate analysis (chemometrics): a novel approach in modern interdisciplinary sciences.* Proceedings of the 12th Australian Wine Industry Technical Conference, Melbourne Vic, 25 - 29th July 2004. Paper presented.

Cozzolino, D.; Smyth, H.E.; Lattey, K.A.; Cynkar, W.U.; Francis, I.L.; Dambergs, R.G.; and Gishen, M., *A preliminary investigation of the potential of near infrared spectroscopy to predict sensory attributes in white wine.* Proceedings of the 12th Australian Wine Industry Technical Conference, Melbourne Vic, 25 - 29th July 2004. Poster presented.

Cozzolino, D.; Smyth, H.E.; Francis, I.L.; Cynkar, W.U.; Janik, L.; Dambergs, R.G.; and Gishen, M., *White wine varietal discrimination using near infrared reflectance spectroscopy.* Proceedings of the 12th Australian Wine Industry Technical Conference, Melbourne Vic, 25 - 29th July 2004. Poster presented.

Siebert, T.E.; Smyth, H.E.; Herderich, M.; Sefton, M.A.; Pollnitz, A.P., *Quantification of 31 important fermentation-derived aroma compounds in wine.* Proceedings of the 12th Australian Wine Industry Technical Conference, Melbourne Vic, 25 - 29th July 2004. Poster presented.

Lattey, K.A.; Smyth, H.E.; D-Costa, N.E.; Liebich, B.K.; Francis, I.L., *Consumer acceptability and sensory properties of a set of commercial Australian Riesling and unwooded Chardonnay wines.* Proceedings of the 12th Australian Wine Industry Technical Conference, Melbourne Vic, 25 - 29th July 2004. Poster presented.

Smyth, H.E.; Cozzolino, D.; Herderich, M.; Sefton, M.A.; Francis, I.L., *Identification of key aroma compounds in Australian Riesling and unwooded chardonnay wines*. Proceedings of the 7th Wartburg Symposium on Flavor Chemistry & Biology, Eisenach, Germany 21st - 23rd April, 2004. Poster presented.

Smyth, H.E.; Cozzolino, D.; Herderich, M.; Sefton, M.A.; Francis, I.L., *Riesling wine aroma: Relating volatile to sensory data using PLS regression models*. Proceedings of the 6th annual scientific meeting of the Australasian Association for ChemoSensory Science (AACSS), Rutherglen Vic, 2nd - 4th October 2003. Paper presented.

Smyth, H.E.; Cozzolino, D.; Francis I.L., *Identification of key aroma compounds in Australian Riesling wines*. Proceedings of the Australian Society of Viticulture and Oenology: Viticulture and Oenology Seminars, Tanunda SA, July 10 - 11th 2003. Paper presented.

External presentations:

Smyth, H.E., *Key aroma compounds in white wine.* Oral presentation for the Department of Dairy and Food Science, The Royal Veterinary and Agricultural University, Copenhagen, Denmark. 26th April 2004

Smyth, H.E., *Key aroma compounds in white wine.* Oral presentation for the Department of Analytical Chemistry, University of Zaragoza, Zaragoza, Spain. 29th April 2004

Press / media interviews:

Goodness, does wine really smell like this? Great drop with fine bouquet of kerosene. By Zac Milbank, The Advertiser, Wednesday December 15th 2004, p 33.

On the nose with a hint of bubblegum. By Rebecca DiGirolamo, The Australian, Wednesday February 11th 2004, p 31.

Choose your own wine aroma. ABC Radio Riverland, Rural Report by Alice Plate, Thursday, 5th February, 2004.

Technology traces essential aromas. Australian Vignerons Volume 3, Number 5, January/February 2004, p 53-55.

The sweet smell of honey, passionfruit and lemon – Uncovering the key aroma compounds. Cooperative Research Centre for Viticulture Newsletter, November-December 2003, Volume 9, Number 6, p 5-7.

Sense of smell drives search for key aroma compounds. Australian and New Zealand Wine Industry Journal: September/October 2003 Volume 18, Number 5, p 82.

Smells OK? It must be the ethyl propanoate. Tim White, The Australian Financial Review: July 19-20th 2003, p 69.

Chapter 1 General introduction

The aroma of a wine is an important aspect of wine quality, and understanding the compositional basis of wine aroma is of great interest to the wine industry. Once the chemical compounds responsible for the aroma nuances of wine have been identified, it is feasible that the formation of these compounds could be manipulated in the vineyard and winery, thus enabling greater control of the aroma of finished wine by the winemaker.

With advances in instrumentation and methodology, wine composition can be studied in greater detail than has been possible in the past. Consequently, there has been much emphasis in the last thirty years on establishing the identity of the volatile compounds found in wine, what sensory attributes individual compounds possess, and where aroma compounds are thought to originate [1]. However, the relationship between the volatile composition of wine and the actual aroma of the wine is still not well understood. Only in the last ten years have multivariate data analysis techniques developed to a level where correlating complex sensory responses with instrumental data can be achieved to explore the complex relationship between wine composition and perceived wine aroma.

The primary purpose of the present work was to study, in detail, the relationship between wine aroma and the chemical composition of Australian commercial Riesling and unwooded Chardonnay wine. A carefully selected set of wines from each variety, with a broad range of aroma properties, was subjected to targeted volatile analysis and sensory descriptive analysis. Subsequent multivariate data analyses were employed to explore the relationship between the volatile compositional and sensory data sets. Through this process, the volatile compounds most important to the aroma properties of each variety were identified and the perception of specific aroma notes were related to particular volatile compounds.

The knowledge gained from this study could be used in the wine industry to characterise wine aroma according to the concentration of particular important aroma compounds present in the wine, as an alternative to expensive and time-consuming sensory analysis. In addition, with the knowledge of the formation of important aroma compounds (and their precursors) during the winemaking process, it might also be possible to manipulate wine aroma, with specific intent, by altering winemaking techniques to favour the production of certain volatile compounds over others to achieve a desired aroma. For example, it is well established that the levels of some norisoprenoids, in particular TDN, are significantly increased in wine made from grapes exposed to sunlight compared to wines made from grapes grown in the shade [2]. At moderate concentrations, TDN is thought to contribute to

the 'developed' aroma of aged Riesling wine [3]. Consequently, it is quite conceivable that regulating the sun-exposure of grapes in the vineyard might translate into controlled levels of norisoprenoids, resulting in a wine with a desired aroma.

With an enhanced understanding of the relationship between composition and wine aroma, it could be feasible in the future to predict the aroma of wine made from particular grape parcels. This might be possible through the analysis of aroma compound precursors that are present in the grape berry that indicate the 'potential' aroma of the wine made from specific parcels of grapes. Consequently, winemakers could tailor the winemaking process to suit the aroma 'potential' of grapes to produce a wine that is best matched to each individual parcel of grapes.

1.1 Volatile compounds important to white wine aroma

Volatile compounds responsible for the aroma of wine are derived from a number of different biochemical and chemical pathways. Compounds are formed during grape berry metabolism, crushing of the berries, fermentation processes (yeast and malolactic) and also from the ageing and storage of wine. Not surprisingly, there are a large number of chemical classes of compounds found in wine which are present at varying concentrations (ng/L to mg/L), exhibit differing potencies, and have a broad range of volatilities and boiling points. The different classes of volatile compounds that have been identified in wine include fatty acids, ethyl esters, alcohols, acetates, carbonyl compounds, furans, lactones, monoterpenes, norisoprenoids, nitrogen-containing and sulfur-containing compounds.

A target list of volatile compounds most likely to be of greatest importance to white wine aroma was compiled from an extensive review of the literature. This list is given in Table 1-1, and includes each compound's respective Chemical Abstracts Service registry number (CAS), reported aroma descriptor (as a neat compound), sensory detection threshold concentration (in model wine, wine, beer or water as indicated), reported concentration ranges in wine (reports in white wine were used unless stated otherwise), and some references where the measurement or relative importance of the compound in wine has been reported. The compounds were selected if they were reported to be important to white wine aroma as deemed by their measurement in white wine at concentrations above sensory threshold, or through the use of gas chromatography olfactometry analysis (GC-O). Additionally, some compounds were included on the list if their importance was undetermined due to insufficient or unreliable sensory threshold and quantitative information.

Although there are many different types of acids found in wine, the fatty acids are considered to be the most likely of this class of compound to contribute to the aroma of wine [4]. Fatty

acids are believed to originate primarily from yeast and bacteria biosynthesis during the fermentation stage in winemaking [5]. As pure compounds the fatty acids generally have cheesy, rancid aromas. Although the sensory contribution of acids to wine aroma is sometimes considered to be negligible [4], they have been measured in wine above their sensory perception threshold concentrations and might contribute to the background aroma of wine.

Table 1-1 Aroma compounds that might be important to white wine aroma

compound	CAS #	descriptor	threshold (µg/L)	wine concentrations (µg/L)	wine references
2-methylbutanoic acid	116-53-0	cheese	3000 [6] [b]	550 – 750 [6]	
3-methylbutanoic acid	503-74-2	blue cheese	3000 [6] [b]	452 – 1500 [6-8]	[4]
2-methylpropanoic acid	79-31-2	acid	200000 [6]	222 – 5800 [6-8]	[4]
acetic acid	64-19-7	vinegar	200000 [6]	255000 – 280000 [6]	[4]
propanoic acid	79-09-4	vinegar	8100 [9]	4 [10]	[4]
butanoic acid	107-92-6	cheese	10000 [6]	546 – 1580	[4]
hexanoic acid	142-62-1	sweaty	3000 [6]	2470 – 3230 [6]	[4]
octanoic acid	124-07-2	rancid cheese	500 [11]	5 – 6400 [8, 10]	[4]
decanoic acid	334-48-5	plasticine	15000 [6]	3 – 3260 [6, 8, 10]	[4]
2-methylbutanol	137-32-6	solvent	65000 [12]	144000 – 248000 [7, 13] [c]	[4]
3-methylbutanol	123-51-3	solvent	30000 [6]	109000 – 127800 [6]	[3, 4]
2-methylpropanol	78-83-1	fusel	40000 [6]	44 – 108000 [6, 7, 10, 13]	[3, 4]
2-phenylethanol	60-12-8	roses	10000 [6]	4000 – 86000 [6, 7, 14]	[3, 4, 15]
butanol	71-36-3	fusel	150000 [16]	2 – 290 [7, 14]	
hexanol	111-27-3	cut grass	8000 [6]	2 – 1890 [6, 10]	[3, 4]
guaiacol	90-05-1	phenolic	10 [6]	2 – 4 [6]	[4]
4-methylguaiacol	93-51-6	smokey	65 [17]		
4-vinylguaiacol	7786-61-0	elastoplast	440 [17]	0.2 – 25 [6, 10]	[4, 15]
4-vinylphenol	2628-17-3	medicinal	770 [17]	35 (red wine) [18]	[4, 15, 19]
4-ethylguaiacol	2785-89-9	leathery	70 [17]	8 – 116 (red wine) [11]	[19, 20]
4-ethylphenol	123-07-9	horse sweat	1100 [17]	0.07 [10]	[4, 19, 20]
3-ethylphenol	620-17-7	smokey	0.5 [6]	0.1 [6]	
eugenol	97-53-0	cloves	5 [6]	0.06 – 5.4 [6, 10]	[4]
vanillin	121-35-5	vanilla	200 [6]	17– 45 [6]	[4, 21, 22]
acetaldehyde	75-07-6	bruised apple	500 [6]	1869 – 41000 [6, 7]	[4]
diacetyl	431-03-8	butter	100 [6]	150 – 180 [6]	[4]
ethyl 2-methylbutanoate	7452-79-1	sweet fruit	1 [6]	4.4 – 4.5 [6]	
ethyl 3-methylbutanoate	108-64-5	berry	3 [6]	3 – 4 [6]	[4]
ethyl 2-methylpropanoate	97-62-1	fruity	15 [6]	150 – 480 [6]	[4]
ethyl propanoate	105-37-3	fruity	1840 [4]	3 [7]	[4]
ethyl butanoate	105-45-4	fruity	20 [6]	1 – 1000 [6, 7, 10]	[4, 23]
ethyl hexanoate	123-66-0	green apple	5 [6]	0.4 – 2000 [6, 7, 10, 24]	[3, 4, 23]
ethyl octanoate	106-32-1	soap	2 [6]	0.5 – 630 [6, 7, 10, 24]	[3, 4, 23]
ethyl decanoate	110-38-3	soap	200 [11]	0.1 – 970 [7, 10, 24]	[3, 4, 23]
ethyl dodecanoate (ethyl laurate)	106-33-2	pears	2000 [25]	66 [7]	[3, 23]
ethyl 2-hydroxypropanoate (ethyl lactate)	97-64-3	milky	150000 [4]	57 – 16600 [7, 10, 24]	[4]
trans-ethyl cinnamate	103-36-6	strawberry cream	1 [6]	2.0 – 2.3 [6]	[4]

compound	CAS #	descriptor	threshold (µg/L)	wine concentrations (µg/L)	wine references
ethyl dihydrocinnamate	2021-28-5	strawberry	1.6 [11]	0.2 – 3 (red wine) [11, 26]	
2-methylbutyl acetate	53496-15-4	banana	5 [27]		[4]
3-methylbutyl acetate	123-92-2	banana	30 [6]	3 – 7000 [6, 7, 10, 24]	[3, 23]
2-methylpropyl acetate	110-19-0	banana	1600 [11]	160 [7]	[4, 23]
ethyl acetate	141-78-6	nail polish	7500 [6]	22500 – 94000 [6, 7, 13]	[4]
hexyl acetate	142-92-7	lolly	670 [4]	0.04 – 1590 [7, 10, 24]	[3, 4, 23]
2-phenylethyl acetate	103-45-7	rose water	250 [6]	0.1 – 219 [6, 10, 24]	[4, 23]
4-hydroxy-2,5-dimethyl-3(2H)-furanone (HDMF, furaneol)	3658-77-3	fairy floss	500 [6]	1.8 – 4.2 [6]	[4, 15, 19]
2-ethyl-4-hydroxy-5-methyl-3(2H)-furanone (homofuraneol)	27538-09-6	caramel	500 [6]	53 – 117 [6]	
sotolon	28664-35-9	spicy	5 [6]	3.3 – 5.4 [6]	[4]
cis-oak lactone	55013-32-6	coconut	23 [28]	8.5 – 121 (red wine) [11]	[4, 29, 30]
(Z)-6-dodeceno-γ-lactone	15456-69-6	soap	0.1 [6]	0.14 – 0.27 [6]	
δ-decalactone	705-86-2	peach	386 [11]	8 – 20 (red wine) [11]	[4, 31]
γ-decalactone	706-14-9	peach	0.7 [32]	0.7 – 3 (red wine) [11]	[31]
γ-nonalactone	104-61-0	coco	30 [33]	3.3 – 41 (red wine) [11]	
α-terpineol	98-55-5	sweet	250 [11]	0.02 – 30 [8, 10]	[3-5, 19, 34, 35]
citronellol	106-22-9	flowery	100 [6]	15 – 188 [6, 8]	[5, 19, 34, 35]
geraniol	106-24-1	flowery	30 [6]	38 – 688 [6, 8]	[3-5, 19, 34, 35]
linalool	78-70-6	baby wipes	15 [6]	0.01 – 307 [6, 8, 10]	[4, 5, 19, 34, 35]
nerol	106-25-2	violets	500 [12]	48 – 224 [8]	[3-5, 19, 34, 35]
hotrienol	20053-88-7	lime tree	110 [5]	3 – 237 [36]	[3-5, 35]
cis-rose oxide	16409-43-1	lychee	0.2 [6]	3 – 21 [6]	[5]
wine lactone	182699-77-0	coconut	0.01 [6]	0.1 [6]	
(E)-β-damascenone	23726-93-4	stewed apples	0.05 [6]	0.8 – 1 [6]	[2, 5, 37]
1,1,6-trimethyl-1,2-dihydronapthalene (TDN)	30364-38-6	kerosene	20 [3]	35 – 189 (heated wine) [37]	[3, 5, 19, 38]
(*E*)-1-(2,3,6-trimethylphenyl) buta-1,3-diene (TPB)	644976-70-5	herbaceous	0.04 [39]	0.05 – 0.2 [39]	[40]
β-ionone	14901-07-6	violets	0.09 [11]	0.032 – 0.2 (red wine) [11]	[5]
α-ionone	127-41-3	sweet fruit	2.6 [4]*	0.02 – 0.5 (red wine) [11]	
2-isobutyl-3-methoxy pyrazine	24683-00-9	capsicum	0.001 [41]	0.0006 – 0.078 [41, 42]	[4, 5, 19, 43, 44]
2-isopropyl-3-methoxy pyrazine	25773-40-4	capsicum	0.002 [41]	0.0002 – 0.007 [42]	[4, 5, 19, 43, 44]
ethyl anthranilate	87-25-2	wet, dirty		0.6 – 4.8 [26]	
methyl anthranilate	134-20-3	wet, dirty	3 [45]	0.06 – 0.6 [26]	
3-mercaptohexan-1-ol	51755-83-0	grapefruit	0.06 [46]	0.4 – 13 [47, 48]	[5, 49]
3-mercaptohexyl acetate	136954-20-6	grapefruit	0.004 [50]	0.0004 – 0.8 [47, 48]	[5, 49]
4-mercapto-4-methylpentan-2-one	19872-52-7	passionfruit	0.0006 [6]	0.0003 – 0.4 [6, 47, 48, 51]	[5, 49]
dimethyl sulfide	75-18-3	asparagus	10 [6]	7 – 14 [6]	[4, 15, 49, 52]
methionol	505-10-2	cabbage	500 [6]	1 – 1415 [6, 10]	[4, 49]

[a] values are sensory detection threshold concentrations determined in either 9.5% ethanol in water (w/w) [9], 10% ethanol in water (w/w) [6], 11% ethanol in water (v/v) model wine [11], 12% ethanol in water (w/w) model wine [46, 50], white wine [17, 28, 33, 39, 41], wine [3-5, 16, 32], beer [4]*[12, 25] or water [27, 45]; [b] the sensory detection threshold of these compounds was determined as a mixture of 2/3-methylbutanoic acid [6]; [c] concentration was determined as a mixture of 2/3-methylbutanol.

Both aliphatic (or so-called fusel) alcohols and phenols are important to the aroma of wine. Fusel alcohols are mainly produced during yeast fermentation of sugars and yeast

metabolism of amino acids and their aroma contribution to wine is not considered to be particularly pleasant [4, 53] (Table 1-1). The fusel alcohol 2-phenylethanol is an exception, as it has a pleasant 'rose-like' aroma [3]. Phenolic alcohols have also been identified in wine and are considered to be possible important contributors to red wine aroma. Some phenols, namely 4-methylguaiacol, guaiacol and eugenol, are formed by chemical degradation of compounds from wood barrels and chips during the storage of wine [22, 54]. Other phenols, including 4-ethylphenol and 4-ethylguaiacol are formed by microbiological transformations of cinnamic acid derivatives originating from the grape berry and are associated with an off-aroma in red wine [55, 56].

A number of aldehydes and ketones have been identified in wine [4]. Aldehydes arise in wine through yeast metabolism of amino acids, and from enzymatic oxidation of unsaturated fatty acids [5]. The majority of ketones in wine are a product of yeast and bacteria metabolism [4]. The carbonyl compounds diacetyl and acetaldehyde have been measured in white wine above their respective model wine (10% ethanol in water w/w) sensory threshold concentrations [6]. Diacetyl has also been identified as particularly important to the aroma of young red wine [11] and has been reported to be responsible for the 'sweet caramel' aroma descriptor often associated with port [57]. Vanillin is considered to be particularly important to the aroma of wines that have been stored in oak barrels as it is known to form from the degradation of lignin during the toasting process [22].

Esters are considered to be the major contributor to the aroma of young wine [58]. Ethyl esters of organic acids are most abundant, followed by acetates and ethyl esters of fatty acids [4]. Consequently there are numerous references describing the presence, and also the importance, of esters to wine aroma [4, 6, 11, 58-60]. Ethyl hexanoate has been highlighted as an important contributor to the aroma of Chardonnay wines [61], and 3-methylbutyl acetate is considered particularly important to the aroma of white wine [4]. The importance of esters to the aroma of young white wine was highlighted in a study by Guth [6], where ethyl 2-methylpropanoate, ethyl butyrate, 3-methylbutyl acetate and ethyl hexanoate were measured in white wine more than ten times higher, and ethyl octanoate more than one hundred times higher, than their respective model wine (10% ethanol in water w/w) sensory threshold concentrations. Esters in wine originate mainly from yeast metabolism during fermentation but some esters are also found in small amounts in the grape berry [4]. The yeast strain chosen for fermentation and the fermentation conditions will influence the concentration and types of esters formed [62]. It is also understood that the concentrations of individual esters both increase and decrease during aging due to hydrolysis and esterification reactions between acids and alcohols, and hydrolysis of the esters [4, 63]. The

rate of change in ester concentrations with ageing is faster for acetates than fatty acid ethyl esters [63, 64].

The furan, furaneol, has been measured in wine above its model wine (10% ethanol in water w/w) sensory threshold concentration [6] and is reported to be responsible for the strawberry-like aroma of some wines [65, 66]. At higher concentrations furaneol is considered an off-flavour [59]. Furaneol and similar furans have been identified in grape juice [66, 67] as well as wine [68], and therefore most likely originate from the grape berry.

Lactones that have been identified in wine are thought to arise from various sources. These include the metabolism of amino acids and keto acids by yeasts [62], *Botrytis cinerea* activity on grapes, aerobic metabolism of flor yeasts on the wine, from precursors extracted from the wood during aging of wine, and as by-products of the metabolism of pantothenic acid [4]. Consequently, some lactones in wine are specific to the style of wine, and to the method of storage used (e.g. wood barrels).

Many terpene compounds have been identified in grapes [5]. However, it is the monoterpene alcohols found in grapes that are considered to be the most important contributors to wine aroma [69, 70]. The aroma thresholds for terpenes in wine and model wine are relatively low, and the aroma contribution of terpene compounds is thought to be additive and perhaps even synergistic [5]. Terpene compounds are known to be important to the aroma of floral varieties including Muscat, Gewürztraminer, Riesling, Auxerrois, Scheurebe, Muller-Thurgau and also other varieties not usually considered to be floral including Pinot Gris and Chardonnay [5, 69, 70]. Although terpenes are found 'free' in grape berries, they are also present in relatively large quantities in glycosidically bound form and these might also release additional free monoterpenes through glycosidase enzyme action, or under acid hydrolysis with storage. The analysis of glycosidic precursors has often been used to examine the potential contribution of terpene compounds to the aroma of particular varieties [5, 69, 71-73]. The very potent monoterpene 'wine lactone' has recently been identified in white wine and was considered to be very important to the overall aroma of white wine [6, 74].

The norisoprenoids are a diverse group of compounds which are thought to contribute to the more complex aromas of wine [59]. Norisoprenoids arise from carotenoid degradation during grape berry ripening. As with monoterpenes, norisoprenoids are found in grapes and wine predominantly as glycosidically bound precursors [75]. The norisoprenoid TDN is of particular importance to the aroma of bottle-aged Riesling wine where is it considered to contribute toward the developed aroma of this variety [3, 38]. The compound TPB is a potent

aroma compound recently identified in white wine [39]. From initial surveys TPB has been measured above its white wine sensory threshold in aged Chardonnay (50 – 100 ng/L), Riesling (60 ng/L) and Semillon (210 ng/L) wines [39]. A norisoprenoid of particular importance is β-damascenone which is extremely potent and has been measured in white wine above its model wine (10% ethanol in water w/w) sensory threshold concentration [6, 74]. The norisoprenoid β-ionone is of importance to the aroma of red wine, where it has been measured at sensorily significant concentrations (e.g. [11, 76]), and is not considered particularly important to white wine.

Methoxypyrazines are considered to be the most important nitrogen-containing wine aroma contributors [4, 43]. They exhibit very low sensory threshold concentrations in wine and need only be present in trace amounts (ppt) to have an influence on wine aroma [43]. Methoxypyrazines are believed to originate from the grape berry, and have been implicated as important contributors to the vegetative and herbaceous aroma of Sauvignon wines [43]. The levels of methoxypyrazines are known to be higher in wines made from cool climate grapes [42]. The anthranilates are also nitrogen-containing compounds. They may be important to the aroma of Pinot Noir wine [26, 77].

Sulfur-containing compounds, including thiols (mercaptans) and sulfides, are believed to be very significant contributors to the aroma of wine. These compounds are thought to be formed during yeast fermentation via the metabolism of sulfur-containing amino acids [62]. Some sulfur-containing compounds might also be derived from degradation of sulfur-containing pesticides, and fungicides used on the grapevines prior to harvest [49]. The compound dimethyl sulfide, which is formed by yeast metabolism of amino-acids and cysteine [49], is considered to be an important contributor to the developed bouquet of Riesling wine and it increases in white wine with ageing [78]. With exceptionally low sensory threshold concentrations (as low as 0.6 ng/L in 10% ethanol in water w/w [6]), the mercaptans 4-mercapto-4methylpentan-2-one [6, 48, 49], 3-mercaptohexanol [48, 49], 3-mercaptohexyl acetate [48, 49] are considered particularly influential on the aroma of some white wines. These potent compounds are derived from odourless precursors in grape must in the form of S-cysteine conjugates, which are cleaved during yeast fermentation to release the free aroma compound [79]. At high concentration in wine, these mercaptans exhibit unpleasant aromas, but at low concentration are reported to have pleasant ‘fruity’ aromas [49]. The quantitation of many sulfur-containing compounds in wine has been hindered by the fact that they are generally present in wine in trace amounts and their measurement requires particularly sensitive instrumental techniques [47].

Although the analysis of the aroma compounds in wine is complicated by the fact that they are often present in trace amounts, sophisticated methods and instrumentation have been developed that can facilitate the accurate and precise measurement of these compounds in wine (refer to Chapter 2 for further discussion).

Even though it is possible to measure the volatiles of a wine, chemical data alone is limited in its ability to describe the aroma contribution of specific compounds, or to identify the relative importance of the compounds measured to the overall aroma of a wine. The aroma properties of wine must be investigated by sensory means to evaluate the actual aroma characteristics of a wine. By then correlating this sensory information with the volatile composition it is possible to determine which volatile compounds influence the aroma of wine and what the nature of that contribution might be.

1.2 Sensory analysis of wine aroma

Sensory analysis by human subjects is a crucial part of wine flavour research as it allows for the perceived aromas and flavours to be accurately defined and quantified. Although instrumental chemical analysis and sensory analysis of wine have developed somewhat independently, important advances in multivariate data analysis techniques enable the chemical composition of a wine to be related to its sensory properties [80].

The most common way to relate compositional data to sensory information in any food or beverage is the use of sensory threshold data. There are different types of sensory threshold information used for a compound and it is important to distinguish between them. A volatile compound's sensory *detection* threshold (also know as absolute threshold or difference threshold) is usually defined as the concentration at which that compound becomes detectable 50% of the time in a certain matrix [81]. A sensory *recognition* threshold is the concentration at which a compound can be identified, and described, in a certain matrix [81]. Different authors use differing methodologies, various matrices in which the threshold is determined (e.g. water, air, model wine) and different criteria by which a sensory threshold is calculated, and these differences will influence the threshold values obtained. Consequently, there is often a broad variation in sensory threshold concentrations obtained from different sources, and care must be taken when comparing threshold information as misinterpretations can occur. A further limitation of sensory threshold information is that the methods used to determine a threshold concentration ignore judge variability, and therefore threshold data should only be treated as indicative rather than absolute values.

A common way of applying sensory threshold data to compositional data is by calculating each compound's odour activity value (or OAV, refer to equation below) which can enable

the chemist to identify the likely importance of each compound in a matrix to the overall aroma. If a compound is measured above its sensory threshold concentration, it will have an OAV of greater than one and is likely to be detectable by sensory means in that matrix. Many wine studies have used this technique to rank the importance of volatile compounds measured in wine [6, 11, 82]. These studies have been useful in pinpointing which compounds are likely to be important to wine aroma and which are not. A limitation of these studies is that application of sensory threshold data to compositional data cannot describe the nature of the aroma that particular compounds contribute to (e.g. do they contribute to a *lemon*, or a *toasty* aroma in wine?).

$$OAV = \frac{[compound]in\ matrix\ a}{[sensory\ threshold]in\ matrix\ a}$$

Techniques that involve a combination of instrumental methods and human subjects include gas chromatography olfactometry (GC-O) and aroma extraction dilution analysis (AEDA). These techniques have been used widely in food research, but also for wine studies [68, 74, 83-92]. Typically, GC-O involves separating volatiles in a sample using GC with a human subject at the tail end of the column recording whether they detect and, if possible, describing the aromas of the volatiles as they elute from the GC. This technique is a useful tool to identify regions or even peaks on the gas chromatogram (by retention time) which are aroma-active. Identification and quantitation of these aroma-active peaks can then be carried out. AEDA is a method which uses GC-O to try to identify the relative importance of volatile aroma compounds. The sample is consecutively diluted and analysed by GC-O. In the dilution process, compounds that were once aroma active become too weak to be detected while others remain detectable by the human nose. Successive dilutions are carried out until no more compounds can be detected by GC-O. This process allows compounds to be 'ranked' in order of their likely importance to the aroma of the sample by how many dilutions they remained detectable.

The major limitation of these techniques (OAV, GC-O and AEDA) is that they investigate the sensory aspect of volatile compounds in a sample as individual entities whereas aroma nuances in a wine are rarely due to a single impact compound but the result of a complex mixture of many compounds [80]. Furthermore, these techniques do not take into account masking, additive or synergistic effects of volatile compounds that are likely to occur in a complex mixture. For example, the aroma contribution of ethyl esters in wine is considered to be additive [93] and while individually each ester may be below its respective sensory threshold concentration, and therefore undetectable, they could act together in a mixture as a group to generate a detectable aroma. Additionally, sensory evaluation methods such as

OAV, GC-O and AEDA presume that there is a linear relationship between odour perception and concentration and they not able to adequately identify, or account for, the non-linear relationships that can exist between odour perception and the concentration of certain volatile compounds [81].

In order to examine the role that complex mixtures of volatile compounds play in the perceived aroma of wine, it is important to study the aroma chemistry of a wine sample as a whole, and not only as a series of individual compounds.

1.2.1 Aroma recombination models

The ultimate test for determining the importance of particular compounds to the aroma of wine is to reconstruct the aroma of a wine according to the relative concentrations of the volatile compounds measured to give an aroma model of the actual wine [94]. Sensory comparison of the aroma model with the actual wine would then allow evaluation of whether all the important aroma-active volatile compounds present in the wine have been identified. Aroma models have been used to successfully support quantitative evaluation of the most important aroma compounds in white wine [6] and have been used to test the representative nature of wine aroma extracts [84].

The question of which volatile compounds are actually contributing to the aroma can sometimes be answered by omission experiments [94]. An omission test involves systematically removing single aroma compounds from an aroma model, followed by sensory comparison to the authentic sample or to the complete aroma model. A degree of 'similarity' is then measured by sensory panel evaluation to determine the effect on the aroma when individual compounds are omitted. If an important volatile compound is missing, then the aroma of the omission model will be significantly different from the complete aroma model (or the original wine). If the compound omitted from the model is not important to the aroma of the original wine, then the omission model will not be different from the complete aroma model. Omission experiments can be a powerful sensory tool in evaluating the importance of individual aroma compounds to the aroma of the authentic sample and have been used successfully to determine the most important volatile compounds to the aroma of Grenache rose wines [95], and Gewürztraminer and Scheurebe wine [6].

Addition tests have been successfully used to explore the sensory contribution of particular compounds in wine from Maccabeo, Spain [91]. In an addition test, a particular compound (or groups of compounds) in an aroma model are increased in concentration and compared, by sensory evaluation, to the original aroma model. A degree of similarity can be used to determine if the elevated concentration of particular compounds change the aroma of the

model. This technique can help to identify compounds that are contributing actively to the aroma of a model and those that are not. Addition models also have the potential to give information about the nature of the aroma (e.g. a *floral* or a *honey* aroma) that a particular compound might be contributing to.

Table 1-2 lists some literature examples of the use of reconstitution experiments, omission and addition models in a range of foods and beverages. A more detailed explanation of both sensory reconstitution and omission experiments is presented in a recent review by Grosch (2001) [94].

Table 1-2 Examples of aroma reconstruction experiments used in the literature

aroma reconstitution experiments only	aroma reconstitution experiments including omission tests	aroma reconstitution experiments including omission and addition tests
Chardonnay wine [96]	Gewürztraminer and Scheurebe wine [6]	wine from Maccabeo [91]
aged red wines from Rioja [84]	Grenache rose wines [95]	
grapefruit juice [97]	orange juice [105]	
coffee [98]	citrus Hyuganatsu [106]	
swiss cheese [99]	strawberry juice [107]	
butter [100]	Jasmine green tea [108]	
sweet cream butter [101, 102]	parsley [109]	
fresh tomato and tomato paste [103]	dill herb [110]	
baguettes [104]	coffee [111, 112]	
	olive oil [113]	
	pepper [114, 115]	
	French fries [116]	
	stewed beef juice [117]	

Although aroma reconstruction experiments have been used for many years in traditional food chemistry, there are few examples of the use of these types of experiments in wine. Wine is a complex medium and it is not possible to reproduce the aroma of wine with two or three compounds as can be the case for some foods and beverages. The effect of the wine matrix makes it difficult to draw precise conclusions from omission and addition experiments and it has been demonstrated that the systematic removal of compounds from an aroma model of wine does not always bring about important changes to the aroma of the model [91]. This indicates that it is the concerted contribution of a number of compounds in a wine that creates the aroma of the wine, rather than just two or three impact aroma compounds. This is particularly the case for wines that have complex aromas (e.g. Chardonnay).

Reconstitution studies are challenging and rely on accurate compositional data for substantial numbers of volatile compounds. As a consequence, the few aroma reconstruction studies published for wine generally involve just one or two wines that are dominated by only a small number of grape-derived aroma compounds. For example, in a study by Guth [6], reconstruction of the aroma of two German white wine varieties, just two

compounds were identified, namely *cis*-rose oxide and 4-mercapto-4-methylpentan-2-one, that singularly dominated and characterised the aroma of a Gewürztraminer and a Scheurebe wine, respectively. Reconstitution studies are very valuable, but due to logistical challenges, and often analytical limitations, they have rarely been used to study the compositional basis of aroma for more complex wine varieties where the aroma of the wine is generated from interactions between, and contribution from, many volatile compounds rather than just one or two impact compounds. An exception to this is an aroma characterisation study of six premium Merlot wines [118]. In this study, addition experiments were conducted to determine the role of different compounds in the aroma of the Merlot wines. This study demonstrated that the complex aroma of Merlot wine is produced from the delicate balance of numerous aroma compounds, and not from the influence of just one or two impact compounds.

1.2.2 Sensory descriptive analysis of wine

Recent studies have attempted to compare sensory data with wine compositional data on a multidimensional level by pairing quantitative compositional data with quantitative sensory descriptive data using multivariate data analysis [7, 18, 87, 88, 119-121]. Techniques such as sensory descriptive analysis allow a robust evaluation of the specific aroma nuances that are perceived in wine and generate a quantitative data set which enables straightforward comparison to instrumental data. In this way, a comprehensive investigation into the diverse aroma properties of a number of wines can be achieved.

Sensory descriptive analysis of wine aims to describe and quantify the intensity of perceived sensory attributes of the wine objectively. Most of the currently used descriptive methods generate quantitative data and hence can be used to define sensory-instrumental relationships [81] (more detailed information on descriptive analysis techniques is provided by Lawless, 1998 [81]; Meilgaard, 1999 [122]; or Stone, 1974 [123]). Like most analytical techniques, there are many variations to sensory descriptive analysis of wine described in the literature, many of which are proprietary methods. In general these techniques proceed with training of panellists and vocabulary formation, followed by the evaluation phase [81]. The purpose of the training phase is to familiarise the panellists with the samples in the study and most importantly to use the panel as a tool to develop a concise list of terms that describe the greatest sensory differences between the wines. During the evaluation phase, panellists rate each of the wines using the developed list of terms. The formal evaluations are usually replicated and carried out under controlled conditions (e.g. constant temperature, sodium lighting, isolated booths). The resulting sensory data describes both the nature and the intensity of the aroma of each wine. This data can be used to compare the wines with

other wines in the set, but also can be used to relate to other information about the wines, such as year of vintage, viticultural region or compositional data.

The main drawback of descriptive sensory analysis techniques is that extensive training of panellists must be employed which can be very expensive and time consuming. Other problems could also arise in these techniques if domination by panel leaders and misunderstanding of terms by panellists occurs. An unavoidable drawback of sensory descriptive analysis is the use of human subjects as measuring instruments. Tasters have been shown to be quite variable over time, demonstrate variability among themselves, and are highly prone to bias [81]. Consequently, a common characteristic of sensory descriptive data is that it contains a relatively high degree of noise [124]. Inadequate training of panellists can also lead to higher levels of noise in sensory data sets.

Another common characteristic of sensory data is that the variables rated are usually highly collinear [125]. The high collinearity can arise from samples having simultaneously higher or lower intensities of a number of distinguishable aroma properties. For example, older wines might have higher intensity of both *honey* and *toasty* aroma, where as younger wines will have higher intensity *citrus* and *floral* aromas. Alternatively, high collinearity in sensory data sets can arise through inadequate choice of attributes where numerous terms are chosen that describe a single aroma property. For example, the terms *buttery*, *butterscotch* and *caramel* may be different words that describe a single aroma feature of a set of samples. Large numbers of similar attribute terms can also contribute noise to sensory data sets due to panellists being split over a number of terms when rating a single aroma property.

These factors must all be taken into account and controlled within a sensory experiment for the results of that experiment to be reliable and therefore meaningful [122]. With the adequate training of panellists and the use of an appropriate descriptive analysis technique, a useful and robust quantitative description of the sensory attributes of a wine can be achieved.

1.3 Relating volatile composition to wine aroma with multivariate data analysis

There are a number of possible multivariate techniques that can be used to relate descriptive sensory data with compositional data. One of the simplest techniques that can be used is linear regression [126]. In linear regression, the concentration of a particular compound may be used to predict the scoring of a particular sensory attribute. If the prediction is good, it indicates the compound used in the prediction might be responsible for the predicted sensory property. Obviously the scope of this approach is limited to foods and beverage matrices with simple aroma structures where only one ‘impact’ compound is responsible for a

particular sensory property. As discussed previously, the aroma of wine is rarely produced by a single impact compound, but is the result of complex interactions between many compounds [80]. Regression techniques have been developed to tackle more complex systems where multiple variables are playing a role in a system. The simplest of these 'multivariate' regression tools is multiple linear regression [126]. Multiple linear regression, as its name suggests, allow multiple variables to be used to predict the scoring of a particular sensory attribute. The 'multivariate' approach is far more useful when complex systems are being explored, such as the case for investigations of the compositional basis of wine aroma. Furthermore, the multivariate approach allows large data sets to be explored, quickly and easily, for variables that relate to each other or that influence each other [127]. In recent years the use of multivariate data analysis has increased due to advances in computer software and hardware capable of dealing with large, complex data sets. Nevertheless there are limited examples in the literature where multivariate data analysis techniques have been successfully used to compare sensory and chemical data sets, particularly in the area of wine aroma research. Table 1-3 gives a summary of different types of multivariate methods which have been used to relate chemical composition to the sensory characteristics of a variety of foods and beverages.

Many different multivariate techniques have been developed to explore relationships between variables in complex data sets and include linear and non-linear methods. Linear methods include multiple linear regression (MLR), general procrustes analysis (GPA), canonical variate analysis (CVA), principal component analysis (PCA and PCR) and partial least squares regression (PLS). The most common of the non-linear methods is artificial neural networks (ANN) [128]. Linear methods are generally limited to systems where there is a linear or approximately linear relationship between the predictor and the response. Some linear methods can cope with minor non-linearities in a data set (e.g. PLS) [129]. Non-linear methods such as ANN are used to explore non-linear relationships between predictor/s and response [128].

The use of cross validation enables PCA, PCR and PLS to avoid the problem of 'overfitting' the data which is a common problem associated with data sets where the number of variables outnumbers the number of samples. In effect, cross validation makes up for shortage of data as it allows a calibration model to be tested without a set of validation samples [130]. With cross validation, the same samples are used both for model estimation and testing. A few samples are left out from the calibration data set and the model is calibrated on the data from the remaining samples. The scoring for the samples that were not used in the calibration can then be predicted, using the model calibrated on the remaining samples, and the prediction ability of the model tested and measured. The

process is repeated with another subset of the samples, and so on until every sample has been left out once. This repetitious process is ideally suited to automation by computer methods. The measure of the prediction ability of the model, by cross validation, is used by the analyst to limit the number of independent variables used in the regression, so that 'overfitting' of the data does not occur, and so that the most realistic and reliable model is achieved [130, 131].

Table 1-3 Multivariate data analysis methods used to relate chemical composition and sensory characteristics of different foods and beverages

method	matrix
Multiple linear regression analysis (MLR, includes stepwise regression)	Spanish Chardonnay, Garnacha and Macabeo wine [7] Spanish white, rose and red wine [23] Tea [132] Tomatoes [133] Boiled prawns [134] Carrots [135]
General procrustes analysis (GPA)	French Chardonnay wine [87] Ice cream [136]
Canonical Variate Analysis (CVA)	No relevant references found Review [128]
Principal component analysis (PCA)	Californian Chardonnay wine [137] Chardonnay wines [138] Fuji apples [139] Carrots [135] Cavourmas (Greek cooked meat product)[140]
Principal component regression (PCR)	Tea [132]
Partial least squares regression (PLS, PLSR)	Spanish red wine [18, 120] Californian Chardonnay wine [88] Californian Sauvignon blanc, Australian Semillon, Austrian Muskat Ottonel [119] Chilean Cabernet Sauvignon wine [121] Chilean Pisco spirit [141] Light beer [142] Tea [132] Carrots [143] Cheese [144-146] Frozen peas [147] Ice cream [136] Boiled prawns [134] Porcine meat patties [148] Review [128]
Artificial Neural Networks (ANN, neural nets)	Blackcurrants [149] Review [128] looks at non-linear effects between variables

1.3.1 Principal component analysis

PCA is an excellent tool for visualisation of data because it is possible to describe a very large proportion of the variability in the data set using just a few of the most significant independent variables. The independent variables constructed using this technique are called principal components (PCs). The PCs constructed can be used to examine any

relevant and interpretable structure in a data set [129]. A PCA plot gives a picture which can be used to illustrate the most important differences between groups of samples, and identifies those variables which have the greatest influence among the samples measured. Furthermore, a PCA plot of sensory and compositional data allow patterns between the data sets to be interpreted and can be used to identify particular compounds that might be related to a particular aroma attribute. PCA has been used in this manner to compare sensory with chemical data for various foods (refer to Table 1-3). It is important to note that misinterpretations can occur using PCA, as variables that relate to each other mathematically do not necessarily indicate a causative relationship. As with any multivariate analysis technique, prior knowledge and understanding of data (including inherent assumptions), experience and intuition must be used to carefully interpret the main relevant phenomena in the data.

1.3.2 Partial least squares regression

The basic PLS concept and algorithm was first developed for applications in the social sciences by Herman Wold [150]. PLS regression was later developed by his son Svante Wold and Harald Martens into a more robust and general purpose technique [151]. In principle, the PLS method maximises the covariance between the latent variable of the *x*-matrix and the *y*-matrix vector. The method is based on a bilinear model with respect to the objects and the variables of the *x* and *y*-matrices [152]. A successful class of applications of PLS regression is 'soft modelling' or exploratory data analysis where the aim is to determine if there are any valid underlying relationships between two blocks of data (e.g. sensory and compositional data) [153]. Using the soft modelling application of PLS regression, models can be developed from compositional data (*x*-variables) so that aroma attribute scores (*y*-variables) can be predicted [128].

There are two variations of PLS regression that can be used to relate chemical and sensory data sets, they are often termed PLS1 and PLS2 [126]. These methods are very similar, the only modification being that where PLS1 relates numerous *x*-variables (chemical compounds) with one *y*-variable (sensory attribute), PLS2 can simultaneously relate many *x*-variables with many *y*-variables [126]. In one model, the PLS2 algorithm not only accounts for collinearity between *x*-variables, but also the collinearity between *y*-variables [126] which might be useful for interpretation of sensory data sets which are highly collinear. For interpretation purposes it can be advantageous to use PLS2, however, for prediction purposes it is usually better to calibrate for each *y*-variable separately (i.e. PLS1) [126]. Overall, it is important to note that PLS1 and PLS2 are fundamentally the same, even though their bilinear model may be written in different ways [154], and that neither can be considered the 'better method' for prediction of sensory properties using compositional data.

Using PLS regression to model the scoring of sensory attributes using chemical data allows selection of those compounds that have the highest loading (or weight) on the regression. PLS regression might then be performed with those selected compounds resulting in efficient models which use a small number of compounds to predict specific sensory properties. In this way, just a few compounds which strongly relate to the scoring of a particular attribute can be extracted from the chemical data set for the prediction of sensory assessments [155]. In other words, PLS regression makes it possible to identify those compounds, or groups of compounds, most likely to be directly responsible for a particular aroma.

Variable selection is important for successful analysis and interpretation of PLS data analysis [155]. Poor variable selection can spoil the PLS regression and lead to misinterpretations of the data analysis. Variable selection methods try to find the most relevant and important variables and base the whole calibration on these variables. The two main questions in variable selection are which strategy to use for the search, and which criterion to use, for optimisation of the number of variables [126].

Techniques involving a 'hands-on' search strategy include forward selection and backward elimination [126, 156]. In forward selection, the strategy is to find the best single variable, the best one to add to it, the next best one to add to those two, and so on. Although computationally this is relatively straightforward, the disadvantage of this method is that it does not guarantee that the best combinations of variables will be found. The backward elimination strategy involves starting with all variables and deleting uninteresting variables successively until only the most influencing variables remain in the model. Although tedious, the backward method is more likely to identify only the most important variables in the PLS regression. With any 'hands-on' approach the operator is being led by finding a model with fewer *x*-variables, and by the criterion used to assess the prediction ability of the model. The major limitation of the 'hands-on' approach is that it is time consuming and often tedious. Furthermore, the results from the different strategies, or even different attempts of the same strategy, can often result in the selection of slightly different *x*-variables giving rise to models with similar prediction ability. This is not surprising as there is seldom one 'perfect' model with the ultimate selection of *x*-variables, rather a number of possible choices which give a model with approximately the same prediction criteria. In these cases, prior knowledge and understanding of the nature of the *x*-variables and *y*-variables is paramount to the interpretation of the variables selected.

A useful 'hands-off' tool, that is readily programmable and automated by multivariate analysis software, that can be used for variable selection is the technique known as jack-knifing (JK)

or 'uncertainty testing'. JK is a versatile technique that was developed for PLS regression to identify non-contributing variables and to optimise regression models [126, 157, 158]. Elimination of non-contributing variables from a model, using JK, results in a model that is simplified (i.e. fewer *x*-variables) and made more reliable [145]. The variables selected to remain in the model are significantly contributing to the regression and are therefore most likely to have a direct relationship with the object they are predicting (e.g. sensory property). The JK technique is based on a similar principle to cross validation as it deletes one sample at a time and the regression coefficients are computed for each subset. The set of regression coefficient vectors gives information about the variability and can be combined in a simple formula to give estimates of the standard errors [126]. In doing so, the software identifies variables that are unstable (i.e. have a large standard error), which can then be made passive and the regression recomputed with only the most stable variables. In effect, the JK technique is a 'software controlled' rather than a 'user controlled' backward elimination technique, but is much faster than the 'hands-on' approach described above. Although automatic procedures, such as JK, are very valuable, if prior knowledge exists that can be used to exclude or include variables then this should be used in preference [126]. It should be noted that the JK technique should only be used in combination with the PLS1 method and is not suited to PLS2 as misinterpretations may occur [159].

In forward and backward variable selection the F-test can be used as a criterion to compare models of different sizes (different number of *x*-variables) by calculation of the F value (or F statistic) for each model. If the F-value of the larger model (with more *x*-variables) is not significant it can be concluded that the extra *x*-variables are not useful [126]. Another useful criterion is the root mean square error of prediction (RMSEP) or the root mean square error of cross validation (RMSECV) as given by the equations below (nomenclature as defined by Næs et al 2002 [126]).

$$RMSEP = \sqrt{\sum_{i=1}^{N_p} (\hat{y}_i - y_i)^2 / N_p} \qquad RMSECV = \sqrt{\sum_{i=1}^{N} \left(\hat{y}_{CV,i} - y_i\right)^2 / N}$$

$$RMSEP^2 \approx SEP^2 + BIAS^2$$

The RMSEP is a measurement of the average difference between predicted and measured response values at the prediction or validation stage. It can be interpreted as the average prediction error, expressed in the same units as the original response values [131]. The RMSEP is calculated using a test set of samples, whereas the RMSECV is calculated by an internal cross validation set. A reasonable model choice will be one that minimised the RMSEP (or RMSECV) and is therefore neither 'underfitted' nor 'overfitted' [126]. If a smaller

model has a similar RMSEP value compared to a larger model, then the smaller will usually be a better choice [126].

Although there is no 'best method' in multivariate data analysis, jack-knifed PLS regression is particularly versatile and transparent [154]. Without the need for a detailed theoretical knowledge of mathematics and statistics, PLS and JK can be used to solve many different data analytical tasks with very good statistical performance [154]. Consequently, this method is considered appropriate and robust for prediction of wine sensory properties using compositional data.

Is it well known that non-linearities exist between perceived intensity and concentration [81] and, although PCR and PLS regression are excellent methods for modelling linear and near-linear relationships, they are not always sufficient where growing non-linear relationships exist in the data set. Some transformations of PLS regression and PCR can be made to allow for the prediction of some more acute non-linear relationships [129], but this requires prior knowledge of the form of non-linearities so that the appropriate transformations can be made. A number of alternative techniques have been developed which can deal with non-linear relationships in multivariate data sets. Artificial neural networks (ANN) is one such method that is growing in use for modelling non-linear relationships in engineering and agricultural disciplines [128, 160, 161]. The potential benefits of using ANN for prediction of sensory-instrumental relationships has been described by Wilkinson and Yuksel, 1997 [162], however, very few papers have been published which describe the prediction of sensory properties with compositional data using ANN. In one example, ANN was used successfully to predict flavour intensity in blackcurrant concentrates [149]. It has been suggested that although ANN cannot replace PLS or PCR for linear relationships it might offer potential for modelling non-linear relationships between sensory and instrumental data [162]. The biggest limitation of ANN is that very large data sets are usually required to ensure that overfitting of the data is less likely to occur [160].

1.3.3 Interpretation of multivariate prediction models

It is important to note that all predictive regressions developed are merely mathematical equations. The variables (volatile compounds) in the models developed using PLS (or other multivariate predictive techniques) should be interpreted as showing association with the sensory attribute predicted, rather than as direct cause and effect relationships [126]. For the most useful and realistic interpretation of the results from the predictive regressions it is imperative to have a solid understanding of the nature of the variables (both sensory and volatile chemical variables), the limitations of the methods by which the variables were obtained (including the standard error of the reference method), and the limitations of the

multivariate method used. With this in mind, careful interpretation of the results of regression models can lead to useful conclusions. The best measure of a causative relationship between sensory perception and volatile compounds is through sensory experiments involving reconstitution of the volatiles in question.

1.4 Aims of this project

The aim of this study was to explore the compositional basis of wine aroma for two Australian commercial varieties, namely Riesling and unwooded Chardonnay.

This project involved:

- Selection of 20 Riesling and 20 unwooded Chardonnay wines with a broad range of sensory characteristics (for Riesling see Chapter 3, and unwooded Chardonnay see Chapter 4);
- Development of analytical methods and application of these, and other published and unpublished analytical methods, to measure targeted volatile compounds in 20 Riesling and 20 unwooded Chardonnay wines (see Chapter 2);
- Sensory descriptive analysis of 20 Riesling and 20 unwooded Chardonnay wines (for Riesling see Chapter 3, unwooded Chardonnay see Chapter 4);
- Multivariate analysis of the volatile and sensory data obtained to identify key aroma compounds in these two varieties (for Riesling see Chapter 3, unwooded Chardonnay see Chapter 4);
- Comparison of the results obtained for the two varieties including volatile chemical, sensory and multivariate analysis (see Chapter 5); and
- Exploration of the relationship between sensory data and rapid instrumental techniques and comparison to volatile chemical analysis (see Chapter 6).

A separate project was also conducted, as part of this thesis, which aimed to quantitatively and qualitatively investigate the formation of wine lactone, from two possible precursors, through hydrolytic studies and subsequent chiral analysis (see Chapter 7).

Chapter 2 Development of analytical methods

2.1 Introduction

High-resolution gas chromatography (GC) techniques coupled with fast-scan mass spectrometers (MS) allow not only the separation but also the structural identification of trace amounts of wine volatiles [1]. Mass spectra of acceptable quality are potentially obtainable for every compound that is separated by the gas chromatograph, even though such compounds might be present in wine in nanogram per litre concentrations only and elute from the GC column over periods of just a few seconds [163].

2.1.1 Stable isotope dilution analysis

In typical gas chromatography-mass spectrometry (GC-MS) quantitation work, an internal standard is used to determine the concentration of the compounds present. Traditionally, the internal standard used is a single compound with a similar structure to the analytes. Ideally, this compound is not present in the matrix to be analysed to start with [29]. However, some internal standard methods have been found to be inadequate for accurate and precise quantitative GC-MS analysis of trace components in wine [164]. Furthermore, great care must be taken when using traditional internal standards together with commonly used headspace techniques or solid phase microextraction (SPME) if accurate quantitation is to be achieved. For this reason stable isotope dilution analysis (SIDA) was developed and has become one of the preferred methods to quantify aroma compounds in wine (the following references are examples of SIDA - GC-MS applied to wine compositional analysis [6, 26, 29, 44, 164-168]). SIDA uses an isotopically labelled (commonly deuterium) analogue of the analyte as the internal standard. A precisely measured amount of the analogue is added to a precisely measured volume of the sample matrix (eg. wine), prior to sample preparation. The volatile organic compounds can then be sampled from the wine, using any appropriate isolation, extraction or concentration sample preparation technique, and injected into the GC-MS. Typical sample preparation techniques include SPME for either liquid or headspace sampling, and also liquid/liquid extraction techniques.

In SIDA, the isotopically labelled compound, which must be added at a concentration similar to that expected of the analyte [169], will act chemically and physically in an almost identical manner to the analyte when sample preparation is undertaken. Any losses experienced by the labelled standard will be experienced in a virtually identical fashion by the unlabelled analyte under the same conditions during extraction, concentration and analysis [165, 168]. Consequently, the accuracy of the analysis is not reduced by inefficiency in isolation or by analyte decomposition. Regardless of what happens during sample preparation and

analysis, the ratio of the isotopically labelled standard to its non-isotopically labelled analogue remains the same [168]. With SIDA, complete extraction of the analyte of interest from the matrix is no longer a necessity. Mass spectrometry has the advantage of being able to determine relative amounts of each compound present in a mixture. This comparison of the ratio of the analyte and the internal standard in samples enables the calculation of the amount of analyte present in a sample. Specifically, this is achieved by measuring the areas of the extracted ion chromatograms for specific ions of the analyte versus specific ions of the unlabelled standard (provided that the labels are not lost from when fragment ions are formed). Furthermore, by using the selected ion monitoring (SIM) technique, the sensitivity (signal / noise ratio) of the MS can be significantly increased as only those ions selected will be monitored.

The main drawback of SIDA as an analytical method is that the stable isotope labelled standards must usually be synthesised and this can be time consuming. Nevertheless, this method is very effective and robust for the analysis of wine volatiles using a broad range of sample preparation techniques and can result in accurate and precise quantitative compositional data.

2.1.2 Development and application of analytical methods

Analytical methods were required to measure a range of volatile aroma compounds targeted as being likely to be important to the aroma of Riesling and unwooded Chardonnay wines. A number of analytical methods had previously been developed for compositional studies at the Australian Wine Research Institute (AWRI) that could be used to measure the study wines for several of the compounds on the target list. Analytical methods were developed for those compounds for which methods of measurement were not available.

This chapter details the development of two analytical methods for a range of wine volatiles which both use headspace solid phase microextraction (HS-SPME), gas chromatography-mass spectrometry (GC-MS) and stable isotope dilution analysis (SIDA). In particular, a method was developed for measuring 31 fermentation-derived volatiles including short chain fatty acids, and the ethyl esters, alcohols and acetates of those acids [170]. A second method was developed for the convenient simultaneous analysis of diacetyl and *trans*-ethyl cinnamate.

This chapter also describes a number of analytical methods, which were developed by others, that were used to measure the study wines for various volatile aroma compounds including a range of grape- and oak-derived compounds [171-173], 4-vinylguaiacol and

4-vinylphenol [174], methionol (unpublished method), low molecular weight sulfur compounds and (E)-1-(2,3,6-trimethylphenyl)buta-1,3-diene (TPB) [39, 40].

2.2 Results and discussion

The methods described in this chapter were all validated by duplicate spiked standard additions to model wine and white wine matrices to determine calibration functions for each analyte. Each method was tested for repeatability to ensure that the methods used for the analysis of wine samples were precise. Prior to using these methods to analyse wines, the standard solutions (of known concentration of analytes), which were used for standard additions, were checked against freshly made solutions to ensure that the concentration of each analyte remained stable. The methods described in subsequent sections were used to analyse the study wines (20 Riesling and 20 unwooded Chardonnay) for a range of targeted volatile compounds. The methods used were accurate and precise and suitable for the number of analyses that were required. The results for the analysis of the study wines are presented for Riesling in Chapter 3 and for unwooded Chardonnay in Chapter 4. The wines that were analysed were all stored under nitrogen at -18ºC following the sensory study, until volatile chemical analysis could take place. Although the freezing process might have slightly changed the composition of the wines, storage at -18ºC was considered the best possible solution for long-term storage as time factors did not allow chemical analysis to take place while the sensory studies were in progress.

2.2.1 Fermentation-derived compounds

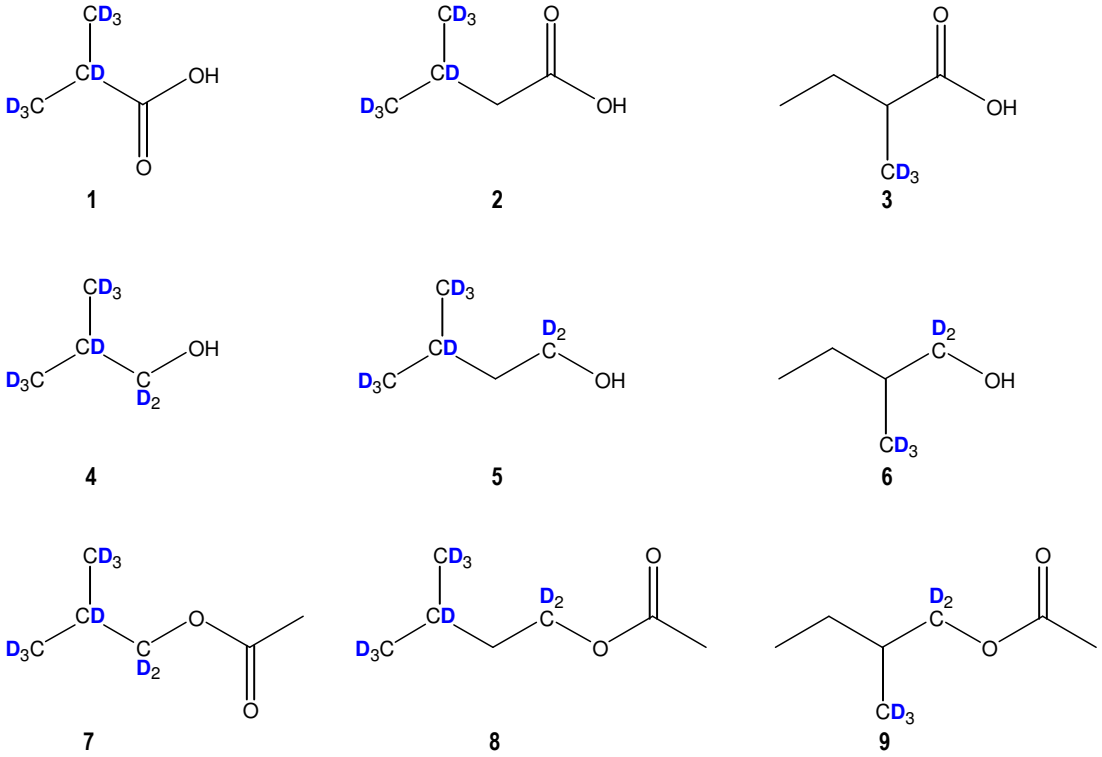
A method was developed to analyse 31 fermentation-derived compounds using a combination of SIDA, HS-SPME and GC-MS. Others from the AWRI, in particular, Tracey Siebert and Alan Pollnitz were also involved in the development of the analytical method together with the author of this thesis. The analytical method development for the fatty acids and alcohols was carried out by the author of this thesis.

2.2.1.1 Synthesis of deuterium labelled standards

In order to use SIDA for each of the 31 analytes, the deuterium labelled analogues of each analyte were required. Some of these deuterium labelled compounds were available commercially and several were prepared synthetically. A range of d_5-ethyl esters were synthesised by Corrina Neuwöhner, George Skouroumounis and Tracey Siebert, d_3-2-phenylethylalcohol and d_3-2-phenylethyl acetate were synthesised by Kevin Pardon [170]. A number of deuterium labelled branched-chain acids, alcohols and acetates were targeted for synthesis by the author as depicted in Figure 2-1 (details of synthesis given in Section 2.4.2.1).

The synthetic strategy used to prepare d_7-2-methylpropanoic acid (**1**), the corresponding d_9-alcohol (**4**) and d_9-acetate (**7**) is shown in Scheme 1; d_7-3-methylbutanoic acid (**2**), the corresponding d_9-alcohol (**5**) and d_9-acetate (**8**) in Scheme 2; and d_3-2-methylbutanoic acid (**3**), the corresponding d_5-alcohol (**6**) and d_5-acetate (**9**) in Scheme 3.

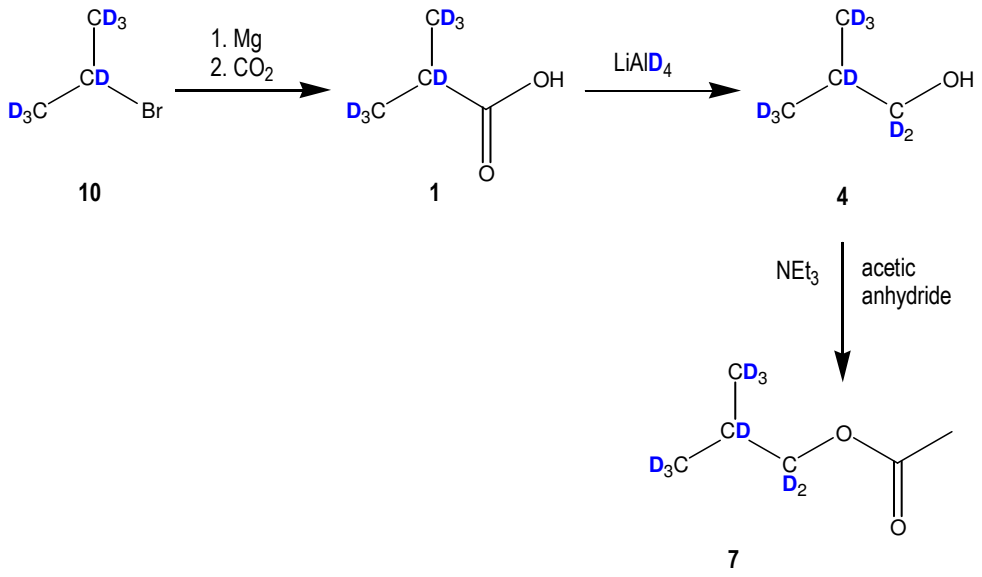
Figure 2-1 Deuterium labelled compounds targeted for synthesis by the author



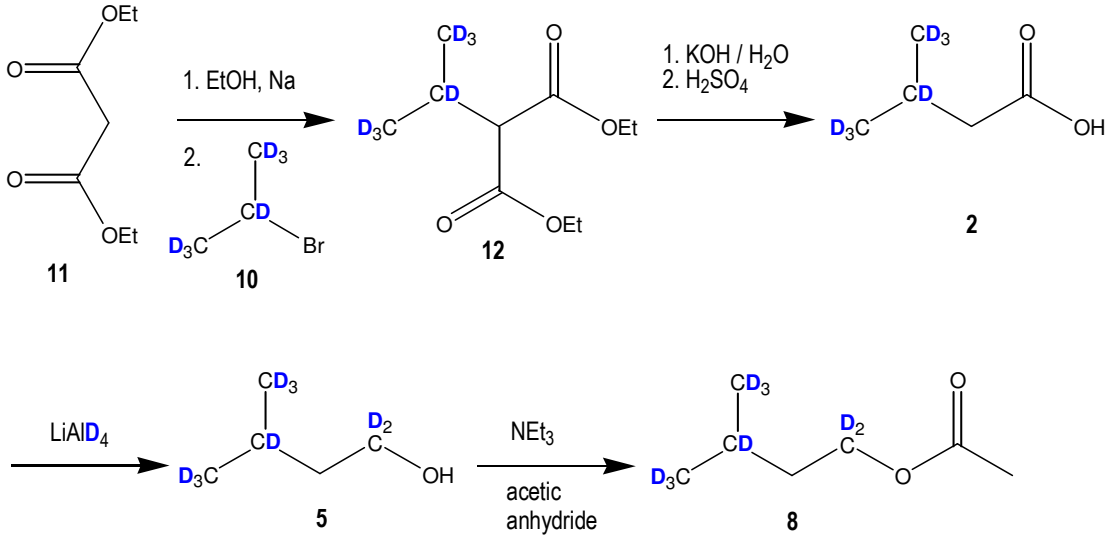
d_7-2-Methylpropanoic acid (**1**) was synthesised from d_7-2-bromopropane (**10**) using similar experimental conditions to that described by Pearson et al for reaction of a Grignard reagent with carbon dioxide [175] (Scheme 1). For the preparation of the corresponding alcohol (**4**) and acetate (**7**) a similar approach was adopted to that of Rowan et al for the preparation of deuterium labelled 2-methylbutanol and acetate [176]. Additional deuteriums were introduced into the d_9-alcohol (**4**) and d_9-acetate (**7**) by using lithium aluminium deuteride to reduce the d_7-acid (**1**) to the d_9-alcohol (**4**).

d_7-3-Methylpropanoic acid (**2**) was prepared in two steps (Scheme 2). The first step involved the preparation of d_7-diethylisopropylmalonate (**12**) from the nucleophilic addition of diethylmalonate (**11**) to d_7-2-bromopropane (**10**) using similar reaction conditions to that used by Adams et al [177]. In the second step, d_7-3-methylbutanoic acid (**2**) was synthesised from d_7-diethylisopropylmalonate (**13**) using similar conditions to those described by Vliet et al [178]. The corresponding d_9-alcohol (**5**) and d_9-acetate (**8**) were prepared in a similar manner to d_9-2-methylpropanol (**4**) and d_9-acetate (**7**).

Scheme 1

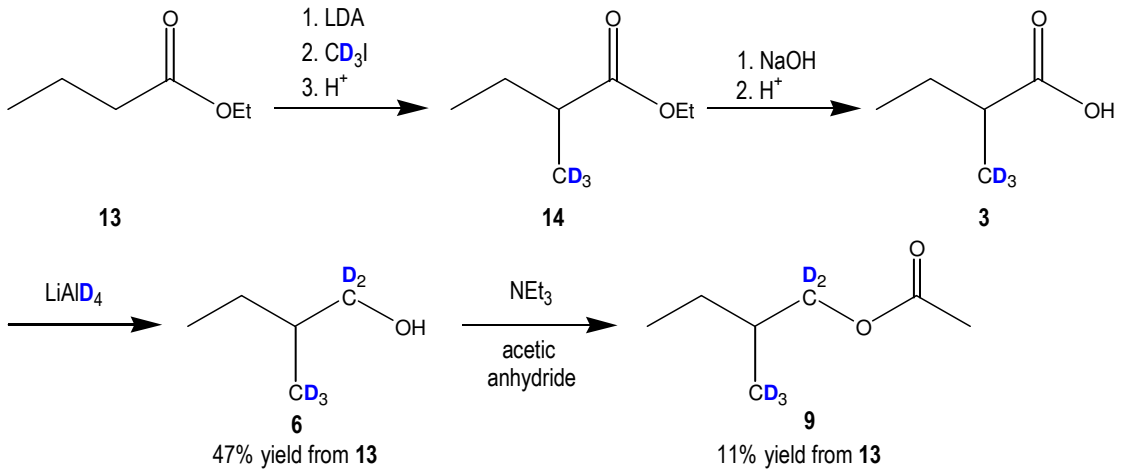


Scheme 2



d_3-2-Methylbutanoic acid (**3**), d_5-2-methylbutanol (**6**) and d_5-2-methylbutyl acetate (**9**) were synthesised as described by Rowan et al [176] with only slight modification (Scheme 3). Additional deuteriums were introduced into the d_5-alcohol (**6**) and d_5-acetate (**9**) by using lithium aluminium deuteride to reduce the d_3-acid (**3**) to the d_5-alcohol (**6**). The advantage of additional deuteriums with SIDA is that greater separation between analyte and labelled analogue is achieved on the GC column (sometimes to baseline, depending on the GC conditions). Additionally, a greater number of distinct ions between analyte and deuterium labelled analogue can often be utilised for quantitation and selected ion monitoring (SIM) when a greater number of deuterium atoms are present.

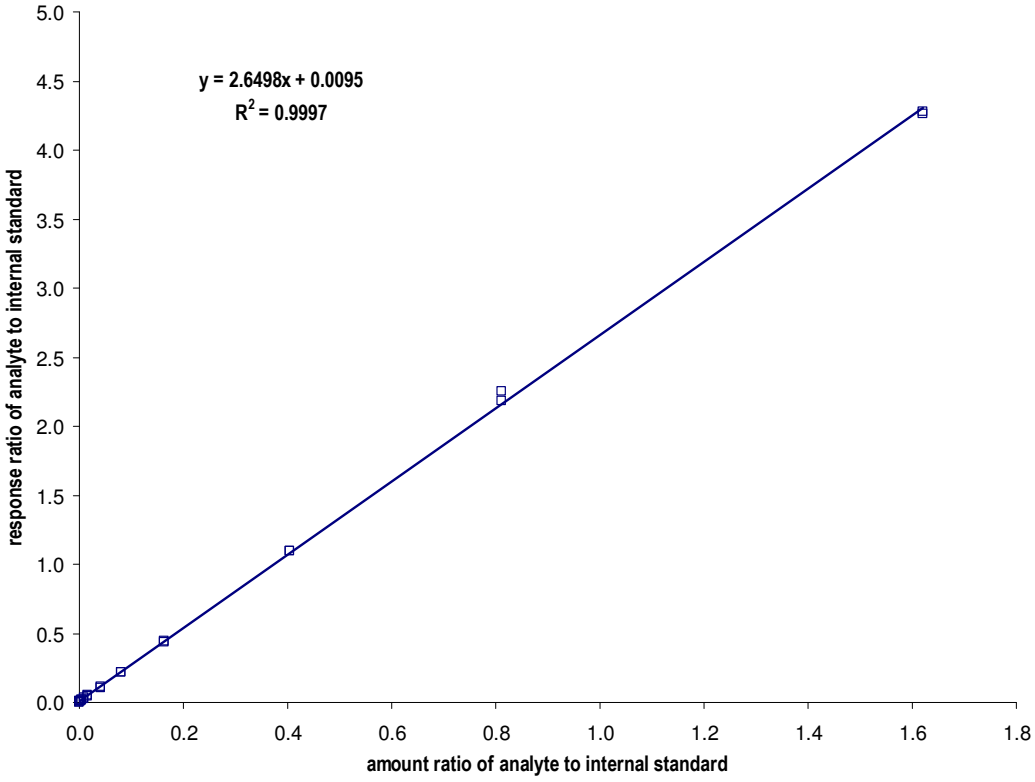
Scheme 3



2.2.1.2 Analytical method development and validation

A standard addition calibration function was developed for model wine and white wine for each analyte (details given in Section 2.4.1 and Section 2.4.2). An example of a typical standard addition calibration function in model wine is depicted for 2-methylpropanol in Figure 2-2.

Figure 2-2 Standard addition calibration function for 2-methylpropanol in 1/10 diluted model wine



concentrations plotted: 0, 5, 10, 25, 50, 100, 250, 500, 1000, 2500, 5000, 10000 (x 2 data points) µg/L

An accurate and precise calibration range was demonstrated for each analyte in both model wine and white wine matrices as shown in Table 2-1. Spiked wines were diluted 1/10 with water prior to addition of labelled standards for analysis. For most carboxylic acids analysed by HS-SPME the calibration equation was quadratic rather than linear, but were nevertheless consistently quantified accurately across the range shown in Table 2-1.

Table 2-1 Calibration range and correlation coefficients for quantitation of fermentation-derived compounds in model wine

analyte	calibration range (1/10 dilution) [a]	equivalent calibration range in wine	coefficient of determination (R^2)	number of data points [b]
ethyl acetate	0, 1 - 5000 µg/L	0, 10 - 50000 µg/L	0.9999	10
ethyl propanoate	0, 0.1 - 500 µg/L	0, 1 - 5000 µg/L	1.0000	12
ethyl 2-methylpropanoate	0, 0.1 - 500 µg/L	0, 1 - 5000 µg/L	0.9999	13
2-methylpropyl acetate	0, 0.1 - 500 µg/L	0, 1 - 5000 µg/L	1.0000	13
ethyl butanoate	0, 0.1 - 500 µg/L	0, 1 - 5000 µg/L	1.0000	13
ethyl 2-methylbutanoate	0, 0.1 - 500 µg/L	0, 1 - 5000 µg/L	0.9991	10
ethyl 3-methylbutanoate	0, 0.2 - 500 µg/L	0, 2 - 5000 µg/L	0.9986	10 [c]
2-methylpropanol	0, 5 - 5000 µg/L	0, 50 - 50000 µg/L	0.9997	10
2-methylbutyl acetate	0, 0.5 - 500 µg/L	0, 5 - 5000 µg/L	0.9999	10 [c]
3-methylbutyl acetate	0, 2 - 500 µg/L	0, 20 - 5000 µg/L	0.9998	7
butanol	0, 25 - 25000 µg/L	0, 250 - 250000 µg/L	0.9966	10 [d]
2-methylbutanol	0, 250 - 10000 µg/L	0, 2500 - 100000 µg/L	0.9998	6 [e]
3-methylbutanol	0, 250 - 10000 µg/L	0, 2500 - 100000 µg/L	0.9984	8 [c]
ethyl hexanoate	0, 0.1 - 100 µg/L	0, 1 - 1000 µg/L	0.9994	11 [c]
hexyl acetate	0, 0.1 - 100 µg/L	0, 1 - 1000 µg/L	0.9999	9 [c]
ethyl lactate	0, 50 - 5000 µg/L	0, 500 - 50000 µg/L	0.9992	6 [c]
hexanol	0, 5 - 500 µg/L	0, 50 - 5000 µg/L	0.9999	6
ethyl octanoate	0, 0.2 - 100 µg/L	0, 2 - 1000 µg/L	0.9996	10 [c]
acetic acid	0, 200 - 200000 µg/L	0, 2000 - 2000000 µg/L	0.9993	10
propanoic acid	0, 20 - 500 µg/L	0, 200 - 5000 µg/L	0.9944	6
2-methylpropanoic acid	0, 10 - 2000 µg/L	0, 100 - 20000 µg/L	0.9999	9
ethyl decanoate	0, 0.1 - 200 µg/L	0, 1 - 2000 µg/L	0.9974	10
butanoic acid	0, 5 - 500 µg/L	0, 50 - 5000 µg/L	0.9994	10
2-methylbutanoic acid	0, 5 - 500 µg/L	0, 50 - 5000 µg/L	0.9996	9
3-methylbutanoic acid	0, 5 - 500 µg/L	0, 50 - 5000 µg/L	0.9949	8 [c]
2-phenylethyl acetate	0, 0.5 - 100 µg/L	0, 5 - 1000 µg/L	0.9998	10
ethyl dodecanoate	0, 0.1 - 200 µg/L	0, 1 - 2000 µg/L	0.9994	12 [c]
hexanoic acid	0, 5 - 500 µg/L	0, 50 - 5000 µg/L	0.9998	13
2-phenylethanol	0, 5 - 2500 µg/L	0, 50 - 25000 µg/L	0.9997	9
octanoic acid	0, 10 - 1000 µg/L	0, 100- 10000 µg/L	0.9995	10 [c]
decanoic acid	0, 5 - 200 µg/L	0, 50 - 2000 µg/L	0.9985	8 [c]

[a] concentrations are of the analyte in the SPME vial (i.e. on the wine diluted 10 times with water); [b] each concentration was measured in duplicate for the calibration function; [c] for one concentration level only one data point was obtained; [d] for three concentration levels only one data point was obtained; [e] for four concentration levels only one data point was obtained.

Repeatability was assessed to validate the precision of the method thoroughly at various levels in model wine and white wine. In one validation exercise, model wine was extracted and analysed in pentuplicate without the addition of analytes, and then with pentuplicate

spiked standard additions of 1, 2, 2000 and 5000 μg/L of all components. In all instances the accuracy and repeatability of the analysis was < 5% RSD (relative standard deviation) for all concentrations investigated within the calibration range shown in Table 2-1. Generally, the method was accurate and precise for all 31 compounds in wine; however, quantitation of all 31 compounds of interest versus their labelled standards in a complex and variable matrix such as wine was not always straightforward for every compound in every wine.

The tenfold dilution of wine had no detrimental effects on the sensitivity of the assay for most compounds, because the dilution also reduced the ethanol content to 1%. The effect of lowered ethanol concentration on improving the sensitivity of SPME has been well documented [179, 180]. Furthermore, an increased sensitivity with dilution was observed for compounds with similar retention to ethanol (e.g. ethyl propanoate, ethyl 2-methylpropanoate and their labelled standards). Due to the relatively high level of particular compounds (e.g. ethyl acetate, ethyl octanoate, hexanoic acid, acetic acid) found in some of the wines analysed, more precise quantitation was obtained from the 1/100 dilution method, otherwise the standard 1/10 method gave the best repeatability and accuracy.

The retention times for 2-methylbutanol, 3-methylbutanol and their labelled analogues were observed to occasionally drift by up to as much as 0.4 minutes during a number of runs. Ethyl hexanoate and its labelled analogue also showed the same phenomena. This becomes a problem if the labelled ethyl hexanoate peak co-elutes with the labelled 2- and 3-methylbutanol peaks or if the unlabelled ethyl hexanoate peak co-elutes with the unlabelled 2- and 3-methylbutanol. It might affect any or all of the four 2- and 3-methylbutanol peaks (two for the labelled internal standards and two for the analytes) due to common ions in the labelled and unlabelled ethyl hexanoate spectra. The least affected ion was chosen as the target ion for each of the labelled and unlabelled 2- and 3-methylbutanol and data from samples that experienced this co-elution problem were not used.

All of the study wines (20 Riesling and 20 unwooded Chardonnay) were analysed, using this method, for fermentation-derived compounds.

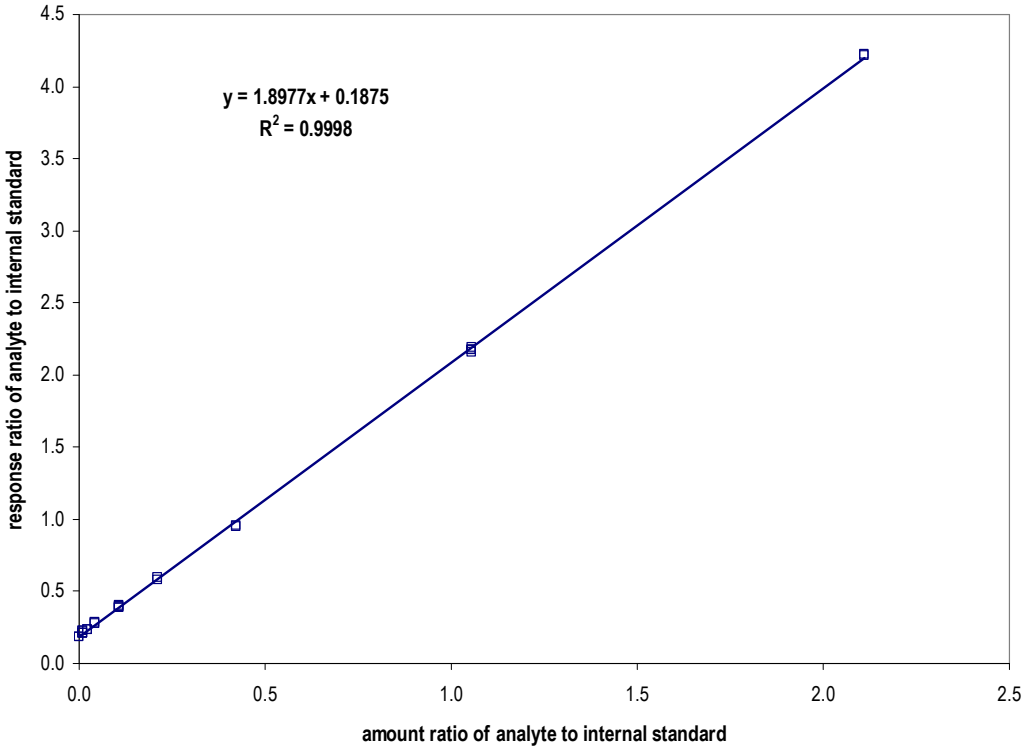
2.2.2 Diacetyl and trans*-ethyl cinnamate*

Both diacetyl and *trans*-ethyl cinnamate were targeted for analysis. Analytical methods have been published for both of these compounds individually using GC-MS and the same GC column type (Carbowax) [77, 181]. For the convenience of analysis, an automated method was developed for the simultaneous analysis of diacetyl and *trans*-ethyl cinnamate in white wine using HS-SPME, GC-MS and SIDA, and validated. According to the literature, diacetyl has been measured in white wine at concentrations of 150 – 180 μg/L and has a sensory

threshold concentration of 100 µg/L in model wine (10% ethanol in water w/w) [6]. *Trans*-ethyl cinnamate has been measured in white wine at 2.0 – 2.3 µg/L and has a sensory threshold concentration of 1 µg/L in model wine (10% ethanol in water w/w) [6]. The analytical method was developed with the aim of covering these concentration ranges. Column type, oven temperatures, general GC conditions, fibre type and sample preparation were adopted from the existing literature methods [77, 181] and optimised for the concerted analysis of these two volatiles (details given in Section 2.4.1 and Section 2.4.3).

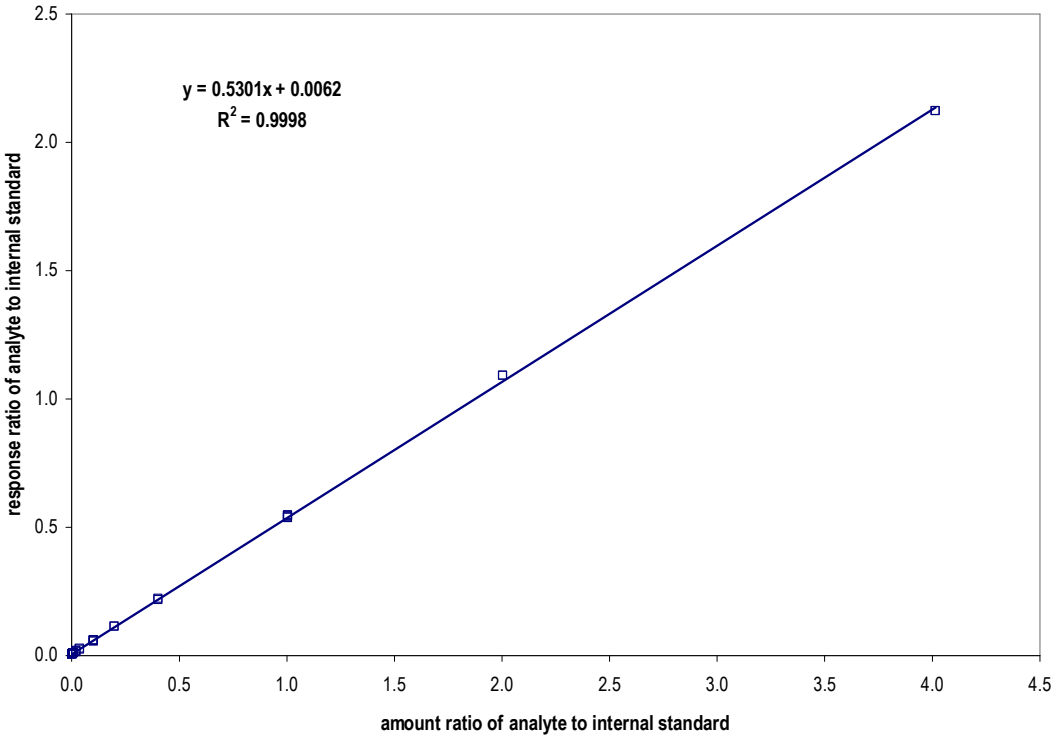
Deuterium labelled d_6-diacetyl was commercially available and deuterium labelled d_5-*trans*-ethyl cinnamate was synthesised by simple esterification of cinnamic acid using d_6-ethanol. Standard addition calibrations in model wine and white wine were developed for each analyte. The diacetyl standard addition calibration developed for white wine (0, 5 - 1000 µg/L) is shown in Figure 2-3. The white wine regression calibration does not go through the origin due to the diacetyl originally present (52 µg/L) in the white wine used for standard additions. The diacetyl standard addition calibration was linear throughout the range 0, 5 - 5000 µg/L for model wine and 0, 5 – 1000 µg/L for white wine with excellent repeatability as tabulated in Table 2-2.

Figure 2-3 Standard addition calibration function for diacetyl in white wine



concentrations plotted: 0, 10, 20, 100, 200, 1000 (x 2 data points) and 5, 50, 500 (x 5 data points) µg/L

Figure 2-4 Standard addition calibration function for *trans*-ethyl cinnamate in white wine



concentrations plotted: 0, 0.1, 0.2, 0.5, 1, 2, 10, 20, 100, 200 (x 2 data points) and 5, 50 (x 5 data points) µg/L

The *trans*-ethyl cinnamate standard addition calibration developed for white wine (0, 0.1 - 200 µg/L) is shown in Figure 2-4. The *trans*-ethyl cinnamate standard addition calibration function was linear throughout the range 0, 0.1 - 250 µg/L for model wine and 0, 0.1 - 200 µg/L for white wine with excellent repeatability as also shown in Table 2-2.

Table 2-2 Repeatability of analysis for diacetyl and *trans*-ethyl cinnamate

analyte	replicates	spike level (µg/L)	model wine			white wine		
			mean (µg/L)	SD (µg/L)	CV (%)	mean (µg/L)	SD (µg/L)	CV (%)
diacetyl	5	529	512	17.5	3	523	2.6	0.5
trans-ethyl cinnamate	5	50.2	50.2	0.33	0.7	50.6	0.29	0.6

SD: standard deviation; CV: coefficient of variation.

The analytical method for diacetyl and *trans*-ethyl cinnamate was used to measure these analytes in the study wines.

2.2.3 Various other yeast, grape- and oak-derived compounds

Various analytical methods were available for use by the author for the measurement of volatile compounds that had been targeted for analysis in the study wines. An analytical method, involved SIDA, liquid/liquid extraction and GC-MS, for the measurement of a number of monoterpenes, norisoprenoids and oak-derived compounds in white wine (13 in

total) had previously been developed [171-173] (details given in Section 2.4.1 and Section 2.4.4). The analysis of 4-vinylguaiacol and 4-vinylphenol was conducted by the author using existing methods [174] and involved SIDA, SPME and GC-MS (details given in Section 2.4.1 and Section 2.4.5). The compound methionol was also measured in the study wines by the author, using an available unpublished analytical method that involved SIDA, liquid/liquid extraction and GC-MS (details given in Section 2.4.1 and Section 2.4.6).

The study wines were also analysed for the compound (E)-1-(2,3,6-trimethylphenyl)buta-1,3-diene (TPB) according to the analytical method described by Janusz et al 2004 [39, 40]. The analysis of TPB in the study wines was not conducted by the author of this thesis.

2.2.4 Low molecular weight sulfur compounds

A method to measure various low molecular weight sulfur-containing compounds in wine was developed by Tracey Siebert, Alan Pollnitz and Markus Herderich. The analytes included in this analytical method are detailed in Table 2-15, Section 2.4.7. This method was applied to the Riesling and unwooded Chardonnay wines by the author and involved HS-SPME and GC-atomic emission detector (AED) instrumentation (details given in Section 2.4.7). Traditional internal standard methods were used rather than SIDA as labelled internal standards were unavailable and the AED cannot discriminate between deuterium labelled standards and the analytes of interest on the sulfur 'channel' used for the analysis. Consequently, great care was taken to prepare and analyse each sample in exactly the same manner and replicate samples were randomised over each sequence.

2.3 Conclusion

The methods described in this chapter were suitably accurate, precise and robust, and fit for the purpose of analysing large numbers of wine samples. The results from the application of these analytical methods to measure a range of important volatile aroma compounds in 20 Riesling and 20 unwooded Chardonnay wines are given in Chapter 3 and Chapter 4 respectively.

2.4 Materials and methods

All reagents used were purchased from SIGMA-Aldrich (Australia) unless otherwise stated. All solvents used were HPLC grade from OmniSolv, with the exception of ethanol, which was freshly distilled bulk ethanol. The water used was purified by a MilliQ system. Positive ion electron impact (EI) mass spectra were recorded over a scan range of *m/z* 35 – 350 (1 second cycle time). ^{1}H and ^{13}C NMR spectra were recorded with a Varian Gemini Spectrometer operating at frequencies of 300 MHz and 75.5 MHz, respectively. Spectra were recorded in deuterated chloroform ($CDCl_3$). Chemical shifts (δ) are reported in parts per million (ppm). The following abbreviations are used in the assignment of ^{1}H spectra: s = singlet; d = doublet; t = triplet; qn = quintet; m = multiplet. All reactions were carried out at room temperature unless otherwise stated. Unless stated otherwise, model wine was 10% ethanol in MilliQ water v/v, saturated with potassium hydrogen tartrate (KHT), and adjusted to pH 3.2 with tartaric acid. For each batch of samples analysed, a quality control wine (spiked with known concentrations of all analytes, and analysed "as is") was also prepared to assess the robustness of the method within each sequence. All standards, quality control samples, and wine samples were prepared for analysis in duplicate unless otherwise stated. All wine samples were stored in glass ampoules under nitrogen at -18°C and thawed to room temperature for analysis.

2.4.1 General Instrumental analysis

All prepared samples were analysed by GC-MS according to the following general instrumental procedure with the exception of those samples prepared for the analysis of low molecular weight sulfur compound analysis using GC-AED. GC-MS instrumental parameters that differed between analytical methods are detailed, for each method, in Table 2-3. Samples were analysed with either an Agilent 6890 gas chromatograph coupled to an Agilent 5973N mass spectrometer and a GERSTEL MPS2 multi purpose sampler, or an HP 6890 gas chromatograph coupled to an HP 5973 mass spectrometer with an autosampler (HP 6890 series injector). The carrier gas was helium (Air Liquide or BOC gases, ultra high purity), vacuum compensated at the mass spectrometer interface. The injector was in pulsed splitless mode.

For SPME injections, a 0.75 mm ID borosilicate glass SPME liner (Agilent) was used. The HS-SPME method was optimised for fibre type, amount of salt, fibre extraction time, incubation temperature, and desorption time and temperature. For liquid injections, the liner used was resilanised borosilicate glass, tapered, with a plug (2 - 4 mm) of resilanised glass wool near the column interface and the residence time for the needle in the injector block was approximately 100 ms.

Table 2-3 Details of instrument, column type and GC-MS instrumental parameters for each analytical method

	fermentation-derived compounds	diacetyl and *trans*-ethyl cinnamate	grape- and oak-derived compounds, method #1	grape- and oak-derived compounds, method #2	4-vinylphenol and 4-vinylguaiacol	methionol
instrument	Agilent 6890 GC Agilent 5973N MS	Agilent 6890 GC Agilent 5973N MS	Agilent 6890 GC Agilent 5973N MS	HP 6890 GC HP 5973 MS	Agilent 6890 GC Agilent 5973N MS	Agilent 6890 GC Agilent 5973N MS
autosampler	GERSTEL	GERSTEL	GERSTEL	HP 6890 series	GERSTEL	GERSTEL
column type	DB-WAX J&W Scientific 122-7062 60 m x 0.25 mm x 0.25 μm	ZB-WAX Phenomenex 7HG-G007-11 30 m x 0.25 mm x 0.25 μm	DB-1701 J&W Scientific 122-0732 30 m x 0.25 mm x 0.25 μm	DB-WAX J&W Scientific 122-7032 30 m x 0.25 mm x 0.25 μm	ZB-WAX Phenomenex 7HG-G007-11 30 m x 0.25 mm x 0.25 μm	DB-WAX J&W Scientific 122-7032 30 m x 0.25 mm x 0.25 μm
linear velocity	33 cm/sec	23 cm/sec	39 cm/sec	39 cm/sec	39 cm/sec	27 cm/sec
flow rate	2.0 mL/min	1.0 mL/min	1.2 mL/min	1.2 mL/min	1.2 mL/min	1.2 mL/min
oven temp	40ºC held for 4 min	40ºC held for 5 min	50ºC held for 1 min	50ºC held for 1 min	50ºC held for 1 min	50ºC held for 1 min
1st ramp	increased to 220ºC at 5ºC/min and held for 20 min	increased to 110ºC at 10ºC/min	increased to 250ºC at 10ºC/min held for 20 min	increased to 220ºC at 10ºC/min held for 20 min	increased to 220ºC at 10ºC/min held for 10 min	increased to 200ºC at 5ºC/min
2nd ramp	N/A	increased to 220ºC at 20ºC/min held for 20 min	N/A	N/A	N/A	increased to 240ºC at 15ºC/min for 5 min
injector temperature	200ºC	200ºC	200ºC	200ºC	220ºC	220ºC
transfer line	250ºC	250ºC	280ºC	250ºC	250ºC	240ºC
splitter	26 : 1	53 : 1	53 : 1	53 : 1	44 : 1	45 : 1
splitter opened	30 sec	36 sec	36 sec	36 sec	36 sec	30 sec
injection type	SPME	SPME	liquid	liquid	SPME	liquid
liquid injection volume	N/A	N/A	1 μL	2 μL	N/A	2 μL
SPME fibre type	65 μm CW/DVB, 'orange' SPME fibre (SUPELCO)	65 μm CW/DVB, 'orange' SPME fibre (SUPELCO)	N/A	N/A	100 μm PDMS, 'red' SPME fibre (SUPELCO)	N/A
mass of salt in SPME vial	2 g	2 g	N/A	N/A	no salt	N/A
HS-SPME extraction time	10 min	10 min	N/A	N/A	20 min	N/A
SPME extraction temp	35ºC	40ºC	N/A	N/A	room temperature	N/A
SPME desorption time	7 min	7 min	N/A	N/A	15 min	N/A
for SIM ions refer to	Table 2-5	Table 2-7	Table 2-9	Table 2-9	Table 2-11	Table 2-13

Positive ion electron impact spectra at 70 eV were recorded in the range *m/z* 35 - 350 for scan runs. For quantification of wine volatiles, mass spectra were recorded in selected ion monitoring mode (SIM).

2.4.2 Method for the analysis of fermentation-derived compounds

2.4.2.1 Preparation of deuterium labelled internal standards

Deuterium labelled compounds used as internal standards were obtained either commercially (SIGMA-Aldrich) or by synthesis as indicated in Table 2-4. The origin of the unlabelled standards is also tabulated in Table 2-4. For details on the synthesis for d_5-ethyl esters, d_{13}-hexyl acetate, d_3-2-phenylethanol and d_3-2-phenylethyl acetate see Siebert et al, 2004 [170].

Table 2-4 Origin of standards for use in method development

analyte	origin of standard	deuterium labelled internal standard	origin of standard
ethyl acetate	Merck EM OmniSolv	d_8-ethyl acetate	Aldrich 99 atom %D
ethyl propanoate	SIGMA, 99%	d_5-ethyl propanoate	Synthesised
ethyl butanoate	Aldrich, 99%	d_5-ethyl butanoate	Synthesised
ethyl 2-methylpropanoate	Aldrich, 99%	d_5-ethyl 2-methylpropanoate	Synthesised
ethyl 2-methylbutanoate	Aldrich, 99%	d_5-ethyl 2-methylbutanoate	Synthesised
ethyl 3-methylbutanoate	Aldrich, 98%	d_5-ethyl 3-methylbutanoate	Synthesised
ethyl hexanoate	Aldrich, 99+%	d_5-ethyl hexanoate	Synthesised
ethyl octanoate	Hopkin & Williams	d_5-ethyl octanoate	Synthesised
ethyl decanoate	Aldrich, 99+%	d_5-ethyl decanoate	Synthesised
ethyl dodecanoate	Synthesised	d_5-ethyl dodecanoate	Synthesised
ethyl lactate	Aldrich, 98%	d_5-ethyl lactate	Synthesised
2-methylpropyl acetate	Aldrich, 99%	d_9-2-methylpropyl acetate	Synthesised *
2-methylbutyl acetate	Aldrich, 99%	d_5-2-methylbutyl acetate	Synthesised *
3-methylbutyl acetate	Aldrich, 94+%	d_9-3-methylbutyl acetate	Synthesised *
hexyl acetate	Aldrich, 99%	d_{13}-hexyl acetate	Synthesised
2-phenylethyl acetate	Merck >99%	d_3-2-phenylethyl acetate	Synthesised
butanol	Merck	d_{10}-butanol	Aldrich 99+ atom % D
2-methylpropanol	Riedel-de Haen	d_9-2-methylpropanol	Synthesised *
2-methylbutanol	Aldrich, 99%	d_5-2-methylbutanol	Synthesised *
3-methylbutanol	Aldrich, 99%	d_9-3-methylbutanol	Synthesised *
hexanol	Aldrich, 99+%	d_{13}-hexanol	Aldrich 98 atom % D
2-phenylethanol	SIGMA	d_3-2-phenylethanol	Synthesised
acetic acid	BDH, glacial	d_3-acetic acid	Aldrich 98 atom % D
propanoic acid	Aldrich, 99.5%	d_5-propanoic acid	Aldrich 98 atom % D
butyric acid	Aldrich, 99%	d_7-butyric acid	Aldrich 98 atom % D
2-methylpropanoic acid	in house	d_7-2-methylpropanoic acid	Synthesised *
2-methylbutyric acid	Aldrich, 98%	d_3-2-methylbutyric acid	Synthesised *
3-methylbutyric acid	Aldrich, 99%	d_7-3-methylbutyric acid	Synthesised *
hexanoic acid	Hopkin & Williams	d_{11}-hexanoic acid	Aldrich 98 atom % D
octanoic acid	Hopkin & Williams	d_{15}-octanoic acid	Aldrich 98 atom % D
decanoic acid	Aldrich	d_{19}-decanoic acid	Aldrich 98 atom % D

* indicates compounds prepared synthetically by the author

Preparation of d_7-2-methylpropanoic acid (1), d_9-2-methylpropanol (4), and d_9-2-methylpropyl acetate (7) (Scheme 1)

Synthesis of d_7-2-methylpropanoic acid (**1**)

Magnesium turnings (0.61 g, 25 mmol) were stirred for 1 hour under nitrogen (N_2). Dry diethyl ether (50 mL) was added followed by the dropwise addition of d_7-2-bromopropane (**10**) (2.5 g, 19 mmol). The reaction mixture was refluxed for 3 hours. The solution was cooled to 0°C and dry carbon dioxide and nitrogen gas was bubbled though the solution for 2 hours while warming to room temperature. The reaction mixture was quenched with water (20 mL), extracted with dichloromethane (4 x 30 mL), the organics dried with magnesium sulfate ($MgSO_4$) and concentrated in vacuo to give the acid (**1**) as a crude oil. A portion was purified by Kugelrohr distillation (~155ºC) to give the title acid (**1**). *m/z* 95 (M^+, 9%), 78 (4%), 77 (30%), 58 (4%), 50 (100%), 49 (10%), 48 (7%), 46 (48%), 45 (14%), 42 (14%); ^{13}C NMR (δ) 17.7 (septet, 2 x CD_3), 33.0 (t, CD), 183.5 (s, CO).

Synthesis of d_9-2-methylpropanol (**4**)

Lithium aluminium deuteride ($LiAlD_4$, 0.74 g, 18 mmol) was added to dry diethyl ether (30 mL) and stirred for 30 minutes under N_2. After this time, d_7-2-methylpropanoic acid (**1**) (1.97 g, 15 mmol) in dry diethyl ether (10 mL) was added dropwise to the stirred solution. The resulting mixture was warmed to reflux for 2.5 hours. The cooled reaction mixture was quenched with hydrous sodium sulfate (25 g) and stirred overnight. The reaction mixture was filtered and the grey-white solid was washed with diethyl ether. The combined organics were gently distilled to approximately 10 mL at atmospheric pressure to give the alcohol (**4**) as a crude oil. Kugelrohr distillation (100 - 110°C) gave the title alcohol (**4**) in 33% yield. *m/z* 83 (M^+, 12%), 65 (3%), 64 (4%), 62 (4%), 51 (3%), 50 (100%), 49 (17%), 48 (51%), 47 (19%), 46 (68%), 45 (9%), 42 (19%), 38 (22%), 37 (28%); ^{13}C NMR (δ) 17.7 (septet, 2 x CD_3), 29.6 (t, CD), 66.8 (qn, CD_2).

Synthesis of d_9-2-methylpropyl acetate (**7**)

d_9-2-Methylpropanol (**4**) (0.76 g, 9 mmol) in diethyl ether (~4 mL) was added to acetic anhydride (2.55 mL, 27 mmol). Triethylamine (NEt_3) (4.45 mL, 32 mmol) was added to the solution which was stirred for 24 hours. Diethyl ether (15 mL) was added and the resulting organics washed with 10% hydrochloric acid (1 x 20 mL), saturated sodium hydrogen carbonate (1 x 20 mL), brine (1 x 20 mL), and water (1 x 20 mL). The organics were dried ($MgSO_4$), and gently distilled to approximately 10 mL. Kugelrohr distillation (100 - 150°C) gave the title acetate (**7**) in 42% yield (calculated over two steps from the acid). *m/z* 125 (M^+, 0.017%), 93 (1%), 86 (1%), 82 (1%), 78 (6%), 75 (16%), 64 (35%), 63 (7%), 50 (6%), 46 (15%), 43 (100%), 42 (9%), 38 (5%), 36 (13%).

Preparation of d_7-3-methylbutanoic acid (2), d_9-3-methylbutanol (5), and d_9-3-methylbutyl acetate (8) (Scheme 2)

Synthesis of d_7-diethylisopropylmalonate (**12**)

Freshly cut sodium pieces (0.97 g, 42 mmol) were refluxed in ethanol (20 mL, 350 mmol) for 30 minutes. Diethylmalonate (**11**) (6.98 mL, 46 mmol) was added dropwise to the cooled stirred solution (<50°C). After 30 minutes, *d*-2-bromopropane (**10**) (5 g, 38 mmol) was added dropwise and the reaction mixture refluxed for 2 hours. The cooled solution was quenched with water (25 mL) and brine (25 mL), and extracted with diethyl ether (5 x 20 mL). The combined organics were dried ($MgSO_4$) and concentrated in vacuo to give the title compound (**12**) as a crude oil (m = 10.9 g). *m/z* 209 (M^+, 0.05%), 191 (1%), 164 (63%), 162 (10%), 161 (100%), 134 (40%), 132 (14%), 119 (24%), 116 (14%), 114 (11%), 106 (12%), 105 (9%), 93 (15%), 91 (35%), 89 (25%), 88 (19%), 86 (14%), 77 (12%), 73 (32%), 64 (10%), 69 (9%), 45 (16%).

Synthesis of d_7-3-methylbutyric acid (**2**)

Potassium hydroxide (8.5 g, 152 mmol) in water (30 mL) was warmed to 70°C and *d*-diethylisopropylmalonate (**12**) (7.95 g, 38 mmol) in tetrahydrofuran (THF) (2 mL) was added dropwise. After refluxing for 30 minutes the ethanol produced in the reaction was removed by distillation. Sulfuric acid (26 mL, 5M, 130 mmol) was added to the cooled solution (0°C) and the resulting reaction mixture refluxed for 3 hours. The cooled mixture was extracted with diethyl ether (5 x 20 mL), dried ($MgSO_4$) and concentrated in vacuo to give the title acid (**2**) as a crude oil (m = 7.5 g). A portion was distilled by Kugelrohr distillation (175 - 185°C) to give the title acid (**2**). *m/z* 109 (M^+, 0.4%), 91 (22%), 72 (4%), 64 (4%), 63 (4%), 62 (24%), 61 (100%), 50 (24%), 49 (13%), 46 (17%), 45 (14%), 44 (15%), 43 (7%), 42 (15%), 41 (7%); ^{1}H NMR (δ) 2.22 (s, CH_2); ^{13}C NMR (δ) 21.0 (septet, 2 x CD_3), 24.6 (t, CD), 42.8 (s, CH_2), 179.3 (s, CO).

Synthesis of d_9-3-methylbutanol (**5**)

$LiAlD_4$ (0.95 g, 23 mmol) was stirred in dry diethyl ether (150 mL) under N_2 for 30 minutes. *d*-3-Methylbutanoic acid (**2**) (2.07 g, 19 mmol), in diethyl ether (50 mL), was added dropwise and the resulting mixture refluxed for 1.5 hours. The cooled reaction mixture was quenched with hydrous sodium sulfate (25 g) and stirred overnight. The reaction mixture was filtered and the grey-white solid was washed with diethyl ether (2 x 20 mL). The combined organics were gently concentrated by distillation with a Vigreux column to less than 10 mL containing the crude alcohol (**5**) (m = 1.4 g). A portion was purified by Kugelrohr distillation (130 - 150°C) to give the title alcohol (**5**). *m/z* 97 (M^+, 0.02%), 79 (6%), 78 (90%), 77 (47%), 76 (7%), 64 (32%), 63 (10%), 62 (17%), 61 (46%), 60 (100%), 59 (12%), 50 (37%), 49 (28%), 48 (68%), 47 (48%), 46 (53%), 45 (33%), 44 (24%), 42 (17%).

Synthesis of d_9-3-methylbutyl acetate (**8**)

Triethylamine (2.53 mL, 15.2 mmol) was added to a stirred solution of d_9-3-methylbutanol (**5**) (0.51 g, 5.2 mmol) and acetic anhydride (1.48 mL, 15.6 mmol) in diethyl ether (~4 mL). After stirring for 24 hours, diethyl ether (50 mL) was added and the resulting organics washed with 10% hydrochloric acid (1 x 100 mL), saturated sodium hydrogen carbonate (1 x 100 mL), brine (1 x 50 mL), and water (1 x 20 mL). The organics were dried ($MgSO_4$), concentrated in vacuo and the product distilled by Kugelrohr (~140°C) to give the title acetate (**8**) with a yield of 15% (calculated over four steps from *d_7*-2-bromopropane). *m/z* 139 (M^+, 0.1%), 91 (2%), 89 (19%), 80 (4%), 79 (51%), 78 (33%), 75 (10%), 63 (8%), 62 (18%), 61 (38%), 60 (18%), 50 (12%), 48 (11%), 46 (13%), 43 (100%), 42 (10%).

2.4.2.2 Preparation of samples for analysis

For quantitation of 31 fermentation-derived compounds, 1 mL of wine was accurately measured directly into a 20 mL SPME vial containing 2 g salt (NaCl) and 9 mL water. A 100 µL volume of a solution of standards containing approximately 1 µg of each labelled ethyl ester and labelled acetate, 10 µg d_8-ethyl acetate, 20 µg d_5-ethyl lactate, 12 µg of each of the labelled alcohols, 2 µg d_{13}-hexanol, 100 µg d_3-acetic acid, 5 µg d_5-propanoic acid, 2.5 µg each of d_7-2-methylpropanoic acid, d_7-butanoic acid and d_{15}-octanoic acid, and 1.2 µg each of d_7-3-methylbutanoic acid, d_{11}-hexanoic acid and d_{19}-decanoic acid in isopropanol, was added to each sample via injection through the seal of the SPME vial cap to give the concentrations of labelled standard shown in Table 2-6.

2.4.2.3 Instrumental analysis

Instrumental analysis was carried out according to the general procedure given in Section 2.4.1 and the instrumental parameters for the analysis of fermentation-derived compounds given in Table 2-3. The SIM ions selected for quantification and qualification of each peak are detailed in Table 2-5.

Table 2-5 Ions monitored in analytical method used to quantify fermentation-derived volatiles

analyte	rt (min)[a]	qt ion (*m/z*)[b]	qf ions (*m/z*)[c]	standard	rt (min)[a]	qt ion (*m/z*)[b]	qf ions (*m/z*)[c]
ethyl acetate	6.31	70	61, 88	d_8-ethyl acetate	6.21	76	66, 45
ethyl propanoate	7.76	102	73, 75	d_5-ethyl propanoate	7.69	107	77, 76
ethyl 2-methylpropanoate	7.96	116	88, 101	d_5-ethyl 2-methylpropanoate	7.88	121	106, 93
2-methylpropyl acetate	9.07	56	73, 86	d_9-2-methylpropyl acetate	8.91	64	75, 78
ethyl butanoate	9.69	88	101, 116	d_5-ethyl butanoate	9.60	93	106, 121
ethyl 2-methylbutanoate	10.15	102	115, 87	d_5-ethyl 2-methylbutanoate	10.06	107	120, 88
ethyl 3-methylbutanoate	10.56	115	88, 130	d_5-ethyl 3-methylbutanoate	10.47	93	120, 85
2-methylpropanol	11.56	43	41, 74	d_9-2-methylpropanol	11.26	46	50, 83
2-methylbutyl acetate	11.94	72	74, 57	d_5-2-methylbutyl acetate	11.85	75	43, 57, 74
3-methylbutyl acetate	11.98	87	88, 69	d_9-3-methylbutyl acetate	11.79	79	89, 78
butanol	13.41	56	41, 55	d_{10}-butanol	13.13	64	46, 48
2-methylbutanol	15.14	56	57, 70	d_5-2-methylbutanol	15.00	59	60, 75
3-methylbutanol	15.22	57	55, 70, 42	d_9-3-methylbutanol	14.99	78	77, 60
ethyl hexanoate	14.94	115	99, 88	d_5-ethyl hexanoate	14.82	93	106, 120
hexyl acetate	16.12	69	84, 73	d_{13}-hexyl acetate	15.79	78	96, 50
ethyl lactate	18.78	75	103, 45	d_5-ethyl lactate	18.61	76	108, 45
hexanol	18.95	56	55, 69	d_{13}-hexanol	18.60	64	62, 78
ethyl octanoate	20.72	101	172, 88	d_5-ethyl octanoate	20.60	106	177, 93
acetic acid	21.49	60	45, 43	d_3-acetic acid	21.48	63	46, 45
propanoic acid	23.84	73	74, 57, 45	d_5-propanoic acid	23.69	79	77, 62, 45
2-methylpropanoic acid	24.53	73	43, 88	d_7-2-methylpropanoic acid	24.37	50	95, 77
ethyl decanoate	26.17	200	157, 101	d_5-ethyl decanoate	26.04	205	162, 106
butanoic acid	25.92	60	73, 45	d_7-butanoic acid	25.81	63	77, 50
2-methylbutanoic acid	27.02	74	87, 73	d_7-3-methylbutanoic acid	26.82	61	91, 62
3-methylbutanoic acid	26.98	87	60, 61	d_7-3-methylbutanoic acid	26.82	61	91, 62
2-phenylethyl acetate	30.15	104	91, 65	d_3-2-phenylethyl acetate	30.09	93	106, 107
ethyl dodecanoate	30.82	228	101, 157	d_5-ethyl dodecanoate	30.68	233	106, 162
hexanoic acid	31.02	60	73, 87	d_{15}-octanoic acid	34.97	77	63, 109
2-phenylethanol	32.28	91	92, 122	d_3-2-phenylethanol	32.21	94	93, 125
octanoic acid	31.02	73	60, 115	d_{15}-octanoic acid	34.97	77	63, 109
decanoic acid	39.33	73	129, 172	d_{19}-decanoic acid	38.91	141	77, 63

[a] rt: retention time of peak; [b] qt ion: ion used for quantitation; [c] qf ion: ion/s used for qualification.

2.4.2.4 Method validation

Calibration functions for each analyte were obtained by spiked standard additions to model wine and white wine. Each analyte was added to give the concentrations detailed in Table 2-6. All spiked samples were prepared and analysed as described for wine samples.

When samples were analysed, they were checked against duplicate standards, at SPME vial concentration, of 0, 5, 50, 250 and 500 μg/L for each of the ethyl esters, acetates and acids; 0, 50, 500, 2500, 5000 μg/L for each alcohol; 0, 50, 500, 2500, 5000, 50000 μg/L for ethyl acetate and ethyl lactate; 0, 1000, 10000, 50000, 100000, 1000000 μg/L for acetic acid; 0, 20, 200, 1000, 2000, 20000 μg/L for propanoic acid, 2-methylpropanoic acid, butanoic acid and octanoic acid, to adjust for mass spectral response factor and ratio drift.

Table 2-6 Concentration of standards in model wine and white wine for analysis of fermentation-derived compounds

analyte	standard addition concentrations (1/10 dilution) [a]	deuterium labelled standard	deuterium labelled standard concentration [a]
ethyl esters	0, 0.1, 0.2, 0.5, 1, 2, 5, 10, 20, 50, 100, 200, 500, 1000 μg/L	d_5-ethyl esters	~ 100 μg/L
ethyl acetate and ethyl lactate	0, 1, 2, 5, 10, 20, 50, 100, 200, 500, 1000, 2000, 5000, 10000 μg/L	d_8-ethyl acetate	~ 1000 μg/L
		d_5-ethyl lactate	~ 2000 μg/L
acetates	0, 0.1, 0.2, 0.5, 1, 2, 5, 10, 20, 50, 100, 200, 500, 1000 μg/L	$d_{3, 5, 9, 13}$-acetates	~ 100 μg/L
alcohols	0, 5, 10, 25, 50, 100, 250, 1000, 2500, 5000, 10000, 25000, 50000 μg/L	$d_{3, 5, 9, 10}$-alcohols	~ 1200 μg/L
		d_{13}-hexanol	~ 200 μg/L
acids	0, 0.1, 0.2, 0.5, 1, 2, 5, 10, 20, 50, 100, 200, 500, 1000, μg/L	d_3-acetic acid	~ 10000 μg/L
		d_5-propanoic acid	~ 500 μg/L
		d_7-2-methylpropanoic, d_7-butanoic, d_{15}-octanoic acids	~ 250 μg/L
		d7-3-methylbutanoic, d_{11}-hexanoic, d_{19}-decanoic	~ 120 μg/L

[a] these approximate concentrations are equivalent to the concentration in the SPME vial (i.e. wine diluted 10 times with water)

2.4.3 Method for the analysis of diacetyl and trans*-ethyl cinnamate*

2.4.3.1 Preparation of deuterium labelled internal standards

Synthesis of d_5-*trans*-ethyl cinnamate

Cinnamic acid (3.79 g, 26 mmol) and thionyl chloride (3.7 mL, 51 mmol) were stirred at 40°C for one hour under N_2. To the cooled solution, d_6-ethanol was added (1 mL, 17 mmol), and the mixture warmed to room temperature over one hour. The reaction mixture was quenched with water (~30 mL) and extracted with dichloromethane (3 x 20 mL). The combined organics were washed with saturated sodium hydrogen carbonate (2 x 20 mL) and water (1 x 20 mL), dried ($MgSO_4$) and concentrated in vacuo. The crude product was purified by silica column chromatography using graduated dichloromethane : petroleum spirit. Subsequent Kugelrohr distillation (100 - 125°C, 1.3 - 1.5 mm Hg) gave the title ester in 73% yield. *m/z* 181 (M^+, 58%), 148 (19%), 131 (100%), 103 (50%), 77 (31%), 51 (12%); ^{1}H NMR (δ) 6.44 (d, 1H), 7.37 (m, 3H), 7.53 (m, 2H), 7.69 (d, 1H); ^{13}C NMR (δ) (m at ~13.0 and 59.8 too weak to clearly define) 118.1 (s), 128.0 (s), 128.8 (s), 130.2 (s), 134.5 (s), 144.5 (s), 167.0 (s).

2.4.3.2 Preparation of samples for analysis

For each sample, 10 mL of wine was measured into a 20 mL SPME vial containing 2 g NaCl. A 100 μL volume of a solution of standards containing approximately 5 μg of d_6-diacetyl and 0.5 μg of d_5-*trans*-ethyl cinnamate in isopropanol was added to each sample via injection through the seal of the SPME vial cap to give the equivalent concentration in wine of labelled standard shown in Table 2-8.

2.4.3.3 Instrumental analysis

Instrumental analysis was carried out according to the general procedure given in Section 2.4.1 and the instrumental parameters for the analysis of diacetyl and *trans*-ethyl cinnamate compounds given in Table 2-3. The SIM ions selected for quantification and qualification of each peak are detailed in Table 2-7.

Table 2-7 Ions monitored in analytical method used to quantify diacetyl and *trans*-ethyl cinnamate

analyte	rt (min)[a]	qt ion (*m/z*)[b]	qf ions (*m/z*)[c]	standard	rt (min)[a]	qt ion (*m/z*)[b]	qf ions (*m/z*)[c]
diacetyl	3.71	86	87	d_6-diacetyl	3.58	92	91
trans-ethyl cinnamate	17.80	176	175, 177	d_5-*trans*-ethyl cinnamate	17.77	181	182, 180

[a] rt: retention time of peak; [b] qt ion: ion used for quantitation; [c] qf ion: ion/s used for qualification.

2.4.3.4 Method validation

Calibration functions for diacetyl and *trans*-ethyl cinnamate were obtained by duplicate spiked standard additions to model wine and white wine. Each analyte was added to give the equivalent concentration in wine given in Table 2-8. All spiked samples were prepared and analysed as described for wine samples. When samples were analysed, they were checked against duplicate standards of 0, 0.5, 50, 500 and 5000 µg/L of each analyte to adjust for mass spectral response factor and ratio drift.

Table 2-8 Concentration of standards prepared in model wine and white wine for analysis of diacetyl and *trans*-ethyl cinnamate

analyte	standard addition concentrations	deuterium labelled standard	deuterium labelled standard concentration
diacetyl and *trans*-ethyl cinnamate	0, 0.01, 0.03, 0.05, 0.1, 0.2, 0.5, 1, 2, 5, 10, 20, 50, 100, 200, 500, 1000, 2000 and 5000 µg/L	d_6-diacetyl	500 µg/L
		d_5-*trans*-ethyl cinnamate	50 µg/L

2.4.4 Method for the analysis of grape- and oak-derived compounds

2.4.4.1 Preparation of samples for analysis

For each sample, a 100 µL volume of a solution of standards containing approximately 1.3 µg each of d_7-linalool, d_7-α-terpineol, d_7-nerol, d_7-geraniol in water (for preparation of labelled monoterpenes refer to [173]), and a 100 µL volume of a solution of standards containing 0.5 µg d_8-napthalene, 0.5 µg d_4-β-damascenone, 0.42 µg d_3-β-ionone, 0.05 µg each of d_3-guaiacol and d_3-4-methylguaiacol, 2.5 µg each of d_4-4-ethylphenol and d_4-4-ethylphenol, 5.3 µg d_4-*cis*-oak lactone and 2.6 µg d_3-vanillin in ethanol, was added to a 15 mL glass screw capped vial followed by 10 mL of wine to give the concentration of deuterium labelled standards at the equivalent concentration in wine shown in Table 2-10. The wine was extracted with pentane / diethyl ether (2 : 1, 3 mL) and the extract transferred to a 2 mL GC-MS vial for analysis.

2.4.4.2 Instrumental analysis

Instrumental analysis was carried out on two different GC-MS instruments, using two different column types, according to the general procedure given in Section 2.4.1 and the instrumental parameters for each method for the analysis of grape-derived compounds given in Table 2-3. The SIM ions selected for quantification and qualification of each peak are detailed in Table 2-9.

Table 2-9 Ions monitored in analytical method used to quantify grape- and oak-derived volatiles

analyte	wax rt (min) [a]	1701 rt (min) [a]	qt ion (*m/z*)[b]	qf ions (*m/z*)[c]	standard	wax rt (min) [a]	1701 rt (min) [a]	qt ion (*m/z*)[b]	qf ions (*m/z*)[c]
linalool	8.77		121	136, 154	d_7-linalool	8.71		100	76, 128, 142
α-terpineol	10.55		121	136, 154	d_7-α-terpineol	10.46		142	99, 127, 69
nerol	11.67		121	139, 154, 69	d_7-nerol	11.58		127	142, 69
geraniol	12.19		121	121, 136, 154	d_7-geraniol	12.09		127	99, 128, 142
cis-rose oxide		7.60	139	154, 140, 69	d_8-napthalene		9.25	136	108
TDN	11.17	11.15	157	142, 172	d_8-napthalene	11.05	9.25	136	108
β-damascenone	11.97	12.08	190	69, 175	d_4-β-damascenone	11.93	12.04	194	73, 193
β-ionone	13.16	13.48	177	192	d_3-β-ionone	13.16	13.45	180	195
guaiacol		8.47	124	109, 81	d_3-guaiacol		8.44	127	109, 81
4-methylguaiacol		9.92	138	123, 95	d_3-4-methylguaiacol		9.90	141	123, 95
4-ethylphenol		10.65	122	107	d_4-4-ethylphenol		10.65	126	111
4-ethylguaiacol		11.07	152	137, 122	d_4-4-ethylphenol		10.65	126	111
cis-oak lactone		12.52	99	114, 128, 156	d_4-*cis*-oak lactone		12.52	90	101, 118, 132
vanillin		13.74	152	151	d_3-vanillin		13.71	154	155

[a] rt: retention time of peak on either the DB-WAX (wax) or DB-1701 (1701) column; [b] qt ion: ion used for quantitation; [c] qf ion: ion/s used for qualification

2.4.4.3 Method validation

Standard addition calibration functions for the analytes were obtained for white wine and model wine by others [173].

Table 2-10 Concentration of standards prepared for analysis of grape- and oak-derived compounds

analyte	standard addition concentrations	deuterium labelled standard	deuterium labelled standard concentration
linalool	0, 156 μg/L	d_7-linalool	~ 131 μg/L
α-terpineol	0, 165 μg/L	d_7-α-terpineol	~ 131 μg/L
nerol	0, 167 μg/L	d_7-nerol	~ 131 μg/L
geraniol	0, 150 μg/L	d_7-geraniol	~ 131 μg/L
cis-rose oxide	0, 55 μg/L	d_8-napthalene	50 μg/L
TDN	0, 49 μg/L	d_8-napthalene	50 μg/L
β-damascenone	0, 60 μg/L	d_4-β-damascenone	50 μg/L
β-ionone	0, 55 μg/L	d_3-β-ionone	42 μg/L
guaiacol	0, 110 μg/L	d_3-guaiacol	5 μg/L
4-methylguaiacol	0, 100 μg/L	d_3-4-methylguaiacol	5 μg/L
4-ethylphenol	0, 274 μg/L	d_4-4-ethylphenol	250 μg/L
4-ethylguaiacol	0, 258 μg/L	d_4-4-ethylphenol	250 μg/L
cis-oak lactone	0, 570 μg/L	d_4-*cis*-oak lactone	526 μg/L
vanillin	0, 260 μg/L	d_3-vanillin	257 μg/L

When samples were analysed, they were checked against duplicate standards of each analyte at the equivalent concentration in wine listed in Table 2-10 to adjust for mass spectral response factor and ratio drift. These standards were prepared directly into 2 mL GC-MS vials together with the addition of 100 µL of the deuterium labelled internal standard solution described in Section 2.4.4.1, and made to volume with dichloromethane. The equivalent concentration in wine for each deuterium labelled internal standard is detailed in Table 2-10.

2.4.5 Method for the analysis of 4-vinylguaiacol and 4-vinylphenol

2.4.5.1 Preparation of samples for analysis

For each sample, 5 mL of wine was measured directly into a 20 mL SPME vial. A 100 µL volume of a solution of standards containing approximately 5 µg each of d_2-4-vinylguaiacol and d_2-4-vinylphenol in ethanol, was added to each sample via injection through the seal of the SPME vial cap to give the equivalent concentrations in wine of labelled standard shown in Table 2-12.

2.4.5.2 Instrumental analysis

Instrumental analysis was carried out according to the general procedure given in Section 2.4.1 and the instrumental parameters for the analysis of 4-vinylguaiacol and 4-vinylphenol given in Table 2-3. The SIM ions selected for quantification and qualification of each peak are detailed in Table 2-11.

Table 2-11 Ions monitored in analytical method used to quantify 4-vinylguaiacol and 4-vinylphenol

analyte	rt (min) [a]	qt ion (*m/z*)[b]	qf ions (*m/z*)[c]	standard	rt (min) [a]	qt ion (*m/z*)[b]	qf ions (*m/z*)[c]
4-vinylguaiacol	16.73	150	135, 107, 77	d_2-4-vinylguaiacol	16.71	152	109, 137, 79
4-vinylphenol	18.52	120	91, 65	d_2-4-vinylphenol	18.50	122	93, 67

[a] rt: retention time of peak; [b] qt ion: ion used for quantitation; [c] qf ion: ion/s used for qualification

2.4.5.3 Method validation

Standard addition calibration functions for 4-vinylguaiacol and 4-vinylphenol were obtained for white wine and model wine by others [174]. When samples were analysed, they were checked against duplicate standards of 4-vinylguaiacol and 4-vinylphenol at concentrations shown in Table 2-12, to adjust for mass spectral response factor and ratio drift. These standards were prepared and analysed as described for the wine samples. The equivalent concentration in wine for each deuterium labelled internal standard is shown in Table 2-12.

Table 2-12 Concentration of standards prepared in model wine for analysis of 4-vinylguaiacol and 4-vinylphenol

analyte	standard addition concentrations	deuterium labelled standard	deuterium labelled standard concentration
4-vinylguaiacol and 4-vinylphenol	0, 250 and 1000 µg/L	d_2-4-vinylguaiacol and d_2-4-vinylphenol	1000 µg/L

2.4.6 Method for the analysis of methionol

2.4.6.1 Preparation of samples for analysis

For each sample, a 20 μL volume of a solution of standard containing approximately 2 μg of d_5-methionol in ethanol, was added to a 4 mL screw capped vial followed by 1 mL of wine, to give the concentration of labelled standard shown in Table 2-14. The wine was extracted with 2 mL of pentane / ethyl acetate (2 : 1) and after settling (30 min) the extract was concentrated (with N_2) to approximately 0.2 mL. The resulting extract was transferred to the 100 μL insert of a 2 mL GC-MS vial for analysis.

2.4.6.2 Instrumental analysis

Instrumental analysis was carried out according to the general procedure given in Section 2.4.1 and the instrumental parameters for the analysis of methionol given in Table 2-3. The ions selected for quantification and qualification of each peak are detailed in Table 2-13.

Table 2-13 Ions monitored in analytical method used to quantify methionol

analyte	rt (min) [a]	qt ion (*m/z*)[b]	qf ions (*m/z*)[c]	standard	rt (min) [a]	qt ion (*m/z*)[b]	qf ions (*m/z*)[c]
methionol	18.26	108	106, 73, 88	d_5-methionol	18.14	111	93, 78, 64

[a] rt: retention time of peak; [b] qt ion: ion used for quantitation; [c] qf ion: ion/s used for qualification

2.4.6.3 Method validation

A standard addition calibration function for methionol was obtained for white wine and model wine by others (unpublished method). When samples were analysed, they were checked against duplicate standards of methionol at the equivalent concentrations in wine given in Table 2-14, to adjust for mass spectral response factor and ratio drift. These standards were prepared directly into 2 mL GC-MS vials by addition of a 20 μL volume of a solution containing 2 μg of methionol in ethanol, and a 20 μL volume of a solution containing 2 μg of d_5-methionol, at the equivalent concentration in 1 mL of wine given in Table 2-14, and topped up with dichloromethane.

Table 2-14 Concentration of standards prepared in model wine for analysis of methionol

analyte	standard addition concentrations	deuterium labelled standard	deuterium labelled standard concentration
methionol	0, 2000 μg/L	d_5-methionol	2000 μg/L

2.4.7 Method for the analysis of low molecular weight sulfur compounds

Model wine used for the analysis of low molecular weight sulfur compounds was 12% ethanol in water (v/v), saturated with potassium hydrogen tartrate (KHT), and adjusted to pH 3.2 with tartaric acid. The model wine prepared also contained fermentation-derived compounds including approximately 50 μg/L of each ethyl ester, 500 μg/L of ethyl acetate and ethyl lactate, 50 μg/L of each acetate, 500 μg/L of each alcohol, and 50 μg/L of each

acid (refer to Table 2-1 for individual compound names) to make the model wine more wine-like.

2.4.7.1 Preparation of samples for analysis

Ampoules of wine (> 50 mL) were thawed to 4°C from storage at -18°C. Each wine was transferred into a pre-chilled 50 mL volumetric flask and made to volume at 4°C. A 100 µL volume of a solution of standards containing approximately 2.1 µg of ethyl methyl sulfide and 4.9 µg of S-n-propyl thioacetate, in ethanol, was added to each 50 mL volumetric flask to give the equivalent concentration in wine of each internal standard shown in Table 2-15. In triplicate, 10 mL of wine was transferred from each flask into a pre-chilled SPME vial (4°C) containing 2 g NaCl, ~1 mg disodium ethylenediaminetetraacetate (Na_2EDTA), and a magnetic stirring flea. All prepared samples were stored in a fridge at 4°C immediately after preparation until instrumental analysis could take place (not more than four days).

For certain wines where the concentration of sulfur compounds measured was very high or the response for the internal standard was unusually low, the wine was diluted, either 1/2 or 1/5 with model wine, prior to addition of standards, and reanalysed.

2.4.7.2 Presentation of sample to the instrument

For each sample, the SPME vial was removed from the fridge (4°C) and warmed to 45°C (in a water bath). Once the salts had dissolved, the sample was stirred at 45°C for 30 minutes. After this time, the needle of a 100 µL air tight syringe was inserted to half headspace height. The syringe was drawn to 80 µL and filled twice with headspace before filling to 70 µL for injection directly onto the GC.

2.4.7.3 Instrumental analysis

The headspace of samples were analysed with an Hewlett-Packard (HP) 6890 gas chromatograph (GC) coupled to an HP G2350A microwave-induced plasma atomic emission detector (AED). The fused silica capillary column fitted to the GC consisted of a long VB-5 fused silica capillary column (ValcoBond, CFS-B06025-050B, 60 m x 0.25 mm x 0.50 µm) preceded by a short SolGel-wax fused silica column (SGE, CC0500, 15 m x 0.32 mm x 0.50 µm). A retention gap between the two column types consisted of a short deactivated column (2 m x 0.53 mm x 0.50 µm). The carrier gas was helium (Air Liquide or BOC Gases, ultra high purity) linear velocity 30 cm/sec, with a constant flow rate of 2.7 mL/min. The oven temperature started with an initial temperature of 30°C held for 5 minutes, ramped at 1°C/min to 45°C, 7°C/min to 180°C and finally 20°C/min to 260°C. The inlet (cool on column) in oven track mode (3°C greater than oven temperature) was pressurised to 22.65 psi with helium gas. The headspace injection volume was 70 µL which was manually injected into the inlet

over 10 seconds. Atomic emission detector (AED) parameters were optimised for sulfur sensitivity (signal to noise, negligible "back amount"). The AED transfer line and the cavity block were held at 250°C. Helium (Air Liquide or BOC Gases, ultra high purity plus SAES Getter) was used for the microwave induced plasma and measured at the cavity vent at 25.0 mL/min. Oxygen (Air Liquide or BOC Gases, ultra high purity) 55.0 psi and hydrogen (Air Liquide or BOC Gases, ultra high purity) 10.0 psi were used as the reagent gases when sulfur (181.40 nm) and Carbon (193.03 nm) emission lines were monitored. A 0.4 L/min spectrometer purge flow of nitrogen was used. The discharge tube was cooled with water at 65°C.

2.4.7.4 Method validation

A standard addition calibration function for each analyte was obtained for white wine and model wine by others (unpublished method). When samples were analysed, they were checked against replicated standards of each analyte and internal standard at the concentrations shown in Table 2-15. The standards were prepared and analysed, as described for the wine samples above, on the same day that the wine samples were prepared, and stored at 4ºC until analysis could take place (not more than four days). To ensure repeatability of the analysis was consistent over the four days of run time, for each set of wines, at least one standard or quality control sample (spiked and un-spiked) was run on every day of analysis.

Table 2-15 Standard addition concentrations and retention times of each analyte for the analysis of low molecular weight sulfur compound

analyte	retention time	standard concentration	internal standard	retention time	internal standard concentration
ethanethiol	10.65 min	0, 173 µg/L	ethyl methyl sulfide	15.31 min	42 µg/L
dimethyl sulfide	11.10 min	0, 196 µg/L			
carbon disulfide	11.42 min	0, 209 µg/L			
diethyl sulfide	21.77 min	0, 173 µg/L			
S-methyl thioacetate	24.94 min	0, 212 µg/L	S-n-propyl thioacetate	32.17 min	97 µg/L
dimethyl disulfide	27.02 min	0, 198 µg/L			
S-ethyl thioacetate	28.20 min	0, 203 µg/L			
diethyl disulfide	33.76 min	0, 205 µg/L			

Chapter 3 The compositional basis of Riesling wine aroma

3.1 Introduction

Riesling is an important grape variety which covers approximately 6.5% of the vineyard area devoted to growing white grape varieties in Australia (including bearing and not yet bearing vineyards in 2002 [182]). Riesling is considered one of the world's classic grape varieties and Australian Riesling is distinctive for being dry with a natural, refreshing acidity and enticing aromas such as floral, citrus, lime, lemon and tropical fruit in a young wine, or developed characters such as toast, honey and sometimes kerosene in an older wine [183]. The volatile compounds, and their precursors, which are responsible for the characteristic aromas of Riesling wine, have been studied for many years [19, 37, 48, 184-186]. Although the volatile aroma compounds present in Riesling wines are generally well known, the relative importance and specific aroma contribution of these compounds is still not well understood for Australian Riesling wine.

This chapter details the selection of 20 commercial Australian Riesling wines with a broad range of sensory properties, and the quantitative sensory descriptive and volatile chemical analysis of these wines. Multivariate analysis of the two data sets was performed with the aim to explore the relationship between volatile composition and sensory perception, and to identify the most important volatile compounds to the aroma of wines of the Riesling variety.

3.2 Results and discussion

From a preliminary screening of 59 commercial Australian Riesling wines, 20 Riesling wines were selected for this study primarily on the basis of having a diverse range of aroma characteristics, and included wines of both low and high intensity aromas. The wines were from a variety of regions, climates, producers, vintages and retail prices and were without obvious faults such as oxidative, reductive or ethyl acetate aromas. The 20 Riesling wines selected for the present work are tabulated in Table 3-1.

The 20 wines covered a range of commercially available Australian Riesling wines in terms of producer, region, climate, style and wine age. The wines chosen for the study were mostly young wines, with eleven wines from the 2002 vintage (~ 6 months of age) and three wines from the 2001 vintage (~ 1.5 years of age). The six remaining wines were older Riesling wines (~ 2.5 – 9.5 years of age) with one wine from each of the 2000, 1999, 1996 and 1993 vintages and two wines from the 1997 vintage. The study wines were from a range of regions across Australia including regions in Victoria, Western Australia, New South Wales and Tasmania, with the majority of wines from regions within South Australia. Additional

information about each wine including details on viticultural fruit origin, varietal purity, method used for harvesting the grapes, type of yeast, fermentation details, fining agents and other winemaking details were provided by winemakers and producers where available. This information was used to ensure the wines were made from 100% Riesling wine grapes, and that the aroma of each wine was not influenced by unconventional viticultural or winemaking practices.

Table 3-1 Identity and basic composition of Riesling wines selected for sensory and chemical analysis

wine code	retail price ($AUD)	year [a]	region [b]	closure [c]	alcohol (% v/v)	pH	TA (g/L) [d]	G + F (g/L) [e]	SO_2 free / total (mg/L) [f]
1	$19.00	2002	Central Vic	cork	12.7	3.08	6.5	4.2	20 / 135
2	$33.00	2002	Clare Valley, SA	screw cap	13.5	3.13	7.2	0.4	24 / 101
3	$10.00	2002	Blend, SA	cork	12.5	3.01	6.4	5.3	11 / 98
4	$7.00	2001	Clare valley, SA / WA	screw cap	12.5	3.05	6.9	0.9	22 / 114
5	$18.00	2002	ACT region, NSW	screw cap	13.7	3.05	6.6	4.0	19 / 118
6	$26.00	1996	Coonawarra, SA	cork	11.4	2.95	6.4	5.1	10 / 108
7	$17.00	2000	Blackwood Park, WA	cork	13.0	3.03	7.0	5.9	15 / 127
8	$9.00	2002	Barossa Valley, SA	cork	12.5	3.11	6.2	2.6	22 / 106
9	$35.00	1993	Eden Valley, SA	cork	12.1	2.98	6.7	6.2	12 / 62
10	$25.00	2002	Northern Tas	screw cap	12.5	3.21	6.3	9.5	20 / 115
11	$14.50	2002	Mount Barker, WA	screw cap	12.8	3.19	7.0	0.7	18 / 88
12	$17.50	2002	Blend, WA	screw cap	12.0	3.05	7.4	4.3	19 / 120
13	$35.00	1997	Eden Valley, SA	cork	13.0	3.11	6.7	3.5	8 / 71
14	$24.00	1997	Clare Valley, SA	cork	12.5	3.10	5.6	3.4	7 / 78
15	$30.00	1999	Grampians, Vic	cork	12.6	3.06	7.4	4.5	17 / 100
16	$16.00	2001	Coonawarra, SA	cork	12.2	2.94	6.2	3.7	25 / 126
17	$20.00	2001	Clare Valley, SA	screw cap	10.8	2.86	6.6	3.6	24 / 84
18	$10.00	2002	Blend, SA	screw cap	11.8	3.04	6.3	5.7	21 / 107
19	$17.00	2002	Eden Valley, SA	cork	12.8	3.07	6.4	3.1	28 / 148
20	$11.00	2002	Eden Valley, SA	cork	12.6	3.11	6.2	4.5	28 / 144

[a] wine age at the time of analysis: vintage 2002 (~ 6 months), 2001 (1.5 years), 2000 (2.5 years), 1999 (3.5 years), 1997 (5.5 years), 1996 (6.5 years), 1993 (9.5 years); [b] Vic: Victoria, SA: South Australia, WA: Western Australia, NSW: New South Wales, Tas: Tasmania; [c] cork refers to natural bark cork; [d] TA: Titratable acidity as tartaric acid (at pH 8.2); [e] G + F: glucose plus fructose; [f] SO_2: sulfur dioxide

Each wine chosen for the study was analysed by the Australian Wine Research Institute's Analytical Services for basic chemical composition (as described in [187]). The results for alcohol (% v/v), pH, titratable acidity (at pH 8.2), glucose plus fructose, and free and total sulfur dioxide (SO_2) are given in Table 3-1. A summary of all the routine chemical variables measured, for each wine, is given in Appendix A. The results for all of the routine chemical parameters for each wine were within the expected range for commercial Australian Riesling wine. The parameters SO_2 free and total and glucose plus fructose showed the broadest ranges, and alcohol, pH and titratable acidity did not vary considerably. The younger Riesling wines (2002 and 2001 vintage) had significantly higher levels of free and total SO_2 than the older Riesling wines as shown in Figure 3-1 (ANOVA, $p < 0.05$). The differences observed for all other parameters did not relate to the vintage of the wines. None of the

basic chemical parameters measured were significantly correlated with retail price, closure type, or viticultural region.

Figure 3-1 Free and total sulfur dioxide for younger (2001 and 2002) and older (1993 - 2000) vintage Riesling wines

Differing alcohol contents have been shown to have an impact on the perception of certain aroma notes in wines [119, 188]. It has been demonstrated that decreasing alcohol content in a reconstituted wine model increases the sensory perception of 'fruity' and 'flowery' notes as well as increasing perception of in-mouth acidity [188]. This indicates that the sensory thresholds of certain volatile aroma compounds could vary according to the ethanol content of wine. Due to the relatively narrow range of alcohol contents observed within this set of Riesling wines (from 10.8% to 13.7%) it is unlikely that the differing alcohol contents have a significant impact on the perception of volatile aroma compounds.

3.2.1 Sensory descriptive analysis

Descriptive analysis was employed to quantify the intensity of the sensory properties of the 20 Riesling wines selected for this study. A trained panel of 16 judges rated each wine, in triplicate, for the intensity of 17 aroma and six flavour attributes on a ten point scale (0 – 9). The results for each sensory attribute rated including mean, minimum, maximum, standard deviation (SD), coefficient of variation (CV) and the standard error of the mean (SEM) are tabulated in Table 3-2. Mean scores of all the sensory attributes rated, for each Riesling wine in the study, are tabulated in Appendix A.

A summary of the analysis of variance (ANOVA) for each attribute for each of the effects tested, as well as the least squares fit for each of the interactions tested, is given in Table 3-3. All sensory attributes were significantly different ($p < 0.05$) between the wines with the exception of the aroma attribute *apricot*, and flavour attributes *overall flavour*, *astringency* and *bitterness*. There was a significant difference between judges, for all attributes which is usual of descriptive analysis data of wine. There was no significant difference between

replicates for any attribute which indicates that the judges did not change the way they rated the wines over time and that bottle to bottle variation was minimal.

Table 3-2 Summary of the descriptive analysis scores for aroma and flavour

aroma attribute	mean	minimum	maximum	SD	CV (%)	SEM
estery	2.64	1.21	4.22	1.00	38	0.066
perfumed floral	2.09	0.56	3.64	1.10	53	0.068
dried rose	1.13	0.46	2.04	0.44	39	0.056
lemon	2.00	0.77	2.97	0.72	36	0.059
grapefruit	1.05	0.41	1.82	0.40	38	0.050
lime	2.13	1.18	3.69	0.76	36	0.063
lychee	0.92	0.31	1.61	0.37	40	0.045
pineapple	1.77	0.86	2.42	0.49	28	0.053
passionfruit	1.22	0.24	4.54	1.03	84	0.061
herbaceous	0.88	0.41	3.04	0.58	66	0.051
stewed apple	0.94	0.50	1.46	0.30	32	0.045
apricot	0.90	0.54	1.30	0.18	21	0.045
honey	1.55	0.34	3.67	1.07	69	0.058
toasty	1.51	0.17	5.21	1.64	108	0.070
caramel	1.05	0.22	2.53	0.77	73	0.055
kerosene	1.21	0.11	3.37	1.27	105	0.061
rubber / plastic	0.34	0.03	1.48	0.37	108	0.033
flavour attribute	**mean**	**minimum**	**maximum**	**SD**	**CV (%)**	**SEM**
sourness	5.03	4.58	5.55	0.30	6	0.049
sweetness	1.35	1.00	1.88	0.26	19	0.037
overall flavour	4.74	4.27	5.49	0.27	6	0.049
flavour persistence	4.82	4.21	5.60	0.35	7	0.050
astringency	1.54	1.33	1.78	0.11	7	0.047
bitterness	0.75	0.40	1.05	0.14	19	0.044

Significant differences were observed for the interaction of (wine x judge) for all attributes with the exception of *lemon*, *sourness*, and *astringency*. This indicates that judges rated the Riesling wines in different ways for almost all of the sensory attributes in the study. This is common of sensory studies of wine, where variability between judges is unavoidable. The interaction of (judge x replicate) did not show significant differences for all attributes with the exception of *grapefruit*, *pineapple*, *flavour persistence*, *astringency* and *bitterness*. This demonstrates that for most attributes, the judges did not rate the replicates differently. For the interaction of (replicate x wine), significant differences were observed for only *estery*, *passionfruit*, *toasty* and *overall flavour*. This shows that there may have been some bottle to bottle variation between replicates for these attributes or that panellist were changing the way that the rated the wines over time for these attributes.

Table 3-3 F ratios and significance for effects of wine, judge, repetition and interactions for each sensory attribute

sensory attribute	wine [a]	judge [a]	replicate [a]	wine x judge [b]	judge x replicate [b]	replicate x wine [b]
estery	14**	24**	0.4^{ns}	2**	1^{ns}	1*
perfumed floral	18**	9**	0.7^{ns}	1**	0.6^{ns}	0.9^{ns}
dried rose	3**	33**	2^{ns}	2**	1^{ns}	0.9^{ns}
lemon	8**	15**	0.2^{ns}	1^{ns}	0.9^{ns}	0.7^{ns}
grapefruit	3**	28**	1^{ns}	2**	2**	1^{ns}
lime	8**	16**	0.7^{ns}	2**	0.8^{ns}	1^{ns}
lychee	3**	16**	1^{ns}	1*	1^{ns}	1^{ns}
pineapple	5**	26**	0.6^{ns}	1**	2*	0.8^{ns}
passionfruit	20**	8**	0.2^{ns}	2**	1^{ns}	2**
herbaceous	7**	11**	2^{ns}	2**	1^{ns}	1^{ns}
stewed apple	2**	27**	0.8^{ns}	1**	1^{ns}	0.9^{ns}
apricot	0.8^{ns}	24**	0.2^{ns}	1*	0.9^{ns}	1^{ns}
honey	25**	6**	0.3^{ns}	3**	1^{ns}	1^{ns}
toasty	60**	4**	0.5^{ns}	2**	1^{ns}	2**
caramel	12**	26**	0.09^{ns}	2**	0.9^{ns}	1^{ns}
kerosene	37**	5**	0.8^{ns}	2**	1^{ns}	1^{ns}
rubber / plastic	7**	8**	2^{ns}	2**	1^{ns}	1^{ns}
sourness	2**	63**	0.1^{ns}	1^{ns}	1^{ns}	0.9^{ns}
sweetness	2**	91**	0.2^{ns}	1**	1^{ns}	0.8^{ns}
overall flavour	2^{ns}	86**	0.2^{ns}	1**	1^{ns}	2**
flavour persistence	2**	37**	0.2^{ns}	1*	2**	1^{ns}
astringency	0.3^{ns}	117**	0.7^{ns}	1^{ns}	2**	0.7^{ns}
bitterness	0.5^{ns}	119**	0.7^{ns}	1*	2**	1^{ns}
degrees of freedom	19	15	2	285	30	38

[a] values from analysis of variance; [b] values from least squares fit effect tests; significance indicated by ** ($p < 0.01$), * ($p < 0.05$), ^{ns} not significant.

Analysis of variance was also conducted separately for the group of younger vintage Riesling wines (2001 and 2001, n = 14) and the older vintage wines (1993 – 2000, n = 6) for each of the sensory attributes rated. All of the sensory attributes were significantly different among the younger vintage Riesling wines with the exception of the aroma attributes *grapefruit*, *stewed apple*, *apricot*, and flavour attributes *overall flavour*, *astringency* and *bitterness*. On the other hand, the only attributes that were significantly different between the older vintage Riesling wines were aroma properties *perfumed floral*, *lime*, *honey*, *toasty*, *caramel*, *kerosene*, and *rubber/plastic*. This is not surprising considering the older Riesling wines were commonly scored very low, if at all, for the *estery*, *floral* and 'fruity' type attributes. The statistically significant differences found for the 'developed' attributes demonstrates that the sensory panel were able to distinguish between the different sensory properties of the older wines in the study.

Although the scale used for descriptive analysis contained ten points (0 – 9) the highest (mean) score of an aroma attributes for any wine was only 5.2 for *toasty* and 4.5 for *passionfruit* (Table 3-2). All other aroma attributes were rated (on mean) no greater than 3.7 (maximum) on a ten point scale. This may indicate that only a few judges were rating these attributes, resulting in a low average score, or that the all panellists were typically using only the lower third of the scale. In comparison to the aroma scores, the variation observed with the flavour scores was very small (all CV < 20%). For the flavour attributes *sourness*, *overall flavour* and *flavour persistence,* panellists tended to use the middle part of the scale (4 – 5), whereas the attributes *sweetness*, *astringency* and *bitterness* were all used to rate the wines using the lowest part of the scale (below 1.5). This may be explained by the fact that all wines have some degree of *sourness* (acidity), and *flavour*, but not all wines are considered to have a degree of *sweetness*, or *bitterness*. The *astringency* property is not normally associated with white wines but with red wines, so it is not surprising that the Riesling wines were all considered to have a score of almost 0 for *astringency*.

The attributes with the broadest variation (CV > 50%) include *perfumed floral*, *passionfruit*, *herbaceous*, *honey*, *toasty*, *caramel*, *kerosene* and *rubber/plastic*. These results indicate that there was good agreement within the panel for the rating of these attributes compared to the attributes with lower variation (CV < 50%). This result was supported by the assessment of judge performance as all the panellists rated *perfumed floral*, *passionfruit*, *herbaceous*, *honey*, *toasty*, *caramel*, and *kerosene* with excellent agreement compared to the other attributes (i.e. scores from each judge were correlated with group mean). Reasonable agreement, where scores from only one judge did not correlate with the group mean, was achieved by the panel for the aroma attributes *estery*, *lime*, *pineapple* and *rubber/plastic* and flavour attributes *sourness*, *sweetness* and *overall flavour*. Moderate agreement was found for the attribute *flavour persistence*, where only two judges were found to be in disagreement with the group mean. The panel scores for the attributes *stewed apple, apricot, astringency* and *bitterness* showed the most disagreement, where scores from seven or more judges were not correlated with the group mean. This result suggests that the terms *astringency* and *bitterness* were probably not appropriate to use for these wines as they were not only scored very low, but the panel were not in agreement about how these terms should be used.

It is important to note that no individual judge was consistently in disagreement with the group mean for the rating of attributes. It may be that more training was required to improve the consistency of the rating of those attributes with low variation and poor judge agreement. Additionally, it may be that there were too many similar 'fruity' attributes, such as *lemon*,

grapefruit, *lime*, *stewed apple,* and *apricot*, which resulted in the panel being split over a number of terms when rating the same sensory property.

The terms selected to describe the Riesling wines in this study are similar in nature to sensory attribute terms used in other studies for descriptive analysis of Canadian [189] and German [190, 191] commercial Riesling wines. In one study, the descriptive analysis of German Riesling wine used fewer terms including only ten aroma and four in-mouth flavour terms [190, 191]. The wines were from two vintages only (1994 and 1993) and were two and three years old at the time of the sensory descriptive study. It may be that more terms would have been used if a broader range of vintages were analysed, including wines from much older vintages. The terms used to describe the Germany Riesling wines that differed notably from the present study include the aroma descriptors *nutty* and *licorice* (liquorice) and the flavour descriptors *body* and *density*. Although the aroma descriptor *nutty* might be considered somewhat similar in nature to *toasty*, the term *licorice* is quite different to any descriptor used in the present study and may be a feature of German Riesling wines. Furthermore, it can sometimes be difficult to compare the sensory attribute terms used across studies considering the panels are influenced by cultural and language differences which might explain any variation observed in the terms used to describe commercial Riesling wine in these studies.

In another descriptive study involving Canadian Riesling wine from four vintages (1994, 1995, 1996 and 1997, aged ~ 1 to 5 years), a similar number of terms were used as in the present study, including ten aroma, six in-mouth flavour, two taste and three mouth-feel sensory terms. Terms that were notably different from the present study include *melon* and *mineral/flint*, and the mouth-feel descriptors *alcohol*, *body* and *finish* [189]. In the present study, the term *melon* was discussed by the panel during training and discarded as it was not considered to identify important differences between the wines compared to the other 'fruity' terms chosen by the panel. The term *mineral/flint* was also discussed during training but was not considered an important descriptor for these wines. It is interesting that in the Canadian study, the only descriptors that were not found to be significantly different between the wines analysed were *peach/apricot*, *melon*, and *alcohol*. This result is similar to the present study where the rating of the term *apricot* was not found to be significantly different between the wines. Unique terms that were used in the present study include *toasty* and *caramel*, both of which are more relevant descriptors for older wines and are not likely to be used for younger Riesling wines.

Pearson correlations (pair-wise) between attributes were assessed for the sensory data and the results of this analysis are tabulated in Table 3-4. High collinearity (correlation) was

observed between attributes, which is common for analyses where many variables are measured, including sensory data sets [125]. Specifically, high positive correlations ($r \geq 0.85$) were found within the aroma attributes between terms that were used to describe younger fresh Riesling wines (for example, between *estery* and *perfumed floral*, $r = 0.97$), as well as between attributes that were used to describe older developed Riesling wines (for example, *caramel* and *toasty*, $r = 0.97$). Within the flavour attributes the only strong correlation ($r \geq 0.85$, $r \leq -0.85$) found was a positive correlation between *overall flavour* and *flavour persistence* ($r = 0.93$) which has also been in other descriptive studies of Riesling wine [190, 191]. There were also strong negative correlations ($r \leq -0.85$) observed between aroma attributes that were used to describe young fresh 'fruity' wines, and attributes which were used to describe older developed Riesling wines (for example, *lemon* and *kerosene*, $r = -0.91$). There were no strong correlations ($r \geq 0.85$, $r \leq -0.85$) found between aroma attributes and flavour attributes for the Riesling sensory data set.

The high collinearity observed between sensory attributes suggests that wines which were high in certain aroma or flavour properties were also always high (or low) in other attributes. For example, aged Riesling wines that were high in *toasty* were also always high in *honey* and *kerosene* characters and always low in young Riesling wine characters such as *estery*, *perfumed floral* and the 'fruity' attributes. Alternatively, this may suggest that there were too many similar attributes used in the sensory study and that panellists were simply not able to distinguish between the 17 aroma and six flavour attributes.

The attributes *passionfruit* and *herbaceous* were highly correlated with each other ($r = 0.92$) and unlike other attributes, they did not have high correlation, either positively or negatively, with any other attribute or group of attributes. When the correlation coefficients among descriptors are not significant, terms can be considered to have been used to describe markedly different sensory characteristics [192]. This implies that together, *passionfruit* and *herbaceous* is a distinctive aroma property which was not confused by the panel with the other young Riesling attributes (e.g. *estery*, *perfumed floral*, *lemon*) or the aged attributes (e.g. *honey*, *toasty*). It is interesting to note that this finding is different to that found in a descriptive study of German Riesling wines, where the *passionfruit* attribute was positively correlated with *floral* and *artificial fruit* (*estery*) and negatively correlated with *grassy / green* (*herbaceous*) [190, 191].

Table 3-4 Pearson correlation coefficient matrix (r) of Riesling sensory attributes

	estery	*perfumed floral*	*dried rose*	*lemon*	*grapefruit*	*lime*	*lychee*	*pineapple*	*passionfruit*	*herbaceous*	*stewed apple*	*apricot*	*honey*	*toasty*	*caramel*	*kerosene*	*rubber / plastic*	*sourness*	*sweetness*	*overall flavour*	*flavour persistence*	*astringency*	*bitterness*
estery	**1.00**																						
perfumed floral	**0.97**	**1.00**																					
dried rose	0.72	0.74	**1.00**																				
lemon	**0.88**	**0.87**	0.58	**1.00**																			
grapefruit	0.67	0.65	0.45	0.76	**1.00**																		
lime	**-0.92**	**-0.88**	-0.76	**-0.83**	-0.71	**1.00**																	
lychee	0.73	0.69	0.61	0.62	0.65	-0.77	**1.00**																
pineapple	0.84	**0.85**	0.61	0.84	0.70	**-0.85**	0.79	**1.00**															
passion fruit	0.42	0.38	0.53	0.45	0.67	-0.56	0.66	0.54	**1.00**														
herbaceous	0.15	0.10	0.26	0.27	0.62	-0.32	0.47	0.30	**0.92**	**1.00**													
stewed apple	-0.79	-0.77	-0.54	-0.79	-0.72	**0.85**	-0.69	-0.70	-0.55	-0.36	**1.00**												
apricot	-0.09	0.01	0.11	0.01	-0.24	0.12	-0.06	0.01	-0.28	-0.31	0.12	**1.00**											
honey	**-0.87**	-0.84	-0.63	**-0.88**	**-0.87**	**0.89**	-0.82	-0.83	-0.67	-0.50	0.83	0.18	**1.00**										
toasty	**-0.88**	**-0.87**	-0.68	**-0.92**	-0.84	**0.90**	-0.76	**-0.86**	-0.59	-0.40	0.81	0.04	**0.97**	**1.00**									
caramel	-0.84	-0.83	-0.62	**-0.88**	**-0.86**	**0.86**	-0.80	-0.84	-0.65	-0.49	0.80	0.06	**0.98**	**0.97**	**1.00**								
kerosene	**-0.90**	**-0.88**	-0.71	**-0.91**	-0.81	**0.96**	-0.77	**-0.90**	-0.63	-0.42	**0.85**	0.07	**0.92**	**0.94**	**0.93**	**1.00**							
rubber / plastic	-0.55	-0.51	-0.64	-0.57	-0.29	0.49	-0.44	-0.53	-0.45	-0.24	0.37	-0.08	0.40	0.43	0.42	0.53	**1.00**						
sourness	-0.05	-0.12	-0.17	-0.07	0.22	0.01	0.39	-0.02	0.35	0.42	-0.13	-0.17	-0.23	-0.09	-0.16	0.02	0.22	**1.00**					
sweetness	0.50	0.51	0.32	0.60	0.20	-0.38	-0.03	0.29	-0.06	-0.18	-0.42	0.12	-0.25	-0.35	-0.25	-0.42	-0.40	-0.56	**1.00**				
overall flavour	-0.49	-0.52	-0.37	-0.37	-0.35	0.55	-0.36	-0.50	-0.05	0.06	0.32	-0.02	0.48	0.55	0.50	0.45	-0.03	0.17	0.09	**1.00**			
flavour persistence	-0.60	-0.62	-0.37	-0.47	-0.33	0.60	-0.36	-0.51	0.06	0.19	0.45	-0.11	0.49	0.56	0.51	0.49	0.06	0.26	-0.15	**0.93**	**1.00**		
astringency	0.19	0.17	0.07	0.08	-0.05	-0.14	0.16	0.03	-0.04	-0.19	-0.13	0.01	-0.16	-0.15	-0.18	-0.14	0.05	0.16	0.01	0.06	0.08	**1.00**	
bitterness	0.37	0.39	0.52	0.19	0.06	-0.32	0.42	0.39	0.20	0.07	0.05	0.26	-0.26	-0.28	-0.28	-0.29	-0.29	0.03	-0.15	-0.36	-0.28	0.02	**1.00**

$r \leq -0.85$ and $r \geq 0.85$ are indicated in bold typeface

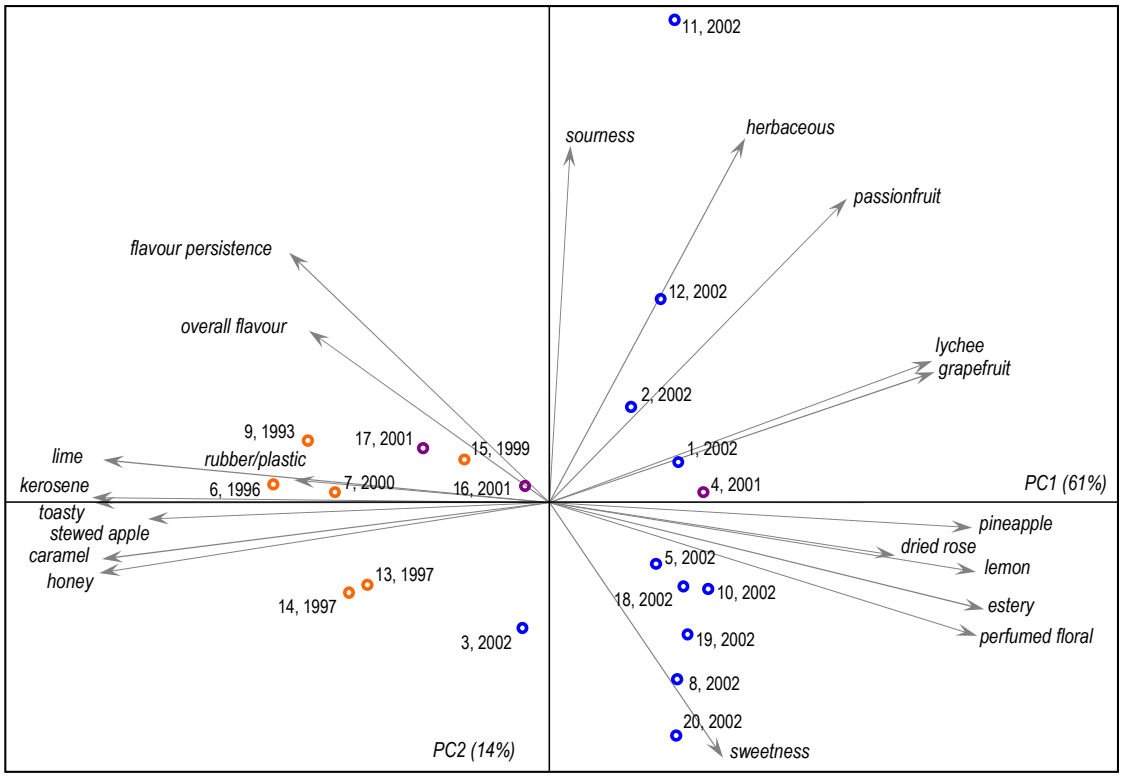
To explore groupings between the wines and to see how each wine was perceived, the sensory data were analysed by principal component analysis (PCA). The first three principal components (PCs) explained 84% of the variation in the data set. Plots of PC1 versus PC2, and PC1 versus PC3 are given in Figure 3-2 and Figure 3-3, respectively.

From the visual observations of the PCA scores, the first PC separated the wines according to age. Wines on the left of Figure 3-2 and Figure 3-3 were predominantly from older vintages, and were rated relatively high for differing intensities of *honey, caramel*, *stewed apple*, *toasty*, *kerosene*, *lime*, *overall flavour*, *flavour persistence* and *rubber / plastic*.

Wines on the right of the plot (in Figure 3-2 and Figure 3-3) were all from younger vintages. These younger wines were separated by PC2 as having either relatively high scores for the *sweetness*, *perfumed floral*, *estery*, *lemon*, *dried rose* and *pineapple* attributes (wine on the bottom right of Figure 3-2) or having relatively high scores for *sourness*, *herbaceous passionfruit*, *lychee*, *grapefruit*, *overall flavour* and *flavour persistence* (wines in the top right of Figure 3-2). The third PC separated the wines according to samples that were rated higher for *flavour persistence*, *overall flavour* and *sweetness* (wine in the top of Figure 3-3) from those that were rated higher for *sourness* and *rubber / plastic* (wines in the bottom part of Figure 3-3). Differences in intensity ratings were also observed within groups as demonstrated by the spread of the wines in both PCA plots (Figure 3-2 and Figure 3-3).

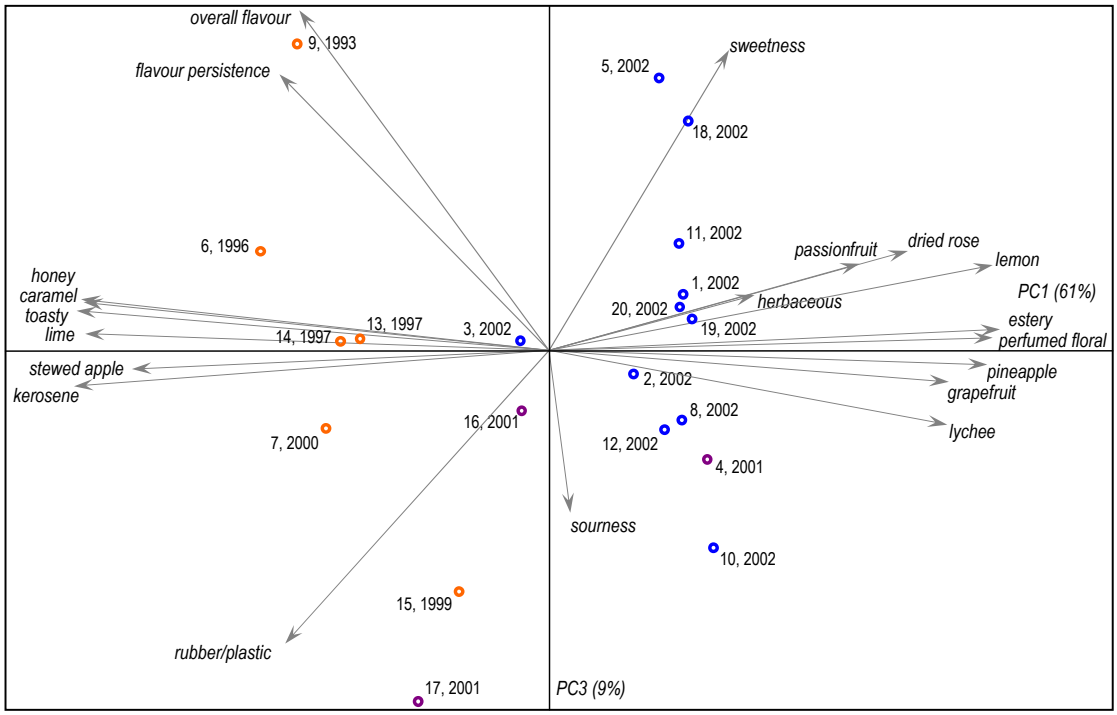
Based on the sensory scores, wines were not grouped according to viticultural origin or closure type. Wines that were rated highly for *caramel*, *honey, stewed apple*, *kerosene*, *toasty* and *lime* were typically more expensive wines from an older ‘reserve’ vintage, and consequently retail price related to these attributes.

Figure 3-2 PCA bi-plot of descriptive analysis results for Riesling wines, PC1 versus PC2



For details on the sample codes refer to Table 3-1, vintages are also indicated. Sample scores are calculated from the mean of 16 judges x three replicates and attribute loadings (vectors) are shown.

Figure 3-3 PCA bi-plot of descriptive analysis results for Riesling wines, PC1 versus PC3



For details on the sample codes refer to Table 3-1, vintages are also indicated. Sample scores are calculated from the mean of 16 judges x three replicates and attribute loadings (vectors) are shown.

3.2.2 Volatile chemical analysis

The 20 Riesling wines selected for this study were analysed for 59 volatile aroma compounds using the analytical methods described in Chapter 2. Overall 47 volatile aroma compounds were quantified and a summary of the results including mean, minimum and maximum concentration, standard deviation (SD), coefficient of variation (CV), standard error of the mean (SEM) and the F ratio for each compound is given in Table 3-5. A summary of the mean measured concentration of each of the volatile compounds analysed, for all of the Riesling wines in the study, are given in Appendix A.

Table 3-5 Summary of volatile chemical analysis results for the Riesling wines

	mean (µg/L)	minimum (µg/L)	maximum (µg/L)	SD (µg/L)	CV (%)	SEM (µg/L)	F ratio [a]
ethyl acetate	**75612**	**49073**	**117130**	17683	23	3985	one rep
ethyl propanoate	191	109	337	53	28	9	8**
ethyl butanoate	**453**	**305**	**599**	84	19	15	3**
ethyl 2-methylpropanoate	**75**	**44**	**143**	26	34	4	14**
ethyl 2-methylbutanoate	**15**	**4**	**38**	11	72	2	29**
ethyl 3-methylbutanoate	**26**	**8**	**57**	17	66	3	21**
ethyl hexanoate	**1313**	**955**	**1704**	173	13	32	3*
ethyl octanoate	**1739**	**821**	**3219**	554	32	124	one rep
ethyl decanoate	**712**	175	**1449**	342	48	56	11**
ethyl dodecanoate	59	nd	160	47	80	8	5**
***trans*-ethyl cinnamate**	0.9	0.2	**2**	0.5	59	0.08	94**
2-methylpropyl acetate	26	7	58	16	62	3	41**
2-methylbutyl acetate	**64**	nd	**191**	66	103	11	33**
3-methylbutyl acetate	**1349**	**38**	**3667**	1301	96	209	69**
hexyl acetate	135	nd	411	140	104	22	178**
2-phenylethyl acetate	**244**	5	**2059**	482	197	76	74**
2-phenylethanol	**20874**	9175	**85393**	19089	91	3001	71**
butanol	854	464	1946	334	39	64	7**
2-methylpropanol	13083	9390	18784	2669	20	487	3**
2-methylbutanol	18345	12511	30606	4680	26	655	6**
3-methylbutanol	**95130**	**66767**	**112432**	11105	12	2376	1[ns]
hexanol	1596	904	2583	461	29	78	6**
acetic acid [b]	**334000**	180000	**620000**	94223	28	21069	one rep
2-methylpropanoic acid	490	339	771	125	25	22	4**
3-methylbutanoic acid	308	221	460	61	20	11	3*
hexanoic acid	**7949**	**5324**	**10001**	1410	18	315	one rep
octanoic acid	**8952**	**7563**	**11230**	975	11	223	1[ns]
decanoic acid	2437	1414	3670	577	24	102	3**
linalool	**45**	nd	**160**	47	104	7	10304**
α-terpineol	63	8	117	32	51	5	3896**
nerol	3	nd	11	3	105	0.5	482**

	mean (µg/L)	minimum (µg/L)	maximum (µg/L)	SD (µg/L)	CV (%)	SEM (µg/L)	F ratio [a]
geraniol	16	7	**36**	8	52	1	48**
***cis*-rose oxide**	0.1	0.06	**0.3**	0.07	50	0.01	78**
TDN	**22**	1	**93**	26	119	4	4219**
TPB	0.01	0.002	**0.04**	0.01	85	0.002	248**
β-damascenone	**3**	**0.7**	**7**	2	56	0.3	451**
guaiacol	0.6	0.2	2	0.4	80	0.07	33**
4-methylguaiacol	0.2	0.03	0.7	0.2	91	0.03	63**
4-ethylphenol	0.2	nd	1	0.3	163	0.05	14**
4-ethylguaiacol	0.04	nd	0.2	0.06	155	0.01	2[ns]
4-vinylguaiacol	0.2	0.03	0.5	0.1	76	0.02	260**
4-vinylphenol	0.3	0.02	0.9	0.3	92	0.04	43**
***cis*-oak lactone**	1	0.02	4	1	103	0.2	6**
vanillin	7	nd	48	13	192	2	158**
methionol	455	278	**1061**	202	44	32	96**
dimethyl sulfide	**27**	4	**81**	23	87	4	148**
carbon disulfide	0.5	nd	1	0.3	68	0.05	21**

[a] significance indicated by ** ($p < 0.01$), * ($p < 0.05$), [ns]: not significant, one rep: only one replicate data point was obtained for each wine; [b] acetic acid measured as volatile acidity; compound not detected (nd); compound concentrations in bold are above their reported sensory detection threshold listed in Table 1-1, Chapter 1.

All compounds measured were found to be significantly different between wine samples (ANOVA, $p < 0.05$) with the exception of 3-methylbutanol, octanoic acid and 4-ethylguaiacol. For certain fermentation-derived compounds, only one data point was obtained for each sample (indicated by 'one rep' in table) and analysis of variance could not be conducted for these compounds. The compounds ethyl lactate, propanoic acid, butanoic acid, β-ionone, ethanethiol, diethyl sulfide, methyl thioacetate, dimethyl disulfide, ethyl thioacetate and diethyl disulfide were not detected in any of the Riesling wines analysed (below detection limit of analysis) and hence are not included in the Table 3-5. Each of the compounds ethyl dodecanoate, 2-methylbutyl acetate, hexyl acetate, linalool, 4-ethylphenol, 4-ethylguaiacol, vanillin, and carbon disulfide were at concentrations below the detection limit of the instrument in at least one wine sample and were not able to be measured (indicated by not detected, 'nd', in Table 3-5). The values obtained from routine chemical analysis for volatile acidity (acetic acid) are used for data analysis. Although diacetyl was measured, only ten Riesling wines were successfully analysed for this compound (due to instrumental difficulties) and the remaining wines were not re-analysed due to time constraints.

As expected, the volatile chemical data ranged from more than 100000 µg/L (for example, ethyl acetate, 3-methylbutanol and acetic acid) to less than 0.1 µg/L (*cis*-rose oxide and TPB). In general, much higher concentrations were found for the fermentation-derived compounds than for the grape-derived or oak-derived compounds. Although some

compounds were measured at relatively low concentrations, their relative variation in concentration was often very broad (high CV). For example, TDN was measured between 1 and 93 μg/L with large variation (CV = 119%) in comparison to 3-methylbutanol which was measured between 66000 and 112000 μg/L with a relatively small variation (CV = 13%). This highlights the fact that although an aroma compound may be measured at high concentration its variation in concentration may not necessarily have a larger influence on wine aroma than a compound which is measured at low concentration. Large variations were observed between the wines for most of the volatile compounds measured which demonstrates the diversity of volatile composition in the Riesling wines selected for the study.

Pearson correlation (pair-wise) between chemical variables were analysed for the volatile chemical data and a summary of these results for the strongest correlations, where $r \geq 0.85$ or $r \leq -0.85$ was found between at least two compounds, is tabulated in Table 3-6.

High collinearity was observed between some of the volatile chemical variables. Specifically, high positive correlations ($r \geq 0.85$) were found between 2-methylpropyl acetate, 2-methylbutyl acetate, 3-methylbutyl acetate and hexyl acetate ($r \geq 0.88$), between ethyl 2-methylbutanoate and ethyl 3-methylbutanoate ($r = 0.93$), between 2-phenylethanol and 2-phenylethyl acetate ($r = 0.94$), between linalool, geraniol and nerol ($r = 0.98$) and between TDN, guaiacol and 4-methylguaiacol ($r \geq 0.91$) (Table 3-6).

The high collinearity observed between volatile compounds may arise from the similarity in biochemical pathways from which these compounds are formed. For example, linalool, geraniol and nerol are all monoterpenes formed by similar pathways and are degraded with wine ageing [193]. As the set of wines includes aged and young Riesling wines, certain compounds may be collinear because they independently, but simultaneously, increase or decrease in concentration with bottle age due to acid hydrolysis or oxidation reactions that occur over time in wine. For example, as wine ages hexyl acetate decreases in concentration due to acid hydrolysis [64], whereas TDN increases in concentration over time due to gradual release from its glycosidic precursor through hydrolytic cleavage [3, 38].

Table 3-6 Pearson correlation coefficient matrix (r) of selected Riesling wine volatile compounds

	2-methylpropyl acetate	ethyl 2-methylbutanoate	ethyl 3-methylbutanoate	2-methylbutyl acetate	3-methylbutyl acetate	hexyl acetate	2-phenylethyl acetate	ethyl octanoate	ethyl dodecanoate	2-phenylethanol	nerol	linalool	geraniol	TDN	β-damascenone	guaiacol	4-methylguaiacol	4-vinylguaiacol	dimethyl sulfide
2-methylpropyl acetate	**1.00**																		
ethyl 2-methylbutanoate	-0.71	**1.00**																	
ethyl 3-methylbutanoate	-0.82	**0.93**	**1.00**																
2-methylbutyl acetate	**0.96**	-0.66	-0.78	**1.00**															
3-methylbutyl acetate	**0.95**	-0.72	-0.82	**0.97**	**1.00**														
hexyl acetate	**0.88**	-0.72	-0.81	**0.91**	**0.97**	**1.00**													
2-phenylethyl acetate	0.68	-0.23	-0.38	0.75	0.62	0.52	**1.00**												
ethyl octanoate	0.65	-0.61	-0.64	0.63	0.60	0.60	0.43	**1.00**											
ethyl dodecanoate	0.70	-0.65	-0.75	0.68	0.63	0.62	0.60	**0.86**	**1.00**										
2-phenylethanol	0.53	-0.06	-0.20	0.64	0.52	0.44	**0.94**	0.32	0.45	**1.00**									
nerol	**0.87**	0.62	-0.76	0.83	0.78	0.72	0.72	0.61	0.80	0.54	**1.00**								
linalool	**0.86**	-0.63	-0.76	0.82	0.76	0.70	0.69	0.59	0.78	0.52	**0.99**	**1.00**							
geraniol	**0.89**	-0.61	-0.75	**0.88**	0.84	0.79	0.74	0.57	0.75	0.58	**0.98**	**0.98**	**1.00**						
TDN	-0.79	0.84	0.84	-0.74	-0.77	-0.74	-0.38	-0.71	-0.69	-0.30	-0.70	-0.70	-0.69	**1.00**					
β-damascenone	0.76	-0.71	-0.76	0.69	0.69	0.63	0.55	0.53	0.69	0.38	0.84	**0.85**	0.83	-0.74	**1.00**				
guaiacol	-0.58	0.81	0.74	-0.54	-0.57	-0.56	-0.25	-0.57	-0.60	-0.22	-0.50	-0.51	-0.48	**0.91**	-0.60	**1.00**			
4-methylguaiacol	-0.72	0.88	**0.85**	-0.68	-0.72	-0.68	-0.30	-0.66	-0.67	-0.22	-0.64	-0.66	-0.63	**0.96**	-0.72	**0.94**	**1.00**		
4-vinylguaiacol	0.84	-0.69	-0.79	0.78	0.79	0.71	0.56	0.43	0.56	0.40	**0.85**	0.81	0.82	-0.72	**0.85**	-0.56	-0.71	**1.00**	
dimethyl sulfide	-0.61	**0.88**	0.74	-0.59	-0.59	-0.57	-0.35	-0.58	-0.65	-0.20	-0.61	-0.64	-0.60	0.75	-0.74	0.76	0.80	-0.61	**1.00**

$r \leq -0.85$ and $r \geq 0.85$ are indicated in bold typeface

To explore the potential sensory impact of each volatile compound measured in the Riesling wines, odour activity values (OAVs) were calculated by dividing each compound concentration by its respective sensory detection threshold value [169, 188]. Table 3-7 shows a summary of each volatile compound's sensory detection threshold concentration (taken from Table 1-1, Chapter 1) and respective mean, minimum and maximum OAV. Literature sensory threshold values that were determined in either white wine or synthetic wine were used in preference where possible. For some compounds the only suitable sensory thresholds found were determined in other media such as beer or water.

Table 3-7 Odour activity values for each volatile compound measured in the Riesling wines

	literature sensory threshold (µg/L)	mean OAV	minimum OAV	maximum OAV
ethyl acetate	7500 [6]	**10**	**7**	**16**
ethyl propanoate	1840 [4]	0.1	0.06	0.2
ethyl butanoate	20 [6]	**22**	**15**	**30**
ethyl 2-methylpropanoate	15 [6]	**5**	**3**	**10**
ethyl 2-methylbutanoate	1 [6]	**15**	**4**	**38**
ethyl 3-methylbutanoate	3 [6]	**9**	**3**	**19**
ethyl hexanoate	5 [6]	**263**	**191**	**341**
ethyl octanoate	2 [6]	**870**	**411**	**1610**
ethyl decanoate	200 [11]	**4**	0.8	**7**
ethyl dodecanoate	2000 [25]	0.03	nd	0.08
***trans*-ethyl cinnamate**	1 [6]	0.9	0.2	**2**
2-methylpropyl acetate	1600 [11]	0.026	0.005	0.04
2-methylbutyl acetate	5 [27]	**13**	nd	**38**
3-methylbutyl acetate	30 [6]	**45**	**1**	**122**
hexyl acetate	670 [4]	0.2	nd	0.6
2-phenylethyl acetate	250 [6]	**1**	0.02	**8**
2-phenylethanol	10000 [6]	**2**	0.9	**9**
butanol	150000 [4]	0.006	0.003	0.01
2-methylpropanol	40000 [6]	0.3	0.2	0.5
2-methylbutanol	65000 [6]	0.3	0.2	0.5
3-methylbutanol	30000 [6]	**3**	**2**	**4**
hexanol	8000 [6]	0.2	0.1	0.3
acetic acid [a]	200000 [6]	**2**	0.9	**3**
2-methylpropanoic acid	200000 [6]	0.002	0.002	0.004
3-methylbutanoic acid	3000 [6]	0.1	0.07	0.2
hexanoic acid	3000 [6]	**3**	**2**	**3**
octanoic acid	500 [11]	**18**	**15**	**22**
decanoic acid	15000 [6]	0.2	0.1	0.2
linalool	15 [6]	**3**	nd	**11**
α-terpineol	250 [11]	0.3	0.03	0.5

	literature sensory threshold (µg/L)	mean OAV	minimum OAV	maximum OAV
nerol	500 [12]	0.006	nd	0.02
geraniol	30 [6]	0.5	0.2	**1**
***cis*-rose oxide**	0.2 [6]	0.7	0.3	**1**
TDN	20 [3]	**1**	0.07	**5**
TPB	0.04 [39]	0.3	0.05	**1**
β-damascenone	0.05 [6]	**63**	**13**	**132**
guaiacol	10 [6]	0.06	0.02	0.2
4-methylguaiacol	65 [17]	0.003	0.0004	0.01
4-ethylphenol	1100 [17]	0.0002	nd	0.001
4-ethylguaiacol	70 [17]	0.0006	nd	0.003
4-vinylguaiacol	440 [17]	0.0005	0.00007	0.001
4-vinylphenol	770 [17]	0.0004	0.00003	0.001
***cis*-oak lactone**	23 [28]	0.05	0.0009	0.2
vanillin	200 [6]	0.04	nd	0.2
methionol	500 [6]	0.9	0.6	**2**
dimethyl sulfide	10 [6]	**2.7**	0.4	**8.1**
carbon disulfide	5 [194]	0.1	nd	0.2

[a] acetic acid measured as volatile acidity; compound not detected (nd); sensory detection thresholds were determined in 10% ethanol in water (w/w) [6], 11% ethanol in water (v/v) model wine [11], beer [25], water [27], wine [3, 4, 194], white wine [17, 28, 33, 39].

As discussed in Section 1.2, Chapter 1, it is difficult to compare sensory thresholds directly when they have been sourced from different authors using different media for threshold determination, different sensory panels and different sensory techniques. It is also important to note that many of the sensory thresholds used for the calculation of OAV's were determined in model wine, and these threshold concentrations might be lower in a white wine matrix (e.g. as observed for TPB [39]). Nevertheless, it can be useful to apply sensory thresholds to provide an estimation of the relative importance of certain aroma compounds measured in the study wines.

Of the 47 compounds quantified, 12 compounds were measured in all wines above sensory threshold, a further eight compounds were on average above sensory threshold, and an additional five compounds were measured in at least one wine above their respective sensory detection threshold concentration (OAV ≥ 1).

Compounds measured at concentrations many times their indicative sensory detection thresholds (OAV > 5) include all the ethyl esters (except for ethyl propanoate, ethyl dodecanoate and *trans*-ethyl cinnamate), 2-methylbutyl acetate, 3-methylbutyl acetate, 2-phenylethyl acetate, octanoic acid, 2-phenylethanol, linalool, β-damascenone and dimethyl sulfide. Although the OAVs calculated for these compounds are only indicative, it is likely

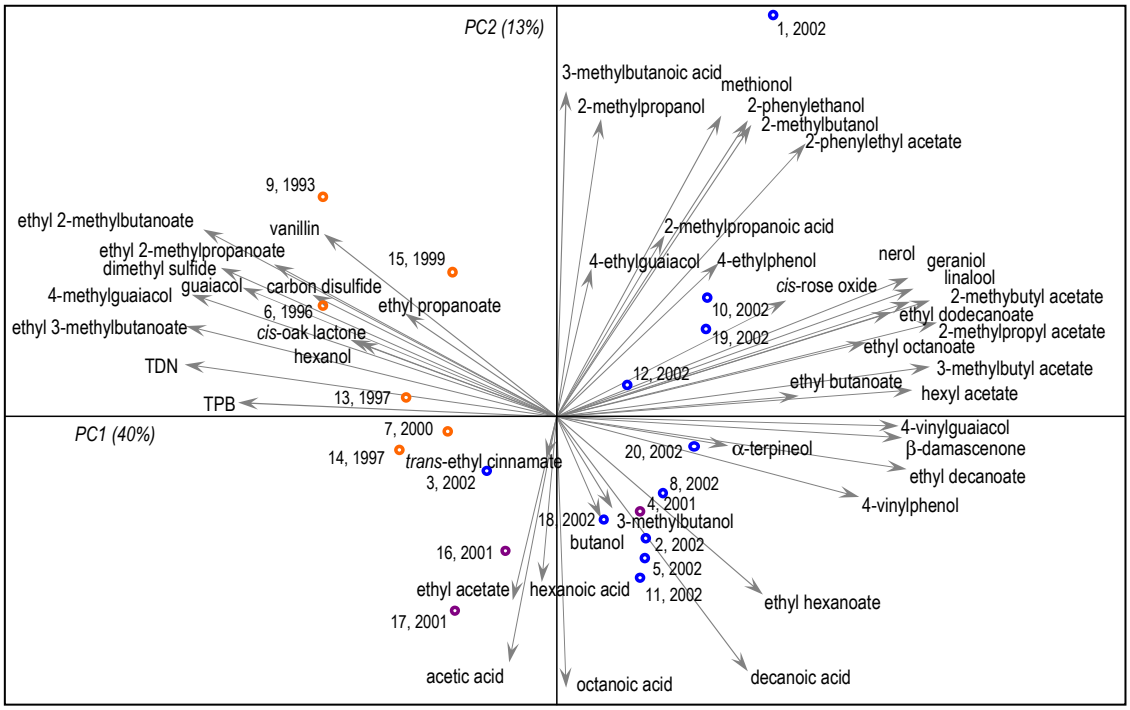
that these compounds are playing an important role in the aroma of the study wines. Some of these compounds, namely ethyl hexanoate, ethyl octanoate and β-damascenone, were measured at concentrations more than 100 times their respective model wine sensory threshold concentration and therefore these compounds could be exceptionally important to the aroma of these Riesling wines.

Compounds that were found at concentrations around their respective sensory threshold (OAV 0.2 - 5) include ethyl propanoate, *trans*-ethyl cinnamate, hexyl acetate, 2-methylpropanol, 2-methylbutanol, 3-methylbutanol, hexanol, acetic acid, 3-methylbutanoic acid, hexanoic acid, decanoic acid, α-terpineol, geraniol, *cis*-rose oxide, TDN, TPB, guaiacol, *cis*-oak lactone, methionol and carbon disulfide. These compounds might play important roles in the aroma of some of the study wines.

Volatile compounds with concentrations well below their indicative sensory detection concentration (OAV < 0.2) are less likely to be playing an important role in the aroma of these wines. These compounds include ethyl dodecanoate, 2-methylpropyl acetate, butanol, 2-methylpropanoic acid, nerol, 4-methylguaiacol, 4-ethylphenol, 4-ethylguaiacol, 4-vinylguaiacol and 4-vinylphenol.

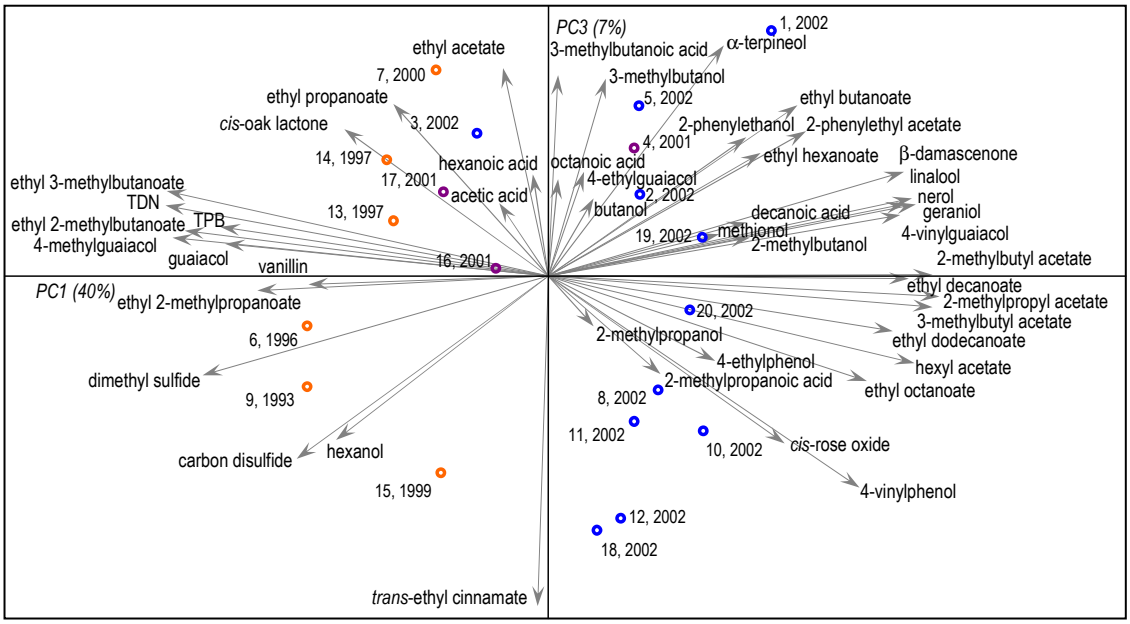
The volatile chemical data were assessed using PCA to further explore relationships between compound variables and to look for groupings among the wines. As discussed earlier, the chemical data is comprised of a large number of volatile compounds, with collinearity between only some of the variables. The compounds measured are derived from a number of independent sources including various biochemical pathways in the grape berry and during yeast fermentation, which are influenced by many different factors such as climate, soil types, viticultural practices and winemaking style. Consequently, a broad diversity of complex information is expressed in the measurement of these volatile compounds. Not surprisingly, PCA was not able to explain a large proportion of the variance of such a rich data set in just three PCs. For this data set, only 61% of variation was explained in the first three PCs which demonstrates the diversity and complexity of the chemical data. Although further PCs were explored only PC1, PC2 and PC3 are presented (Figure 3-4 and Figure 3-5).

Figure 3-4 PCA bi-plot of volatile analytical results for the Riesling wines, PC1 versus PC2



For details on the sample codes refer to Table 3-1, vintages are also indicated. Sample scores are calculated from the mean of two replicates and volatile compound loadings (vectors) are shown.

Figure 3-5 PCA bi-plot of volatile analytical results for the Riesling wines, PC1 versus PC3



For details on the sample codes refer to Table 3-1, vintages are also indicated. Sample scores are calculated from the mean of two replicates and volatile compound loadings (vectors) are shown.

As with the sensory data, the first PC constructed from the volatile chemical data divided the older Riesling wines (on the left, Figure 3-4) from the young Riesling wines (on the right,

Figure 3-4). Many similarities in the grouping of wines between the sensory and volatile chemical data were observed visually by PCA. For example, the 2001 vintage Riesling wine (4, 2001) which grouped with the young 2002 wines in the sensory data set (refer to Figure 3-2), again grouped with the young 2002 wines according to the volatile chemical data. Similarly, the 2002 vintage wine (3, 2002) which in the sensory data PCA grouped toward the older Riesling wines (refer to Figure 3-2) also grouped toward the older Riesling wines in the volatile chemical data plot.

The similarities observed between sensory and chemical data PCA plots indicate that the sensory differences between the wines could be explained by the variation in the volatile composition. The major differences observed in grouping of wines between the sensory and volatile chemical PCA involved wine 11, 2002, and wine 1, 2002. Wine 11, 2002, which was scored highly for *passionfruit* and *herbaceous* in the sensory analysis (refer to Figure 3-2), did not differ from the other 2002 wines according to the volatile chemical data (Figure 3-4). This indicates that the compound/s that may explain the sensory variation observed for wine 11 may not have been measured. On the other hand, wine 1, 2002, which was scored in a similar way to the other 2002 wines in the sensory analysis (refer to Figure 3-2), was not similar to the 2002 wines according to the volatile chemical analysis (Figure 3-4). The difference observed by PCA in the volatile chemical analysis was due to high concentrations of 2-phenylethanol and 2-phenylethyl acetate for wine 1. This could suggest that the variation of these two volatiles did not have a large impact on the aroma of the wines for the attributes scored by the sensory panel.

Older Riesling wines, grouped on the left had side of the plot (Figure 3-4 and Figure 3-5) were characterised by higher concentrations of ethyl 2-methylbutanoate, 3-methylbutanoate, ethyl 2-methylpropanoate, ethyl propanoate, vanillin, TDN, TPB, *cis*-oak lactone, guaiacol, 4-methylguaiacol, dimethyl sulfide, carbon disulfide, and hexanol. The younger Riesling wines, grouped on the right side of the plot (Figure 3-4 and Figure 3-5) had higher concentrations of geraniol, linalool, nerol, *cis*-rose oxide, 2-methylbutyl acetate, 3-methylbutyl acetate, 2-methylpropyl acetate, hexyl acetate, ethyl dodecanoate, ethyl octanoate, ethyl butanoate, ethyl decanoate, ethyl hexanoate, β-damascenone, 4-vinylguaiacol and 4-vinylphenol. The young wines differed across PC2 in having either higher concentration of acetic acid, octanoic acid, hexanoic acid, decanoic acid and ethyl acetate (on the lower half of Figure 3-4), or having high concentration of 3-methylbutanoic acid, 2-methylpropanoic acid, methionol, 2-phenylethanol, 2-phenylethyl acetate, 2-methylbutanol, 2-methylpropanol, 4-ethylphenol and 4-ethylguaiacol (on the top half of Figure 3-4). The third PC, which only explains 7% of variation, is strongly dominated by differences in concentration of *trans*-ethyl cinnamate for both older and young Riesling wines.

To examine the distribution of volatile compound concentrations found within the aged and young Riesling groups of wines further, the wines were divided into two groups according to their age. The 2002 and 2001 vintage wines, which were 6 months and 18 months of age respectively, were included in the 'young Riesling' group and the wines from the 2000 vintage and older, which were more than 2 years old, were included in the 'aged Riesling' group. The average, minimum and maximum concentrations of each compound for the two groups of wines are given in Table 3-8. Compound concentrations that were above sensory detection threshold (OAV > 1) are indicated in bold typeface. As discussed previously, cautious interpretations must be made when comparing compound concentrations against threshold data that is sourced from different authors and/or that are determined in different media.

Table 3-8 Summary of the volatile chemical analysis results for the 'aged' and 'young' Riesling wines

	'young Riesling' group (n=14)			'aged Riesling' group (n=6)		
	mean (µg/L)	minimum (µg/L)	maximum (µg/L)	mean (µg/L)	minimum (µg/L)	maximum (µg/L)
ethyl acetate	**75109**	**49073**	**117130**	**76787**	**58706**	**97754**
ethyl propanoate	182 *	109	261	212 *	136	337
ethyl butanoate	**469** *	311	**599**	**414**	**305**	**520**
ethyl 2-methylpropanoate *	**64** *	44	**102**	**100** *	**74**	**143**
ethyl 2-methylbutanoate *	**8** *	4	**17**	**29**	**23**	**38**
ethyl 3-methylbutanoate *	**16** *	8	**32**	**49**	**45**	**57**
ethyl hexanoate	**1356** *	1159	**1704**	**1214**	**955**	**1417**
ethyl octanoate *	**1940**	1266	**3219**	**1271**	**821**	**1486**
ethyl decanoate *	**871**	528	**1449**	**340** *	175	**632**
ethyl dodecanoate *	81	26	160	9 *	nd	46
***trans*-ethyl cinnamate**	0.9 *	0.2	**2**	0.8 *	0.4	**2**
2-methylpropyl acetate *	33 *	7	58	11 *	8	12
2-methylbutyl acetate *	**90** *	nd	**191**	1	nd	**9**
3-methylbutyl acetate *	**1895** *	80	3667	**75**	**38**	**101**
hexyl acetate *	192 *	0.8	411	0.2	nd	0.8
2-phenylethyl acetate	**344** *	9	**2059**	10 *	5	18
2-phenylethanol	**22639** *	9175	**85393**	**16755** *	**10001**	**29464**
butanol	856 *	529	1473	852 *	464	1946
2-methylpropanol	12661 *	9390	18784	14069	10728	18268
2-methylbutanol	18737 *	12511	30606	17430	14287	21052
3-methylbutanol	95554	66767	105628	**94140**	**71069**	**112432**
hexanol *	1451 *	904	2254	1936	1339	2583
acetic acid [a]	**335000** *	180000	**620000**	**331667**	**260000**	**400000**
2-methylpropanoic acid	501 *	339	771	466	346	644
3-methylbutyric acid	295 *	221	460	337 *	283	385
hexanoic acid	**8116**	**5324**	**10001**	**7560**	**5798**	**9046**

	'young Riesling' group (n=14)			'aged Riesling' group (n=6)		
	mean (µg/L)	minimum (µg/L)	maximum (µg/L)	mean (µg/L)	minimum (µg/L)	maximum (µg/L)
octanoic acid	**9074**	**7578**	**11230**	**8669**	**7563**	**9986**
decanoic acid *	2673	1696	3670	1885	1414	2466
linalool *	**64** *	2	**160**	0.9 *	nd	3
α-terpineol *	73 *	39	117	40 *	8	107
nerol *	4 *	nd	11	nd	nd	nd
geraniol *	19 *	7	**36**	9	7	10
***cis*-rose oxide**	0.2 *	0.06	**0.3**	0.11 *	0.07	0.2
TDN *	10 *	1	**56**	**51** *	**24**	**93**
TPB *	0.01 *	0.002	0.03	0.02	0.01	0.04
β-damascenone *	**4** *	**2**	**7**	**1** *	**0.7**	**2**
guaiacol *	0.3 *	0.2	0.7	1 *	0.3	2
4-methylguaiacol *	0.1 *	0.03	0.3	0.4 *	0.2	0.7
4-ethylphenol	0.2 *	nd	1	0.07	nd	0.3
4-ethylguaiacol	0.04	nd	0.2	0.04 *	nd	0.1
4-vinylguaiacol *	0.2 *	0.05	0.5	0.05 *	0.03	0.07
4-vinylphenol *	0.4 *	0.02	0.9	0.04 *	0.02	0.07
***cis*-oak lactone**	0.9 *	0.02	4	2 *	0.2	4
vanillin *	1 *	nd	9	20 *	nd	48
methionol	461 *	285	**1061**	441 *	278	**781**
dimethyl sulfide *	**15** *	4	**25**	**55** *	**23**	**81**
carbon disulfide *	0.3 *	nd	0.60	0.8 *	0.3	1

[a] acetic acid measured as volatile acidity; compound not detected (nd); compound concentrations in bold are above sensory detection threshold (threshold data in Table 3-7); compounds labelled with * indicate a statistically significant difference was found between 'young Riesling' and 'aged Riesling' groups (ANOVA, $p < 0.05$); mean values labelled with * indicates a statistically significant difference was found within that group of wines (ANOVA, $p < 0.05$).

The variations in concentrations of volatile compounds within the group of young wines and within the group of older wines is likely to be due to differences in viticultural regions and climates, winemaking practices, yeast type used, and the varying qualities of the fruit used for winemaking. The compositional differences between the 'young Riesling' and the 'aged Riesling' groups may also be influenced by these factors, but more importantly from the compositional changes that occur through acid hydrolysis and oxidative reactions occurring over time during bottle ageing.

The 'aged Riesling' wines were found to have significantly higher concentrations of ethyl 2-methylpropanoate, ethyl 2-methylbutanoate, ethyl 3-methylbutanoate, hexanol, TDN, TPB, guaiacol, 4-methylguaiacol, 4-vinylguaiacol, 4-vinylphenol, vanillin, dimethyl sulfide and carbon disulfide (ANOVA, $p < 0.05$). The 'young Riesling' wines had significantly higher concentrations of 2-methylpropyl acetate, 2-methylbutyl acetate, 3-methylbutyl acetate, hexyl acetate, ethyl octanoate, ethyl decanoate, ethyl dodecanoate, decanoic acid, linalool,

α-terpineol, nerol, geraniol and β-damascenone (ANOVA, $p < 0.05$). It might be that these compounds have a major influence on the distinguishing aromas of an aged or a young Riesling wine. The remaining compounds were not significantly different between the two groups.

A comparison of wines within each group showed that, for the young Riesling group, most volatile compounds were found to have statistically significant differences between wines. Similarly, several of the compounds were found to have significant differences between wines in the aged Riesling group (ANOVA $p < 0.05$).

Overall, the variation in composition observed between the study wines appeared to be sufficient for the purpose of this study, and was expected to perform well in the prediction of sensory properties during multivariate data analysis.

3.2.3 Multivariate analysis of sensory and chemical data

Compared to the use of OAVs, which has many limitations (refer to discussion in Section 1.2, Chapter 1), multivariate data analysis is a far more useful tool for exploring the importance of individual volatile compounds, from a body of volatile chemical data, to explain the aroma properties of a wine.

It is possible that not all of the volatile compounds measured in the Riesling wines are actively contributing to the aroma of the wines. As a consequence the volatile chemical data set could contain redundant or useless information. To minimise the data set for multivariate analysis, compounds that were not contributing useful information to the data set were identified and excluded where possible to reduce the possibility of modelling redundant information or overfitting the model.

A conservative approach was used to reduce redundant variables from this data set. Groups of compounds that were either collinear (as investigated by Pearson's correlation and PCA), or shared similar biochemical origins, or had similar aroma properties (as a pure compound) or were likely to be acting additively were combined to give a new single 'grouped variable'. The new 'grouped variable' was calculated by dividing each compound's concentration, for each wine, by its own sensory detection threshold followed by adding them together as reported by Aznar [18] and as shown in the equation below.

$$x = \frac{[compound_1]}{[threshold\ of\ compound_1]} + \frac{[compound_n]}{[threshold\ of\ compound_n]}$$

Dividing each compound's concentration by its sensory threshold concentration allowed the mathematical contribution of each compound in the group to be adjusted by its probable sensory contribution to the group additively. Although every attempt was made to use threshold data from the same source and matrix within groups, this was not possible in all cases.

It is important to note that some groups of compounds may act in an additive or synergistic manner to create aroma in wine and will have an impact on the aroma even though all compounds within the group may be present at sub-threshold concentrations. In this study, compounds below their respective sensory detection threshold in model wine or white wine have not been excluded from multivariate analysis. It could be assumed, that compounds which are not influencing the aroma of wine will not be highlighted as important to the scoring of sensory attributes during multivariate data analysis.

Compounds that were grouped are tabulated in Table 3-9. These new grouped variables were used for multivariate data analysis instead of the single compound concentrations.

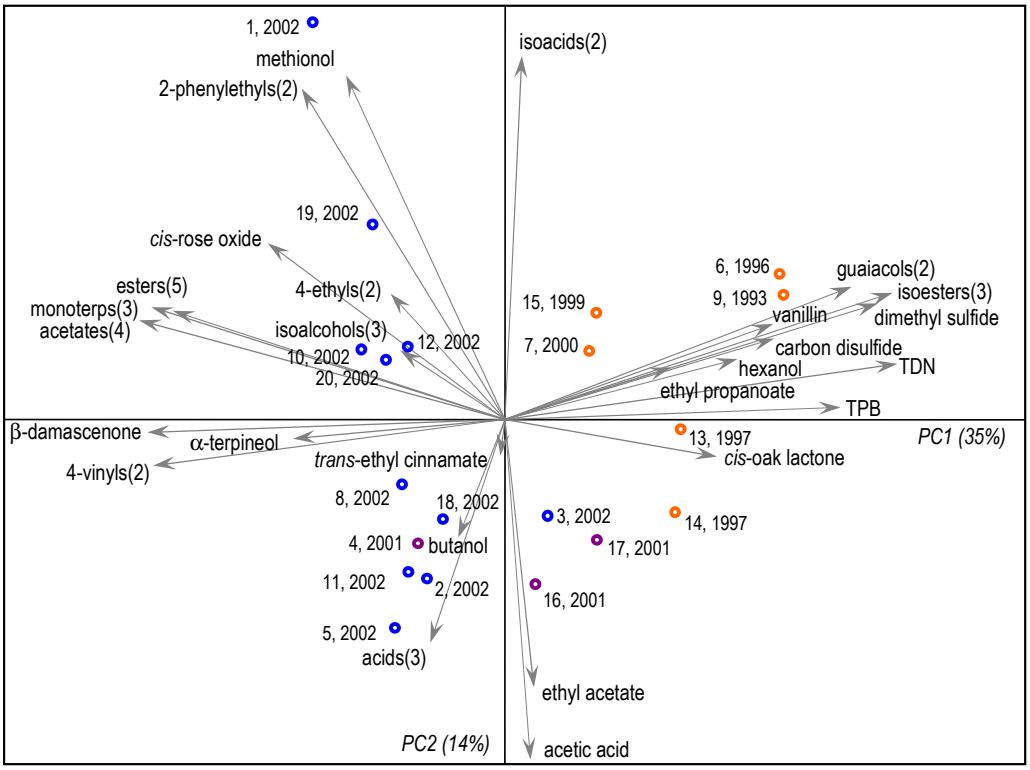
Table 3-9 Volatile chemical variables that were grouped into a single variable

new group (number of compounds)	compounds included in group
isoesters(3)	ethyl 2-methylpropanoate, 2-methylbutanoate, ethyl 3-methylbutanoate
esters(5)	ethyl butanoate, ethyl hexanoate, ethyl decanoate, ethyl octanoate, ethyl dodecanoate
acetates(4)	2-methylpropyl acetate, 2-methylbutyl acetate, 3-methylbutyl acetate, hexyl acetate
2-phenylethyls(2)	2-phenylethyl acetate, 2-phenylethanol
isoalcohols(3)	2-methylpropanol, 2-methylbutanol, 3-methylbutanol
isoacids(2)	2-methylpropanoic acid, 3-methylbutanoic acid
acids(3)	hexanoic acid, octanoic acid, decanoic acid
monoterpenes(3)	linalool, geraniol, nerol
guaiacols(2)	guaiacol, 4-methylguaiacol
4-vinyls(2)	4-vinylguaiacol, 4-vinylphenol
4-ethyls(2)	4-ethylguaiacol, 4-ethylphenol

the sensory threshold data used for each compound in this table to calculate the new variables are given in Table 3-7

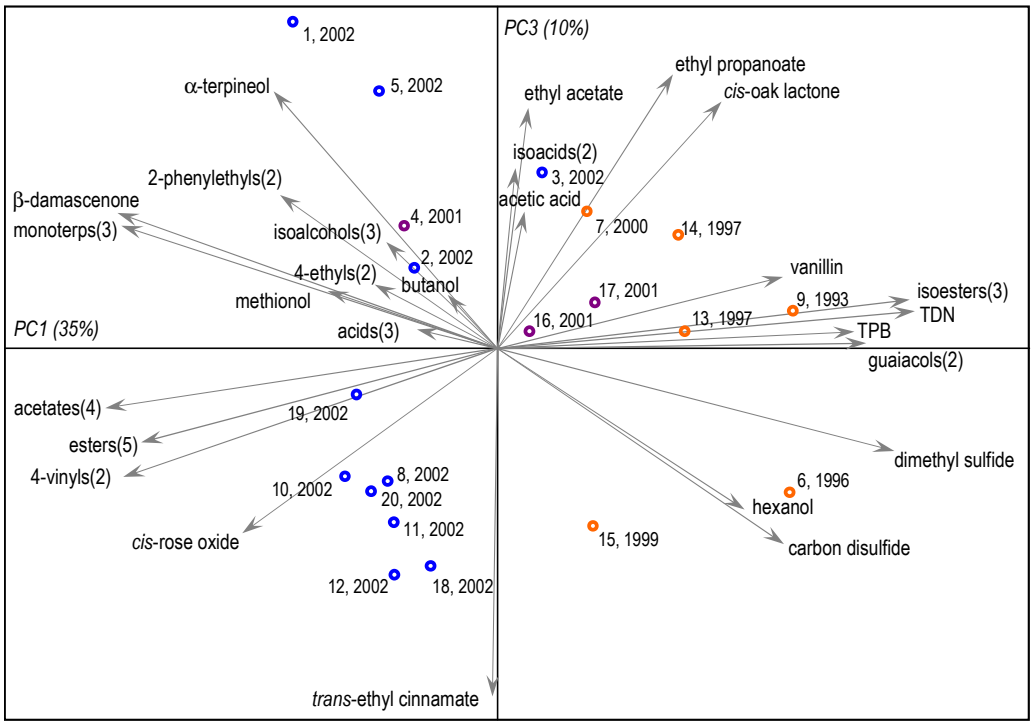
PCA bi-plots of the volatile chemical data set including the new grouped variables are shown in Figure 3-6 and Figure 3-7. The first three PCs explained 59% of variation in the data set which was almost the same amount of variation explained as was accounted for by the PCA of the ungrouped volatile compound data (61%). The grouping of the wines and the relationships between variables remained very similar to the original PCA of the volatile chemical data (refer to Figure 3-4 and Figure 3-5), with the advantage of a simplified data set (27 variables rather than 47) and the removal of some redundant information.

Figure 3-6 PCA bi-plot of volatile compounds and grouped variables for the Riesling wines, PC1 versus PC2



For details on the sample codes refer to Table 3-1, vintages are also indicated. Sample scores are mean values and volatile compound loadings (vectors) are shown.

Figure 3-7 PCA bi-plot of volatile compounds and grouped variables for the Riesling wines, PC1 versus PC3



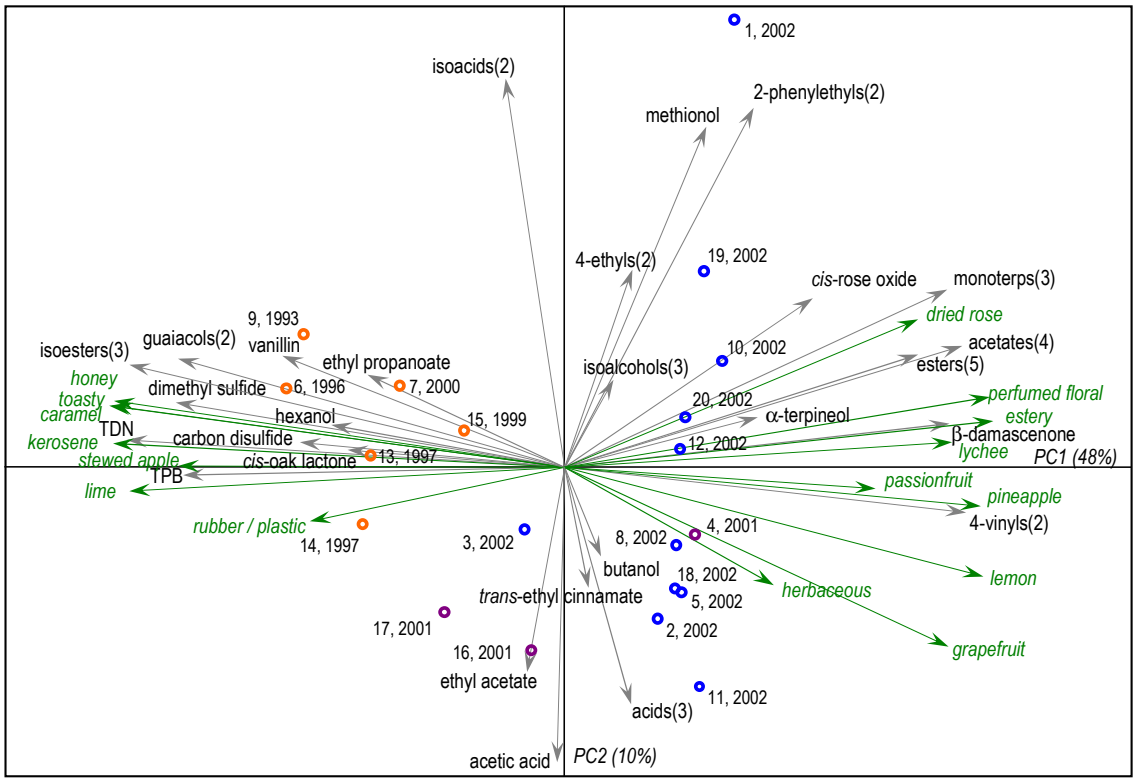
For details on the sample codes refer to Table 3-1, vintages are also indicated. Sample scores are mean values and volatile compound loadings (vectors) are shown.

The routine chemical variables (e.g. pH, alcohol, SO_2, etc), and the in-mouth flavour attributes rated in descriptive analysis, were not included in the multivariate data analysis presented in this thesis.

3.2.3.1 Relationships between the sensory and compositional data

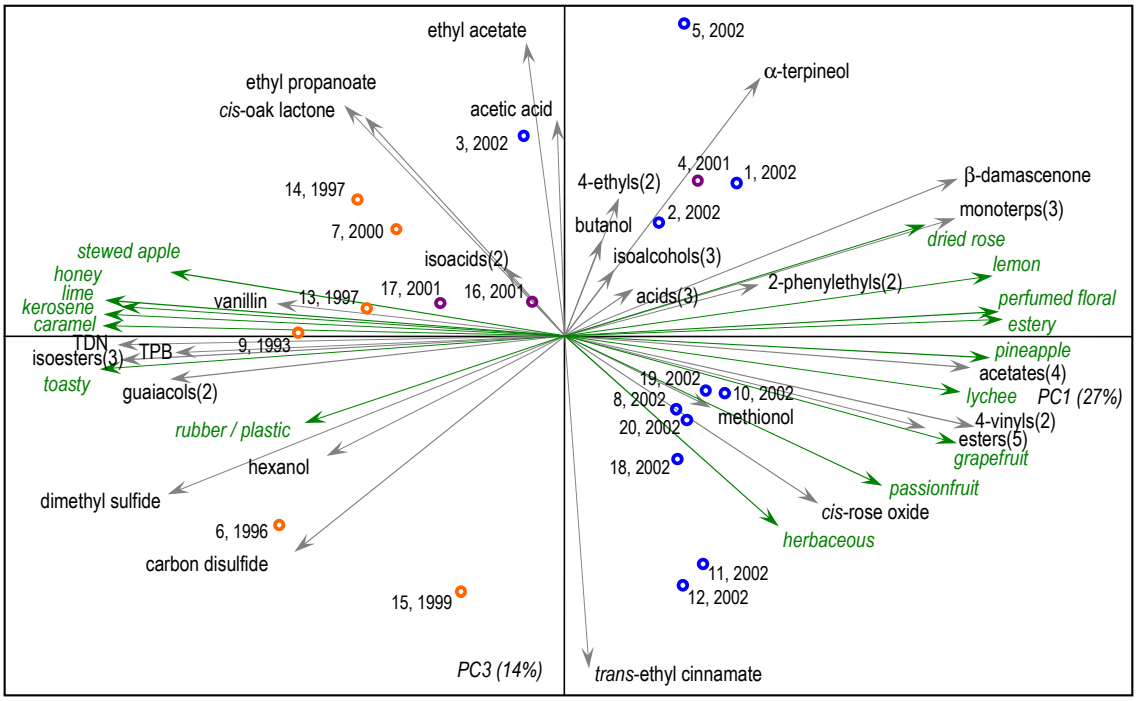
An initial explorative investigation was employed to assess how the compositional data set, as a whole, related to the sensory properties of the wines. In order to reduce the dimensionality of the data set, principal component analysis (PCA) was used to investigate relationships between sensory attributes and volatile compounds. The PCA bi-plots of the combined sensory data and volatile chemical variables are given in Figure 3-8 and Figure 3-9. By PCA, 65% of variation was explained by the first three PCs. As was observed previously with the PCA of the separate sensory (refer to Figure 3-2 and Figure 3-3) and volatile chemical data sets (refer to Figure 3-6 and Figure 3-7), the first PC constructed from the combined data set separated the older wines (on the left of the plot in Figure 3-8 and Figure 3-9) from the young wines (generally on the right).

Figure 3-8 PCA bi-plot of sensory and volatile chemical data for the Riesling wines, PC1 versus PC2



For details on the sample codes refer to Table 3-1, vintages are also indicated. Sample scores are mean values. Volatile compound loadings and sensory attribute loadings are shown.

Figure 3-9 PCA bi-plot of sensory and volatile chemical data for the Riesling wines, PC1 versus PC3



For details on the sample codes refer to Table 3-1, vintages are also indicated. Sample scores are mean values. Volatile compound loadings and sensory attribute loadings are shown.

By visual inspection of the loadings and scores in the bi-plot, wines that were scored higher for aged attributes including *kerosene*, *caramel*, *toasty*, *honey*, *lime* and *stewed apple* were typically also higher in concentration of guaiacols(2), isoesters(3), TDN, TPB, dimethyl sulfide, vanillin, hexanol, *cis*-oak lactone and carbon disulfide. Wines with higher scores for young 'floral' and 'fruity' attributes including *dried rose*, *perfumed floral*, *estery, lychee*, *passionfruit*, *pineapple*, *lemon*, *grapefruit* and *herbaceous* also had higher concentrations of *cis*-rose oxide, monoterpenes(3), acetates(4), esters(5), β-damascenone and 4-vinyls(2).

3.2.3.2 Prediction of sensory properties using compositional data

Partial least squares (PLS) regression was employed to analyse the data set with the aim to identify specific compounds related to specific aroma attributes. PLS1 was used to develop predictive models of each aroma attribute (*y*-variable) using the volatile chemical data including the grouped variables (*x*-variables). Two different sets of models were developed to explore the ability of the volatile compositional data to explain the variation of the scoring of sensory attributes.

For the first set of models, a regression was built using all *x*-variables (27 in total, refer to Appendix C for the results of these models) and the jack-knifing (JK) technique was used to identify non-contributing *x*-variables. These non-contributing variables were made passive, and models from subsequent iterations were developed that used only the *x*-variables that

were significantly contributing to the model [126, 142, 145, 195]. This approach was used to demonstrate overall how well the chemical data could perform in explaining the variation observed for each sensory attribute (*y*-variable).

The calibration statistics including coefficient of determination (R^2), root mean square error of cross validation (RMSECV), F value, optimum number of components used (C_{opt}), and the number of *x*-variables used (*x*-var) for the models are given in Table 3-10. The *x*-variables identified as significantly contributing to the models, either positively (+) or negatively (-) loaded, are also given in Table 3-10.

Table 3-10 PLS model results using jack-knifing for the prediction of Riesling aroma attribute scores

attribute	R^2	RMSECV	F value	C_{opt}	*x*-var	(+) loaded *x*-variable	(-) loaded *x*-variable
estery	0.79	0.44	2^{ns}	1	13	acetates(4), esters(5), 2-phenylethyls(2), monoterpenes(3), 4-vinyls(2), *cis*-rose oxide, β-damascenone	isoesters(3), guaiacols(2), TDN, TPB, dimethyl sulfide, carbon disulfide
perfumed floral	0.79	0.48	2^{ns}	1	12	acetates(4), esters(5), monoterpenes(3), 4-vinyls(2), *cis*-rose oxide, β-damascenone	isoesters(3), guaiacols(2), TDN, TPB, dimethyl sulfide, carbon disulfide
dried rose	0.53	0.30	1^{ns}	1	12	acetates(4), esters(5), monoterpenes(3), 4-vinyls(2), α-terpineol, β-damascenone	isoesters(3), guaiacols(2), TDN, TPB, dimethyl sulfide, carbon disulfide
lemon	0.88	0.24	13**	2	7	isoalcohols(3), β-damascenone	isoesters(3), guaiacols(2), TDN, TPB, dimethyl sulfide
grapefruit	0.66	0.23	7*	1	4		isoesters(3), guaiacols(2), TDN, TPB
lime	0.76	0.37	2^{ns}	1	12	isoesters(3), guaiacols(2), TDN, TPB, dimethyl sulfide, carbon disulfide	acetates(4), esters(5), monoterpenes(3), 4-vinyls(2), *cis*-rose oxide, β-damascenone
lychee	0.53	0.25	4^{ns}	1	4	acetates(4), esters(5), monoterpenes(3), β-damascenone	TDN
pineapple	0.74	0.24	3^{ns}	1	10	acetates(4), esters(5), monoterpenes(3), 4-vinyls(2), β-damascenone	isoesters(3), guaiacols(2), TDN, TPB, dimethyl sulfide
passionfruit	0.24	0.88	0.3^{ns}	1	10	acetates(4), esters(5), monoterpenes(3), β-damascenone	isoesters(3), guaiacols(2), TDN, TPB, dimethyl sulfide, carbon disulfide
stewed apple	0.56	0.19	1^{ns}	1	10	isoesters(3), guaiacols(2), TDN, TPB, dimethyl sulfide	esters(5), acetates(4), monoterpenes(3), 4-vinyls(2), β-damascenone
honey	0.86	0.39	6**	1	10	isoesters(3), guaiacols(2), TDN, TPB, dimethyl sulfide	esters(5), acetates(4), monoterpenes(3), 4-vinyls(2), β–damascenone
toasty	0.96	0.36	33**	1	8	isoesters(3), guaiacols(2), TDN, TPB, dimethyl sulfide	esters(5), acetates(4), β-damascenone
caramel	0.90	0.24	10**	1	9	isoesters(3), guaiacols(2), TDN, TPB, dimethyl sulfide	esters(5), acetates(4), 4-vinyls(2), β-damascenone
kerosene	0.90	0.40	26**	1	5	guaiacols(2), TDN, TPB	4-vinyls(2), β-damascenone
rubber / plastic	0.23	0.32	0.5^{ns}	1	7	TDN, TPB	esters(5), acetates(4), monoterpenes(3), 4-vinyls(2), β-damascenone

^{ns} not significant, * significant ($p < 0.05$), ** significant ($p < 0.01$)

In general, the chemical data performed quite well to predict the scores of a number of the Riesling wine sensory attributes. Attributes, for which models were be developed with

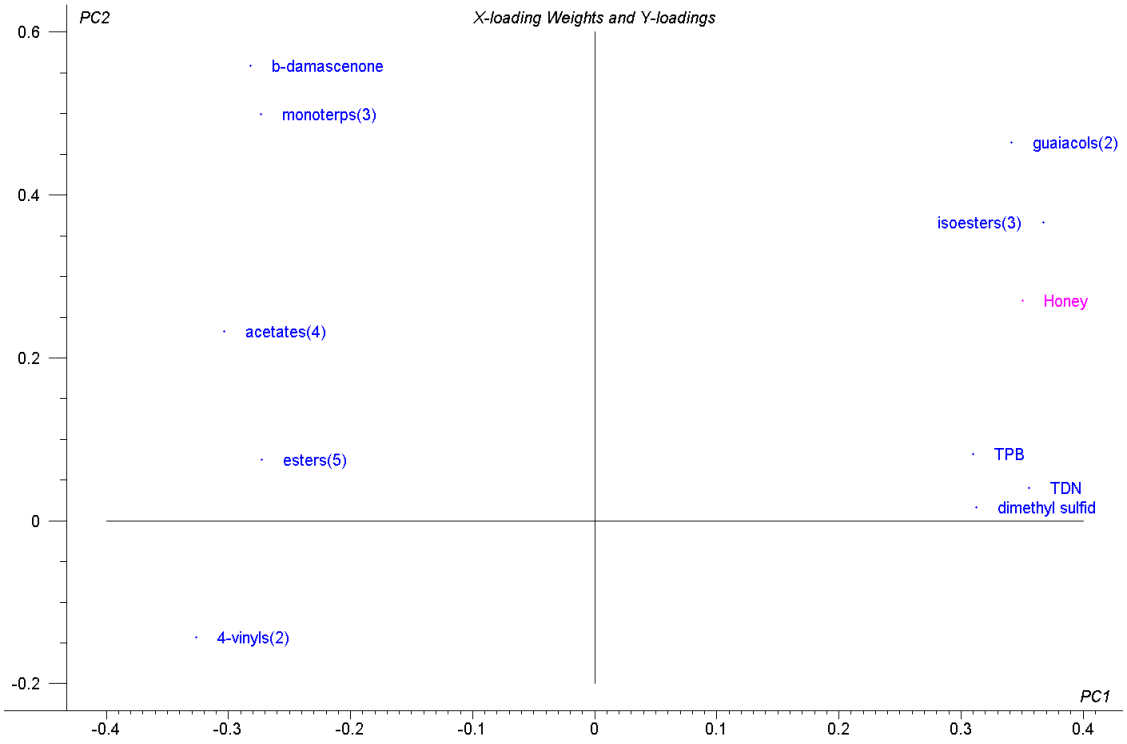
excellent calibration statistics, where more than 85% of variation was explained by the model ($R^2 > 0.85$), include *lemon*, *honey*, *toasty*, *caramel* and *kerosene*. The similarity in the results for *honey*, *toasty*, *caramel* and *kerosene* may be attributed to their inherent collinearity. Good calibration statistics were achieved ($R^2 > 0.75$) in the models for *estery*, *perfumed floral*, and *lime*, moderate calibrations statistics ($R^2 > 0.5$) were achieved in the predictions for *dried rose*, *grapefruit*, *lychee*, *pineapple* and *stewed apple*, and poor results ($R^2 < 0.5$) were obtained in the models developed for *passionfruit* and *rubber / plastic*. A meaningful model could not be obtained for the *herbaceous* attribute through the use of JK.

Overall, the RMSECV values for the models developed ranged from 0.19 to 0.88. The RMSECV is expressed in the same units as the original sensory variables (i.e. on the scale of 0 – 9) and reflects the prediction error expected in new samples and the performance of the models. The ratio of the SD of the reference data (i.e. the SD of the sensory descriptive data) to the RMSECV enables the evaluation of the predictive error of the models, in comparison to the error associated with the reference data used to build the prediction models. This ratio is commonly known as the 'ratio of standard error of performance to standard deviation' or the RPD [196, 197]. Ideally, the RPD should be 5 or higher [197]. For most of the attributes an acceptable prediction ability was achieved (RPD = 2 - 5) with the exception of *lychee*, *passionfruit*, *herbaceous* and *rubber / plastic*, which were found to have very poor prediction ability (RPD = 1).

The attributes associated with aged wine were generally much better predicted by the chemical data (high R^2, low RMSECV) than the attributes of younger wines. The descriptive analysis data for the aged wine attributes typically had much broader variation in scoring across the wines (high CV, refer to Table 3-2) which would aid in building a calibration with good prediction statistics. It also might be that, chemically, older wines become more similar as they age, whereas younger wines show more chemical diversity. This could mean that the aroma of older wines is more straightforward to predict with chemical data, resulting in better prediction statistics. This idea is supported by the fact that the *x*-variables used to predict the older wine attributes are almost identical, although the loading weights for each variable were different. The positive contribution of isoesters(3), guaiacols(2), TDN and TPB were common to the prediction of aged wine attributes *lime*, *stewed apple*, *honey*, *toasty*, *caramel*, and *kerosene*, and dimethyl sulfide was common to all but *kerosene*. The negative contribution of the esters(5), acetates(4) and β-damascenone were also common to most of the four aged wine attributes.

An example of positively and negatively contributing variables is given in the plot of *x*-loading weights and *y*-loadings for the prediction model of the *honey* attribute Figure 3-10.

Figure 3-10 Loadings of PLS model (10 *x*-variables) to predict *honey*

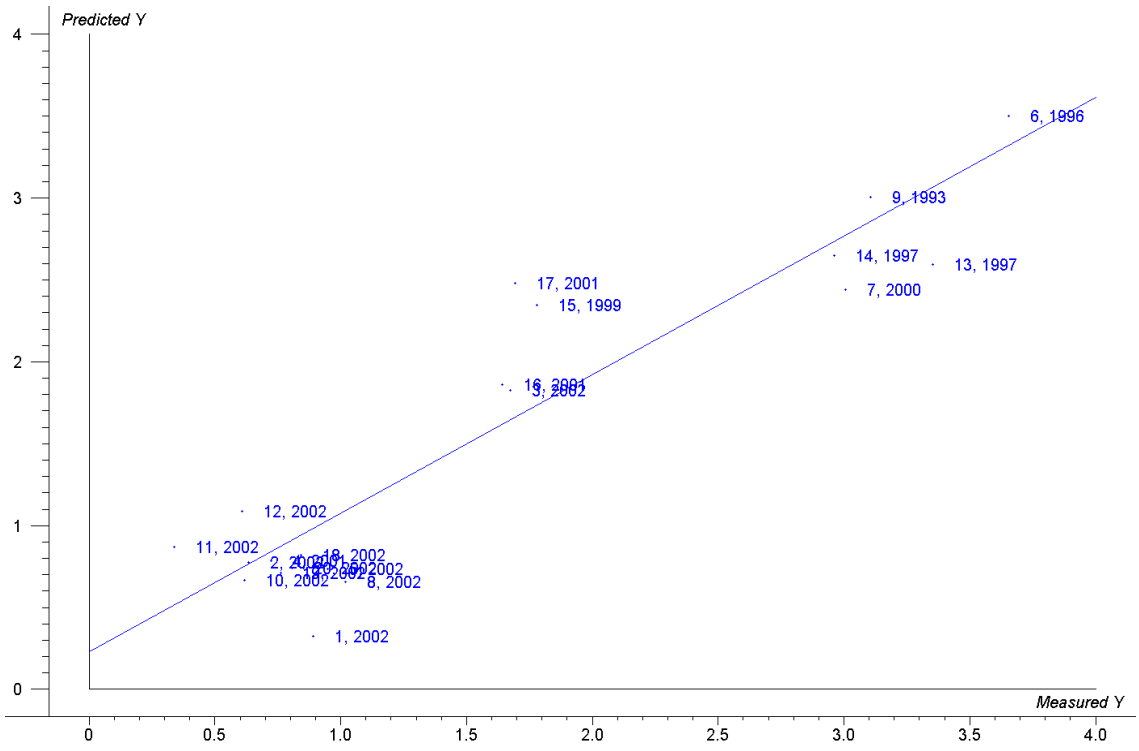


Variables in the model that were positively contributing to the *honey* attribute are on the right hand side of the plot (in Figure 3-10) with the *honey* attribute, and include guaiacols(2), isoesters(3), TPB, TDN, and dimethyl sulfide. Compounds in the regression that were negatively contributing to the prediction of *honey* are situated on the left hand side of the plot away from *honey*, and include β-damascenone monoterpenes(3), acetates(4), esters(5) and 4-vinyls(2). In general, the loadings can be interpreted that positively contributing compounds are likely to be responsible for the *honey* aroma attribute in wine, and negatively contributing compounds may be masking the perception of the *honey* aroma attribute in wine.

As was observed for the aged wine attributes, similar patterns of volatile compounds were observed between the models developed for the prediction of many of the young Riesling wine attributes. The prediction models for the 'fruity' and 'floral' attributes almost always included the positive contribution of the acetates (4), esters(5), monoterpenes(3), 4-vinyls(2) and β-damascenone, and the negative contribution of isoesters(3), guaiacols(2), TDN, TPB and dimethyl sulfide. This relationship appears to be the reverse of the pattern observed for the aged attributes in that many of the compounds positively loaded in models for the 'fruity' and 'floral' attributes are negatively loaded in the models for the prediction of the aged wine attributes, and the compounds negatively loaded in the 'fruity' and 'floral' attribute models are positively loaded in the prediction models for the aged wine attributes.

This inverse relationship can be explained by the fact that older wines are relatively high in compounds that are specific to aged wines and lower in those compounds typical of young wines (and vice versa). For example, Figure 3-11 shows a plot of the model of predicted versus measured for *honey*, with aged wines (2.5 – 9.5 years old) in the top right of the plot and young wines (6 – 18 months of age) on the bottom left of the plot.

Figure 3-11 PLS model to predict the scoring of *honey*



The aged wines in the top right of the plot, with higher scores for *honey*, have generally higher concentrations of isoesters(3), guaiacols(2), TDN, TPB and dimethyl sulfide and lower concentrations of acetates(4), esters(5), monoterpenes(3), 4-vinyls(2) and β-damascenone. These wines were also scored highly for *kerosene*, *caramel* and *toasty*. Consequently, strong positive relationships exist between compounds at higher concentration in older wines and the typical aged aroma properties of the older wines and strong negative relationships also exist between compounds typical of younger wines and the aged wine sensory attributes. The opposite is true for the 'floral' and 'fruity' attributes typical of the young Riesling wines. This may be the reason why the predictions of either aged or young wine sensory properties rely on similar compositional variables.

Like the aged wine attributes, the terms *passionfruit*, and *herbaceous* were also scored with high variation (high CV, refer to Table 3-2), however, the predictions of these attributes by the chemical data were very poor, indicating that the compounds responsible for these attributes were not measured.

The attributes *dried rose*, *lychee* and *stewed apple* were not predicted as well as some of the other young wine attributes. These attributes were scored both with low variation between the wines and with relatively low scores (less than 2) compared to the other young wine attributes. As discussed previously (Section 3.2.1), these attributes are perhaps superfluous to the already large number of 'fruity' or 'floral' terms that were included in the descriptive study. Consequently, the poor calibration statistics for these attributes are a reflection of the limitations and problems associated with the method used to obtain scores for these attributes, rather than the compositional data's lack of ability to explain the scoring of sensory attributes. For the young wine attribute *grapefruit*, there were no compounds positively contributing to the predictive model, although good calibration statistics were achieved from the inclusion of eight negatively contributing variables. It might be that the compounds responsible for the *grapefruit* aroma in wine, which were not measured, are being masked by the negatively contributing variables included in the model. Consequently, there appears to be a reasonable mathematical relationship between the concentration of the negatively contributing variables and the scoring of the *grapefruit* attribute, leading to reasonable prediction statistics without the need for analytical data on the actual compound/s responsible for *grapefruit* aroma in wine.

Compounds that were excluded from all of the regression models developed using the JK technique included ethyl acetate, ethyl propanoate, hexanol, butanol, acetic acid, acids(3), isoacids(2), methionol, 4-ethyls(2), α-terpineol and *trans*-ethyl cinnamate. All of these compounds were measured in the wines below their respective model wine or white wine sensory detection threshold concentrations, with the exception of ethyl acetate (mean OAV 10), and *trans*-ethyl cinnamate which was measured above its model wine sensory threshold in eight Riesling wines (mean OAV 0.9, max OAV 2). The low indicative OAVs for most of the volatiles included in these 12 variables support the elimination of the variables by JK, as they are not expected to be important contributors to the prediction of sensory attributes in this set of wines.

The models generated using the conservative JK approach were limited in terms of identifying just a small number of the most important aroma compounds for the prediction of each attribute. A main goal of this research was to identify the smallest number of compounds possible that could be used to explain the sensory perception of this set of wines. For this reason, a second set of models was developed from the JK models as an improvement toward selecting the smallest number of compounds possible to predict each sensory attribute. For the second set of models an iterative process was employed to remove *x*-variables, that were not critical to the power of the regression, from the JK models built with all significantly contributing *x*-variables (in Table 3-10). This process aimed to

achieve the simplest (fewest number of *x*-variables possible), most powerful (best calibration statistics) and most reliable predictive model [18, 145]. The *x*-variables identified in this process gave the most valuable information to the predictive model and may be more likely to have a causative relationship with the aroma attribute they predict. The major limitation of the iterative approach is the risk associated with collinearity where variables might be chosen that do not have a causative relationship with the sensory attribute in place of those that do, resulting in a model that might be misinterpreted.

The calibration statistics for the simplified models are given in Table 3-11 together with the identity of the minimum number of *x*-variables which gave the best calibration statistics.

Table 3-11 Simplified PLS model results for the prediction of Riesling aroma attribute scores

attribute	R^2	RMSECV	F value	C_{opt}	*x*-var	(+) loaded *x*-variable	(-) loaded *x*-variable
estery	0.88	0.33	28**	1	4	acetates(4), monoterpenes(3)	isoesters(3), TPB
perfumed floral	0.85	0.41	10**	1	7	acetates(4), monoterpenes(3), *cis*-rose oxide, β-damascenone	isoesters(3), guaiacols(2), TPB
dried rose	0.71	0.23	13*	1	3	acetates(4), α-terpineol, monoterpenes(3)	
lemon	0.90	0.22	20**	1	6	isoalcohols(3), β-damascenone	isoesters(3), guaiacols, TPB, dimethyl sulfide
grapefruit	0.66	0.23	10*	1	3		isoesters(3), guaiacols(2), TPB
lime	0.83	0.32	14**	1	5	guaiacols(2), TPB	acetates(4), esters(5), monoterpenes(3)
lychee	0.69	0.20	12*	1	3	esters(5), monoterpenes(3)	TDN
pineapple	0.81	0.21	12**	1	5	esters(5), monoterpenes(3), 4-vinyls(2)	guaiacols(2), TDN
passionfruit	0.29	0.87	2ns	1	4	esters(5), monoterpenes(3)	isoesters(3), TDN
stewed apple	0.66	0.17	7*	1	4	guaiacols(2), TPB	esters(5), β-damascenone
honey	0.92	0.30	43**	1	4	isoesters(3), guaiacols(2), TPB	4-vinyls(2)
toasty	0.98	0.28	184**	1	4	isoesters(3), guaiacols(2), TPB, dimethyl sulfide	
caramel	0.94	0.19	34**	1	6	isoesters(3), guaiacols(2), TPB, dimethyl sulfide	esters(5), 4-vinyls(2)
kerosene	0.90	0.38	25**	1	5	guaiacols(2), TPB	esters(5), 4-vinyls(2), β-damascenone
rubber / plastic	0.28	0.31	3ns	1	2		monoterpenes(3), 4-vinyls(2)

ns not significant, * significant ($p < 0.05$), ** significant ($p < 0.01$)

The simplified models (Table 3-11) all had better calibration statistics (higher R^2, lower RMSECV and more significant F values) than the models from which they were developed (Table 3-10). Furthermore, several of the original prediction models which were not previously significant according to the F value, were found to be statistically significant in the optimised models which is not surprising considering fewer *x*-variables were used to build better models. With the exception of *passionfruit* and *rubber / plastic*, all models were found to be significant.

Attributes for which models were developed with excellent calibration statistics, where more than 85% of variation was explained ($R^2 > 0.85$) included *estery*, *perfumed floral*, *lemon*, *honey*, *toasty*, *caramel* and *kerosene*. Good calibration statistics were achieved ($R^2 > 0.75$) for the predictions of *lime* and *pineapple*, and moderate calibration statistics were found ($R^2 > 0.5$) for the models of *dried rose*, *grapefruit*, *lychee* and *stewed apple*. Poor calibration results ($R^2 < 0.5$) were again obtained in the models developed for *passionfruit* and *rubber / plastic*. RMSECV values ranged from 0.17 to 0.87 which was an improvement on the previous results (Table 3-10). Calculation of RPD values, showed that the prediction ability of all models was improved (RPD ≈ 2 – 6) with the exception of *passionfruit*, and *rubber / plastic* which remained the same.

The most important difference between the JK models and the simplified models was that substantially fewer volatile compounds could be used to predict the same attributes with better predictive power. This could mean that the most important compounds related to specific aroma properties have been identified in the simplified models. Unlike the JK models which generally grouped the compounds into two types of model for 'young' and 'aged' attributes, more interesting differences were observed between the attributes in the simplified models.

For example, the models for the *floral* and *estery* attributes used the positive contribution of acetates(4) and monoterpenes(3), whereas the prediction of the 'fruity' attributes *lychee*, and *pineapple* used the positive contribution of esters(5) and monoterpenes(3). The acetates typically have confectionary banana type aromas which logically relate to the *estery* character of wine. The acetate 3-methylbutyl acetate has been proposed as being probably the most important of the acetates and is thought to contribute a 'fruity' or 'estery' aroma to wine [4, 64]. The 'floral-smelling' monoterpenes which are considered to be important for the varietal aroma of Riesling wine [35] are likely to be related to the 'floral' aromas of these study wines. Monoterpenes are reported to be associated with 'floral' and 'citrus' attributes and their sensory contribution is thought to be additive [5]. On the other hand, the ethyl esters, which have long been implicated as important contributors to the aroma of white wine [3, 4], are described as more 'fruity-smelling' and it is logical that these esters might generate a *pineapple* aroma in wine. The model for the *pineapple* attribute also included the positive contribution of 4-vinyls(2) which were always well below their white wine sensory threshold concentration in the wines analysed and are not likely to be playing an important role in the *pineapple* aroma of these wines.

The prediction model for the *lemon* attribute included the positive contribution of the isoalcohols(3) and β-damascenone. Of the isoalcohols(3), the compound 3-methylbutanol

has been reported by others as important to white wine aroma [64] and this compound might be contributing to the aroma of the study wines. The compound β-damascenone was measured above its model wine sensory threshold in all of the Riesling wines and was measured in some of the wines more than one hundred times its respective model wine sensory threshold concentration. Similar concentrations have been reported by others [5] and it is very likely that this compound is playing an important role in the *lemon* aroma of these wines. The only other model that included the positive contribution of β-damascenone was the model for the attribute *perfumed floral*. The compound β-damascenone was originally isolated as a constituent of Bulgarian rose oil [198], and hence it is logical that this compound be significant to the prediction of a *floral* aroma in wine. The compound *cis*-rose oxide also contributed positively to the model for *perfumed floral*, which is, as the name suggests, not surprising considering the aroma of neat *cis*-rose oxide is 'rose-like'. It is possible that β-damascenone and *cis*-rose oxide are, together, contributing to the difference in the perceived *estery* and *perfumed floral* aromas of the study wines.

Typically, the variables isoesters(3), guaiacols(2) and TPB or TDN were negative contributors to the predictive models for the younger wine attributes. Although it is feasible that the concentrations of TPB, TDN, guaiacol and 4-methylguaiacol may mask the perception of younger attributes in wine, the masking role of the isoesters(3), to these attributes, is questionable. It is known that the concentration of esters both increase and decrease in wine during ageing [4, 63]. In these Riesling wines the concentrations of ethyl 3-methylbutanoate, ethyl 2-methylbutanoate and ethyl 2-methylpropanoate appeared to be higher in the older vintage wines. Considering these compounds have 'fruity' types of aromas it is not likely that these compounds are masking the younger fresher aromas of wine, or contributing to the developed aroma of wine, rather their contribution to these models is possibly merely mathematical rather than causative.

It is important to note that the occurrence of TPB and TDN was found to be relatively interchangeable throughout the set of models developed, and usually one or the other was used in an optimised model and not both. This is not surprising as these two compounds were found to be correlated ($r = 0.81$), making the mathematical contribution of these two compounds to the models developed almost exactly the same. These two variables were not combined into a single grouped variable because they were considered to have different aromas (as pure compounds) and they probably do not share similar biochemical origins. For this reason, caution must be taken in the interpretation of the role that these two compounds play in the models developed. TDN was measured above its sensory threshold concentration (determined in wine [3]) in many of the wines in this study and this compound has long been implicated as important to the developed aroma of aged Riesling wines [3, 19,

37, 184, 199]. It is very likely that TDN is playing an important role in the aroma of the study wines. The role of TDN in the *kerosene* aroma of wine is well established, so it could be interpreted that for the model developed for *kerosene*, TDN should be in place of TPB, even though TPB gave slightly better calibration statistics. Furthermore, it is quite feasible that TDN plays a role in masking the fresh 'fruity' and 'floral' aromas of young fresh wines, therefore TDN could be included in the models for *estery*, *perfumed floral*, *lemon*, and *grapefruit*. The compound TPB ((E)-1-(2,3,6-trimethylphenyl)buta-1,3-diene), a recently identified wine constituent, was also found above its white wine sensory detection threshold in some of the Riesling wines. This compound has also been measured above its white wine sensory threshold in other white wines, including Riesling, Chardonnay and particularly for Semillon wine [39, 40]. The nature of the contribution that TPB gives to the aroma of wine is yet to be determined and it is difficult to make clear conclusions about whether or not this compound is actually playing a masking role or contributing to the 'bottle-aged' aroma of these wines.

For the older wine attributes, the positive contribution of the guaiacols(2), TPB or TDN, and the occasional inclusion of dimethyl sulfide is common to *lime*, *stewed apple*, *honey*, *toasty*, *caramel* and *kerosene*. Although guaiacol and 4-methylguaiacol were not measured above their model wine and white wine detection thresholds in the wines, it is possible that they may be playing a role in the developed aroma of the wines through an additive effect. The inclusion of dimethyl sulfide as a contributor to the 'developed' aroma of wine agrees with work published by others [78].

Multivariate analysis has proved to be an excellent tool for identification of specific compounds, among the numerous potential odorants, that are most likely to be responsible for specific aroma notes of different Riesling wines. The results obtained from the PLS models developed for the Riesling sensory attributes indicate that the compounds most important to the aroma of Riesling wine include linalool, geraniol, nerol, ethyl 3-methylbutanoate, ethyl 2-methylbutanoate, ethyl 2-methylpropanoate, ethyl butanoate, ethyl hexanoate, ethyl octanoate, ethyl decanoate, ethyl dodecanoate, hexyl acetate, 2-methylpropyl acetate, 3-methylbutyl acetate, 2-methylbutyl acetate, β-damascenone, TDN, TPB, dimethyl sulfide, 4-vinylguaiacol, 4-vinylphenol and the occasional importance of *cis*-rose oxide and α-terpineol. It is also possible that guaiacol, 4-methylguaiacol, 2-methylpropanol, 2-methylbutanol and 3-methylbutanol may also be important contributors to the aroma of Riesling wine.

3.3 Conclusion

The use of quantitative sensory and accurate, precise chemical analytical methods, together with the application of multivariate techniques, have allowed the identification of the volatile aroma compounds likely to contribute to the aroma properties of a set of 20 commercial Australian Riesling wines. The volatile compounds identified include a number of yeast fermentation-derived compounds, and grape-derived monoterpenes and norisoprenoids. The results suggest that the measurement of a relatively small number of volatile compounds in a Riesling wine may allow a good indication of the aroma properties of that wine. The models presented are useful to understand the complex relationships between volatile aroma compounds and the sensory perception of wine. If these relationships are confirmed in subsequent studies, such as sensory reconstitution studies or through the use of a separate validation set of wines, the application of a relatively straightforward instrumental analysis may allow an objective assessment of wine quality for Riesling wine, through use of predictive models.

This study has reinforced the view that, for Riesling wine, no single compound or class of compounds has an overriding influence on aroma. The aroma properties of a Riesling wine are likely to arise from contributions from, and interactions among, a range of aroma compounds. Together these components, when present in differing proportions in individual wines appear to confer the range of aroma characteristics observed.

3.4 Materials and methods

3.4.1 Wines

To select suitable wines for the study, an informal preliminary sensory assessment of a broad range of commercial Australian Riesling wines was conducted. Fifty nine Riesling wines were selected by reference to tasting notes from wine show information and current reports on commercial wine in the wine press. The wines were sourced, according to availability, from a range of producers, regions (64% SA, 5% NSW, 5% Tas, 14% Vic, 12% WA), vintages (2% 1993, 3% 1995, 3% 1996, 3% 1997, 2% 1998, 3% 1999, 8% 2000, 47% 2001, 27% 2002), retail prices ($7 - $36 / bottle) and closure types (59% natural bark cork, 41% screw cap). Wines were presented in coded glasses as sets of four - six wines to a panel of seven - twelve AWRI staff, with extensive wine tasting experience, over a number of sessions spanning four weeks (25th Sept – 16th Oct 2002). Tasters were asked to independently comment on the appearance, aroma and flavour of each wine and also to score the wines according to the 20 point wine quality scoring system involving colour, aroma, and palate. Discussions with the panellists at the end of each tasting helped to identified wines that were possible candidates for the study as well as wines that were spoiled with winemaking faults or were otherwise unsuitable. At the end of the screening process, 20 suitable Riesling wines were chosen that were deemed to encompass the range of sensory characteristics observed across all of the Riesling wines screened for the study, and included wines that had both high and low intensity aromas. The wines selected for the study were analysed by the Australian Wine Research Institute's Analytical Services for a number of chemical variables including alcohol, specific gravity, pH, free and total sulfur dioxide, titratable acidity (at pH 8.2), total dry extract, glucose and fructose, and volatile acidity (as acetic acid) [187].

3.4.2 Sensory descriptive analysis

Conventional quantitative sensory descriptive analysis was employed for the sensory analysis of the wine samples [81]. A 16-membered panel of judges was selected, comprising six male and ten female panellists, aged 21 - 51 years (average age 34 years), all of whom were staff and students of The Australian Wine Research Institute with previous experience in wine sensory studies. Training sessions were conducted over four weeks (17 sessions, 21st Oct – 18th Nov 2002) and involved six discussion sessions, four individual booth sessions and seven practice sessions using the computers in the booths. During training, the judges generated a set of descriptive terms using the study wines. By consensus, 17 aroma terms and six in-mouth flavour terms were selected to rate during the formal sessions (see Table 3-12). Sensory reference standards were developed for each aroma term during the training and usually consisted of a neutral wine doctored with food stuffs or spiked with

aroma compounds (Table 3-12). An 'other' attribute was also included for both the aroma and flavour (taste) terms, for panellists to use if they could smell or taste a character that was not covered by the agreed upon list of terms. A number of practice booth sessions were carried out prior to formal sessions to ensure that panellists were confident in rating the wines, were familiar with the set-up that would be used during the formal sessions and to assess that the panel was sufficiently well trained to progress to the formal sessions. Every wine was presented to the panel at least once during the training phase.

Table 3-12 Composition of sensory reference standards

aroma attribute	sensory reference standard composition
estery	2 mL stock estery mix [a] in base wine [b]
perfumed floral	0.5 mL of rosewater (Queen brand, Flavouring Essence, Natural Rosewater) in base wine
dried rose	0.2 mL of a stock solution of 2-phenylethanol (1 mL / 10 mL ethanol) in base wine
lemon	Small piece (1 cm^2) of fresh lemon zest soaked in base wine for 30min before use.
grapefruit	Small triangle (4 x 1.5 cm) of fresh grapefruit skin and pulp straight into glass (no base wine)
lime	7 mL of lime cordial (Bickfords brand) in base wine
lychee	3 mL lychee syrup (UFC lychees in syrup) in base wine
pineapple	7 mL pineapple juice (Golden Circle pineapple juice) in base wine
passionfruit	Small piece of fresh passionfruit skin (0.5 cm^2) plus pulp (~3 seeds) straight into glass (no base wine)
herbaceous	0.2 mL of 2-isobutyl-3-methoxypyrazine stock solution (53 ppb) in base wine
stewed apple	1 teaspoon of stewed granny-smith apple (not canned) in base wine
apricot	7 mL of tinned apricot syrup plus one tinned apricot half (Goulburn Valley) in base wine
honey	1 mL honey (Capilano) dissolved into 5mL hot water and added to base wine
toasty	small piece (5 cm^2) of freshly buttered and toasted bread straight into glass (no base wine)
caramel	one caramel (Pascal, creamy éclairs) cut into pieces added straight into glass (no base wine)
kerosene	1,1,6-trimethyl-1,2-dihydronapthalene (TDN) in ethanol (0.05 mL, 1 mg/mL) in base wine
rubber / plastic	no standard. The aroma of rubber tyres or plastic
flavour attribute	
sourness	no standard
sweetness	no standard
overall flavour	no standard. The overall intensity of retronasal flavour experienced after spitting
flavour persistence	no standard. The length of time that retronasal flavour persisted.
astringency	no standard. The degree of drying experienced in the mouth after spitting
bitterness	no standard

[a] 0.5 g 2-methylpropyl acetate, 0.09 g ethyl butyrate, 0.2 g ethyl hexanoate, 0.2 g ethyl octanoate, in 100 mL redistilled ethanol; [b] 100 mL of Yalumba Chenin Blanc, 2002, 2 L cask wine (11% alcohol / volume).

Formal rating sessions were held in which judges evaluated the 20 Riesling wines in triplicate (15 sessions, 19th Nov – 13th Dec 2002). Four wines were presented to each panellist each session in randomly ordered coded glasses. The wines were randomised within each replicate giving five blocks of four wines using a latin square design [200]. Formal sessions

were conducted in the sensory laboratory at the AWRI which contains five isolated booths equipped with sink, computer, and under sodium lighting to mask colour differences among the wines. During the formal sessions, the panel were also presented with the set of freshly prepared sensory reference standards (Table 3-12). The reference standards and wines (30 mL) were presented in covered ISO standard wine tasting glasses, together with a glass of spring water for rinsing between wines. Panellists were asked to smell the reference standards and then to evaluate each wine and rate the intensity of the aroma and flavour (taste) attributes using a structured ten point line scale (0 – 9), anchored from none to high. The data acquisition software used was *FIZZ* (Fizz for Windows, 2.00 E, Biosystemes, Couternon, France).

During the sensory study, the bottled wines were stored in the dark at constant humidity and temperature (16ºC) prior to use. For all sensory sessions, the wine bottles were opened and freshly poured no more than 30 mins before the beginning of the session. Wines were checked for possible taints (oxidation or cork taint) by informal sensory evaluation prior to each session, and replaced with a new bottle where necessary. To attempt to avoid bottle to bottle variation between replicates, wines that were from an older vintage (2000 or older) were scanned in a cuvette (1 cm) at 420 nm prior to each session. Replicate bottles were replaced with a new bottle if a 420 nm measurement for that bottle differed from previous measurements of bottles from the same wine label. Wines were also scanned by VIS-NIR, after each session, to enable later evaluation of bottle to bottle variation between replicates. The NIR methodology is described in greater detail in Chapter 6.

3.4.3 Volatile chemical analysis

After the sensory study was complete for the Riesling wines, two bottles of each wine were each divided into glass ampoules (1 x 20 mL, 4 x 50 mL) and a screw cap bottle (1 x 500 mL, sealed with foil), sealed under nitrogen, and stored at -18˚C on the 28th December 2002, until chemical analysis could take place. Analytical methods, as described in Chapter 2, were applied to measure a number of volatile compounds in the wines. Storage time for the wines prior to the application of each analytical method is shown in Table 3-13.

Table 3-13 Storage time for Riesling wines prior to chemical analysis

analytical method	time spent in storage at -18ºC (months)	storage period
fermentation-derived compounds	8 month	December 2002 – August 2003
diacetyl and *trans*-ethyl cinnamate	16 months	December 2002 – April 2004
grape- and oak-derived compounds	3 months	December 2002 – March 2003
4-vinylguaiacol and 4-vinylphenol	9 months	December 2002 – September 2003
methionol	16 months	December 2002 – April 2004
low molecular weight sulfur compounds	20 months	December 2002 – August 2004

3.4.4 Statistical and multivariate data analysis

The statistical software package used for univariate analysis of the sensory and volatile chemical data was *JMP* (version 5.0, SAS Institute Inc., Cary, NC, USA).

3.4.4.1 Statistical analysis of sensory data

Analysis of variance (ANOVA) was performed on the raw data set (16 judges x three replicates x 20 wines) for each attribute to determine if there were significant differences among the wines, judges or replicates ($p < 0.05$) using a mixed model treating judge as a random effect. Interactions between effects, including (wine x judge), (wine x replicate) and (replicate x judge), were assessed using least squares fit. The standard error of the mean (SEM) for each attribute was also calculated from the raw data set (16 judges x three replicates x 20 wines). The mean, minimum, maximum, standard deviation (SD) and the coefficient of variation (CV) were calculated from the summarised data set. Pearson's correlations between attributes were also calculated. Judge performance was assessed by repeated measurement ANOVA for each judge for each attribute using the software *Senstools* (OP&P Product Research B.V., Utrecht, The Netherlands). In addition, agreement among the judges for each attribute was assessed by determining Pearson's correlation coefficients (r) for each judge with the panel mean, excluding that judges data. Judges were deemed to be in agreement if the correlation was positive (i.e. $r > 0$).

3.4.4.2 Statistical analysis of volatile chemical data

The raw GC-MS data were processed using *Advanced ChemStation* (G1701DA version D.00.00.38, Agilent Technologies). One way ANOVA was performed on the raw data set (number of replicates x 20 wines) for each volatile compound to determine if there were significant differences among the wines ($p < 0.05$). The standard error of the mean was calculated from the raw data (number of replicates x 20 wines). The mean, minimum, maximum, standard deviation (SD) and the coefficient of variation (CV) were calculated from the summarised data. Pearson's correlations between volatile compounds were also calculated.

3.4.4.3 Multivariate data analysis

For multivariate data analysis of the volatile chemical data and sensory data both *JMP* and *The Unscrambler* (version 7.8, CAMO ASA, Oslo, Norway) software were used. The data tables were structured so that the wines were in rows and the variables in columns (volatile compound concentrations, routine chemical data, sensory attribute scores). Prior to PCA or PLS, all variables (sensory and chemical data) were autoscaled by dividing each value of each variable (concentration or score) for each wine by that variable's standard deviation, such that all variables had a standard deviation of 1 [125, 129, 201]. Volatile compounds

that were below the detection limit of the instrument in some of the Riesling wines analysed were given a value of 0 for multivariate analysis.

3.4.4.3.1 Principal component analysis

PCA was performed before PLS models were developed to examine any relevant and interpretable structure in the data [129]. PCs were constructed and plotted using both *The Unscrambler* and the *JMP* software.

3.4.4.3.2 Partial least squares regression

Calibration models between sensory properties (aroma) and volatile chemical data were developed using PLS1 regression with full cross validation using *The Unscrambler* software. The calibration statistics used to assess the power of each model were the coefficient of determination (R^2), root mean square error of cross validation (RMSECV), F value [126, 202], optimum number of components used in the PLS model (C_{opt}), and the number of *x*-variables used (*x*-var). Note that *The Unscrambler* software uses the abbreviation RMSEP when referring to the RMSECV. The optimum number of components in the PLS calibration models was determined in cross validation [126] as indicated by the lowest number of components that gave the closest to minimum value of the PRESS (prediction residual error sum of squares) function in order to avoid overfitting of the models.

Chapter 4 The compositional basis of unwooded Chardonnay wine aroma

4.1 Introduction

Chardonnay is a very important Australian variety as it has the highest annual production of all white grape varieties produced and covers 35% of vineyard area growing white grape varieties in Australia (including bearing and not yet bearing vineyards, 2002 [182]). In Australia, winemaking for Chardonnay has been fine-tuned in a number of different climates such that a diversity of styles has been established [183].

The primary fruit characters of a young Chardonnay wine include grapefruit, lemon, pineapple, melon, stone fruit, and tropical fruit, whereas a more developed wine exhibits flavour characteristics such as toast, honey, fig and nuts. Winemaking often plays an important role in contributing to the aroma of Chardonnay wine with lees contact, malolactic fermentation (MLF) and barrel storage all adding distinct flavours and aromas to the finished wine.

The volatile compounds responsible for the characteristic aromas of Chardonnay wine have been widely studied [8, 14, 24, 61, 69, 203-206]. A handful of studies have also been published which use various multivariate data analysis techniques, to explore the relationships between compositional and sensory characteristics of Spanish, French and Californian Chardonnay wine [7, 87, 88, 136, 137] (refer to Table 1-3, Chapter 1, for the multivariate technique used). Although these studies have provided some insight into the compositional basis of commercial Chardonnay wine aroma, the results from these studies are limited due to either the small number of wines analysed (e.g. only six wines [87]), too few compounds measured (e.g. only four volatile compounds [137]), lack of accuracy in the volatile chemical analysis due to inadequate internal standards (e.g. [88]) and ambiguous sensory terms rated (e.g. 'quality of aroma' [7]). One of these studies does not actually deal with relating the sensory properties of wine to quantitative composition, but rather attempts to relate GC-O data of wine to sensory data [87]. Furthermore, these studies involve commercial oaked Chardonnay wine, and wine that is likely to have undergone MLF. Consequently, the aroma of the wines studied could be substantially influenced by the volatiles derived from these two winemaking practices and the purely grape-derived aroma of Chardonnay wine was not explored.

The compositional basis of the aroma of unwooded Chardonnay wine has not been explored using multivariate data analysis and the most important volatile compounds and their aroma contribution are still not well understood for Australian Chardonnay wine.

4.2 Results and discussion

From a preliminary screening of 76 Australian commercial unwooded Chardonnay wines, 20 unwooded Chardonnay wines were selected for this study on the basis of having a diverse range of sensory properties with both high and low intensity aromas and being fault-free. Additionally, Chardonnay wine that had not been in contact with oak-wood and that had not intentionally undergone malolactic fermentation or spent considerable time on yeast lees was preferentially selected for this study. In this way, only the volatile compounds derived from the fruit or from yeast fermentation would need to be considered for chemical analysis. The wines selected were from two vintages (2002 and 2001), and a range of regions, climates, producers and retail prices were included. The 20 unwooded Chardonnay wines selected for the present work are tabulated in Table 4-1.

The wines chosen for the study were all young wines, with 17 wines from the 2002 vintage and three wines from the 2001 vintage. Australian commercial unwooded Chardonnay wine is not typically sold as a 'reserve' vintage product or as a wine that is intended to be bottle-aged by the consumer. The wines selected were from a range of viticultural regions across Australia including regions in Victoria, Western Australia, New South Wales and Tasmania, with the majority of wines from regions within South Australia.

Each wine chosen for the study was analysed by the Australian Wine Research Institute's Analytical Services for basic chemical composition (as described in [187]), and the results for alcohol (% v/v), pH, titratable acidity (at pH 8.2), glucose plus fructose, and free and total sulfur dioxide (SO_2) are given in Table 4-1. A summary of all the routine chemical variables measured, for each wine, is given in Appendix B. The results for all of the routine chemical parameters for each wine were within the expected range for commercial Australian unwooded Chardonnay wine. The parameters SO_2 free, SO_2 total and glucose plus fructose showed the broadest ranges, and alcohol, pH and titratable acidity did not vary considerably. The alcohol contents of the wines were all relatively high for white wine except for three wines (wines 2, 17 and 18). Wine 6 had unusually high alcohol content, low pH, high titratable acidity, high residual sugar and low free and total SO_2 compared to the other unwooded Chardonnay wines analysed. None of the routine chemical parameters measured related to vintage, retail price, closure type, or viticultural region.

Additional information about each wine including details on viticultural fruit origin, varietal purity, method used for harvesting the grapes, winemaking details, type of yeast, fermentation details and fining agents was provided by winemakers and producers where available. A summary of the winemaking details obtained, for the unwooded Chardonnay wines in the study, is given in Appendix B. The only wines for which this information could not be obtained were wines 10, 17, 18 and 20 (Table 4-1). This information was used to determine if the wines were made from 100% Chardonnay wine grapes, if the wine had been in contact with oak-wood, if the wine had undergone malolactic fermentation and if the aroma of each wine might be influenced by unconventional viticultural or winemaking practices.

Table 4-1 Identity and basic composition of unwooded Chardonnay wines selected for chemical and sensory analysis

wine code	retail price ($AU)	year [a]	region [b]	closure	alcohol (% v/v)	pH	TA [c] (g/L)	G + F [d] (g/L)	SO_2 free / total (mg/L) [e]
1	$18.00	2001	Adelaide Hills, SA	cork	13.6	3.26	6.3	3.5	23 / 149
2	$13.00	2001	Hunter Valley, NSW	cork	12.7	3.23	6.4	3.6	24 / 122
3	$14.00	2001	McLaren Vale, SA	cork	13.7	3.32	6.6	2.7	15 / 143
4	$9.99	2002	North-West Vic	cork	13.4	3.39	6.6	1.6	30 / 126
5	$15.99	2002	McLaren Vale, SA	cork	13.1	3.23	6.5	3.6	24 / 115
6	$20.69	2002	Pipers Brook, Tas	cork	14.6	3.09	8.9	7.5	9 / 53
7	$11.99	2002	Swan Valley, WA	cork	13.3	3.32	6.7	5.7	34 / 147
8	$13.60	2002	Mount Barker, WA	cork	13.4	3.42	6.1	3.5	25 / 135
9	$9.56	2002	Gingin, WA	cork	13.5	3.41	6.4	3.6	27 / 126
10	$35.00	2002	Eden Valley, SA	cork	13.8	3.30	6.5	3.7	24 / 122
11	$16.60	2002	Adelaide Hills, SA	screw cap	13.9	3.37	6.6	1.7	26 / 135
12	$16.15	2002	Pemberton, WA	cork	13.4	3.17	5.6	4.6	36 / 136
13	$14.00	2002	McLaren Vale, SA	cork	13.2	3.27	6.9	6.6	19 / 153
14	$13.60	2002	Limestone Coast, SA	cork	13.7	3.31	6.8	5.3	31 / 132
15	$10.70	2002	Clare Valley / Limestone Coast, SA	cork	13.1	3.48	6.5	4.3	13 / 98
16	$10.99	2002	Limestone Coast, SA	cork	13.9	3.21	6.5	1.9	26 / 113
17	$9.99	2002	Blend, SA	cork	12.0	3.39	6.4	5.6	25 / 142
18	$12.99	2002	Barossa Valley, SA	cork	12.6	3.42	6.1	3.4	27 / 109
19	$10.99	2002	Blend, SA	cork	13.7	3.29	6.4	2.1	24 / 136
20	$14.00	2002	Clare Valley, SA	screw cap	13.8	3.19	7.1	2.8	8 / 100

[a] wine age at the time of analysis: vintage 2002 (~ 1 year), 2001 (~ 2 years); [b] Vic: Victoria, SA: South Australia, WA: Western Australia, NSW: New South Wales, Tas: Tasmania, Blend: a blend of multiple regions; [c] TA: Titratable acidity (at pH 8.2); [d] G + F: glucose plus fructose; [e] SO_2: sulfur dioxide

Only nine of the unwooded Chardonnay wines were made from 100% Chardonnay grapes (wines 1, 2, 3, 4, 6, 9, 13, 14 and 19, Table 4-1), seven wines were identified as not varietally pure (85% to > 98% Chardonnay) and four wines of unknown varietal purity (wines 10, 17, 18 and 20, Table 4-1). Australian law does not require producers to place grape varietal information on the label of commercially produced wine. If producers choose to label wines with the varietal information, Australian law requires that they must specify no less than 85% of the wine variety on the label [207]. Wines labelled 'unwooded Chardonnay' in this study, for which detailed information on the varietal purity could not be obtained from the winemaker

or producer, should therefore contain no more than 15% of a different variety in the final blend. Wines that were not varietally pure (wines 5, 7, 8, 11, 12, 15, and 16 Table 4-1) were blended with small levels of various other white grape varieties including Semillon (up to 15%), Sauvignon Blanc (< 3%), Riesling (up to 4%), Chenin Blanc (up to 13%), Sultana (< 2%), Gewürztraminer (< 0.5%), Verdelho (< 2%). These low levels were not considered to be likely to have a major influence the aroma of the unwooded Chardonnay wines. Consequently, this study was not of varietally pure wines, but of commercial wines which were labelled 'unwooded Chardonnay'.

From the information provided for 16 of the wines, one wine contained less than 5% of a lightly wooded Chardonnay (oak added at 2 mg/L) in the final blend (wine 14, Table 4-1). From the preliminary tasting session, the low level of oaked-wine content was not considered to strongly influence the aroma of that wine. For the wines from some of the larger wine producers the wine used in the final blends was commonly sourced from other wine producers, and therefore it could not be confirmed that the final wine blend was 100% unwooded wine.

Wines 2 and 15 were reported to have, in the final blend, a small portion of wine that had undergone malolactic fermentation (MLF) (no more than 15%). Some winemakers were not certain if the wine had undergone MLF but reported that their wine had probably not undergone MLF (wines 5, 8 and 12). From the preliminary screening none of the wines selected were considered to be strongly influenced by typical MLF sensory characters.

4.2.1 Sensory descriptive analysis

Descriptive analysis was employed to quantify the intensity of the sensory properties of the 20 unwooded Chardonnay wines selected for this study. A trained panel of 20 judges rated each wine, in triplicate, for the intensity of 14 aroma and six flavour attributes on a scale of 0 - 9. The results for each of the sensory attributes, rated by the panel, including mean, minimum, maximum, standard deviation (SD), coefficient of variation (CV) and the standard error of the mean (SEM) are listed in Table 3-2. Mean scores of all the sensory properties rated, for each unwooded Chardonnay wine, are tabulated in Appendix B.

Most of the sensory attributes were rated with relatively low scores on the ten point scale (0 - 9) with the highest mean scores for any of the individual wines being 5.6 for *passionfruit* and more than 5 for *sourness*, *overall flavour* and *flavour persistence*. For most of the other attributes, the highest average score was between two and three on the scale. Sensory attributes with the broadest variation across the wines (CV > 50%) include *passionfruit*, *herbaceous*, *sweaty*, *butterscotch* and *spicy*. Although the flavour attributes *sourness*,

overall flavour and *flavour persistence* were scored on a higher part of the scale than many of the other attributes they showed relatively narrow variation across the wines (CV < 10%). Assessment of judge performance showed that there was excellent agreement for each judge with the group mean for the attributes *passionfruit* and *butterscotch* (all judges in agreement), and very good agreement for the attributes *honey*, *woody*, *overall flavour* and *flavour persistence* (only one judge not in agreement). For the attributes *herbaceous* and *sweaty* three or four judges were in disagreement with the group mean, but those judges that were in agreement typically had very high correlation with the group mean ($r > 0.6$) compared to other attributes. Reasonable agreement was found between judges and the group mean for the attributes *estery*, *stewed apple / pear*, *spicy*, *sourness* and *sweetness* (two judges not in agreement). The attributes *floral*, *lychee*, *citrus*, *pineapple*, *astringency* and *bitterness* all showed moderate judge agreement with group mean (3 – 5 judges not in agreement) and *stone fruit* showed the poorest judge agreement (8 judges not in agreement).

Table 4-2 Summary of the descriptive analysis scores for aroma and flavour

aroma attribute	mean	minimum	maximum	SD	CV (%)	SEM
estery	2.59	1.93	3.39	0.37	14	0.058
floral	1.50	0.75	2.17	0.38	25	0.053
lychee	1.24	0.70	2.33	0.38	31	0.051
citrus	1.53	1.11	2.02	0.28	18	0.048
pineapple	2.15	1.59	2.82	0.34	16	0.054
stewed apple / pear	1.46	0.40	2.00	0.41	28	0.053
stone fruit	1.84	1.27	2.20	0.28	15	0.058
passionfruit	1.44	0.40	5.60	1.50	104	0.065
herbaceous	0.68	0.23	2.08	0.50	74	0.040
sweaty	0.99	0.26	2.87	0.69	70	0.051
honey	1.48	0.68	2.60	0.52	35	0.053
butterscotch	1.05	0.24	2.34	0.59	56	0.050
woody	1.21	0.47	2.36	0.55	45	0.057
spicy	0.67	0.30	1.91	0.38	57	0.047
flavour attribute	**mean**	**minimum**	**maximum**	**SD**	**CV (%)**	**SEM**
sourness	4.67	4.28	5.12	0.22	5	0.046
sweetness	1.79	1.29	2.28	0.24	13	0.051
overall flavour	4.45	4.09	5.41	0.34	8	0.043
flavour persistence	4.56	4.18	5.53	0.38	8	0.044
astringency	1.70	1.37	2.10	0.19	11	0.050
bitterness	1.23	1.01	1.56	0.16	13	0.048

A summary of the analysis of variance (ANOVA) for each of the effects tested is given in Table 4-3. All attributes were significantly different ($p < 0.05$) between the wines with the exception of the aroma attribute *stone fruit* and the flavour attributes *sourness*, *sweetness*, *astringency* and *bitterness*. There was a significant difference between judges, for all

attributes which is usual for descriptive analysis data [81]. There was no significant difference between replicates for each attribute, with the exception of *astringency*. It might be that the significant differences between replicates for *astringency* were due to the differences in the sets of wines presented to panellists in each replicate block. This could have caused certain wines to seem more or less *astringent*, depending on the wines they were tasted next to. Alternatively, the panel may have changed the way they rated in-mouth *astringency* over time.

Table 4-3 F ratios and significance for effects of wine, judge, repetition and interactions for each sensory attribute

sensory attribute	wine [a]	judge [a]	replicate [a]	wine x judge [b]	judge x replicate [b]	replicate x wine [b]
estery	2**	31**	0.5^{ns}	1^{ns}	0.9^{ns}	0.9^{ns}
floral	3**	14**	1^{ns}	1**	1^{ns}	2**
lychee	3**	20**	1^{ns}	1*	2**	1^{ns}
citrus	2**	39**	0.1^{ns}	1**	1^{ns}	0.7^{ns}
pineapple	2**	26**	0.08^{ns}	1**	0.7^{ns}	2*
stewed apple / pear	3**	19**	0.8^{ns}	1^{ns}	1^{ns}	1^{ns}
stone fruit	1^{ns}	10**	2^{ns}	1**	2*	1^{ns}
passionfruit	46**	5**	0.05^{ns}	2**	0.7^{ns}	2**
herbaceous	9**	8**	0.6^{ns}	2**	2*	0.9^{ns}
sweaty	10**	10**	0.7^{ns}	2**	2*	0.8^{ns}
honey	5**	26**	0.03^{ns}	2**	0.8^{ns}	1^{ns}
butterscotch	8**	13**	0.6^{ns}	1**	2**	0.8^{ns}
woody	5**	28**	0.8^{ns}	2**	0.9^{ns}	0.9^{ns}
spicy	3**	18**	0.9^{ns}	1^{ns}	0.8^{ns}	0.8^{ns}
sourness	1^{ns}	92**	0.2^{ns}	1^{ns}	1^{ns}	0.7^{ns}
sweetness	1^{ns}	123**	0.3^{ns}	1*	3**	0.7^{ns}
overall flavour	3**	75**	0.6^{ns}	1**	2**	0.9^{ns}
flavour persistence	4**	47**	1^{ns}	1**	2**	1^{ns}
astringency	1^{ns}	101**	3*	1*	2**	1^{ns}
bitterness	1^{ns}	91**	0.1^{ns}	0.8^{ns}	2*	1^{ns}
degrees of freedom	19	19	2	361	38	38

[a] values from analysis of variance; [b] values from least squares fit effect tests; significance indicated by ** ($p < 0.01$), * ($p < 0.05$), ns: not significant.

Significant differences were observed for the interaction of (wine x judge) for all attributes with the exception of *estery*, *stewed apple / pear*, *spicy*, *sourness*, and *bitterness*. This indicates that judges rated wines in different ways for most of the sensory attributes in the study. The interaction of (judge x replicate) also showed significant differences for the aroma attributes *lychee*, *stone fruit*, *herbaceous*, *sweaty*, *butterscotch*, and flavour attributes *sweetness*, *overall flavour*, *flavour persistence*, *astringency* and *bitterness*. This result demonstrates that for these attributes, different judges rated replicates differently. No significant differences were observed for the interaction of (replicate x wine), except for

aroma attributes *floral* and *passionfruit*, which shows that there was minimal bottle to bottle variation between replicates. It could be that panellist changed the way that the rated *floral* and *passionfruit* over time, resulting in significant differences for these attributes between replicates. Alternatively, it might be that those particular wines with high *floral* and/or *passionfruit* characters were different between replicate bottles.

Numerous studies have been carried out to profile the sensory properties of commercial Chardonnay wine made from American, Australian, French and Canadian grapes [88, 137, 138, 208-213] and Chardonnay wine made under experimental conditions [87, 214-217]. These studies include the use of descriptive analysis, free choice profiling and variations of these techniques. The descriptors used in the present study are generally similar to those used to describe the aroma of Chardonnay wine in other studies involving commercially produced Chardonnay wine with the exception of the terms *sweaty* and *flavour persistence* which appear to be unique to the present work. Terms used in other studies that were not used in the present work include *melon*, *green apple*, *aldehyde*, *earthy aroma*, *rubber*, *tea/tobacco*, *neutral* (or *vinous alcohols*), *yeasty* (or *microbiological*), *hydrogen sulfide* (H_2S), *chemical* and *nutty*. Some of these terms are perhaps associated with wines that have winemaking faults (e.g. *aldehyde*, *chemical*, *rubber* and H_2S), wines that have been stored in oak barrels or have been bottle-aged (e.g. *nutty*), wines that have undergone MLF, or wines that have been in contact with yeast lees (e.g. *yeasty*). None of these other terms were deemed appropriate by the sensory panel to use for describing the commercial unwooded Chardonnay wines in this study.

Pearson correlations (pair-wise) between attributes were analysed for the sensory data and the results of this analysis are tabulated in Table 4-4. Some collinearity (correlation) was observed between sensory attributes, although the collinearity across the data set was not as extensive as might be expected for sensory data. High positive correlations were found between *passionfruit*, *sweaty*, *herbaceous*, *overall flavour* and *flavour persistence* ($r > 0.85$). Strong positive correlations were also found between *woody* and *spicy* ($r = 0.86$), between *honey* and *butterscotch* ($r = 0.80$), between *butterscotch* and *woody* ($r = 0.80$), and between *estery* and *floral* ($r = 0.82$).

Table 4-4 Pearson correlation coefficient matrix (r) of unwooded Chardonnay sensory attributes

	estery	*floral*	*lychee*	*citrus*	*pineapple*	*stewed apple / pear*	*stone fruit*	*passionfruit*	*herbaceous*	*sweaty*	*honey*	*butterscotch*	*woody*	*spicy*	*sourness*	*sweetness*	*overall flavour*	*flavour persistence*	*astringency*	*bitterness*
estery	**1.00**																			
floral	0.82	**1.00**																		
lychee	0.37	0.05	**1.00**																	
citrus	0.46	0.36	-0.07	**1.00**																
pineapple	0.62	0.66	0.04	0.36	**1.00**															
stewed apple / pear	0.13	0.28	-0.47	0.56	0.22	**1.00**														
stone fruit	0.34	0.33	0.18	-0.01	0.30	0.18	**1.00**													
passionfruit	0.14	-0.17	0.59	-0.30	-0.04	-0.84	-0.28	**1.00**												
herbaceous	0.02	-0.26	0.57	-0.31	-0.07	**-0.86**	-0.36	**0.97**	**1.00**											
sweaty	-0.01	-0.35	0.59	-0.37	-0.17	-0.83	-0.24	**0.91**	**0.93**	**1.00**										
honey	-0.46	-0.30	-0.50	0.06	-0.42	0.64	-0.01	-0.73	-0.73	-0.69	**1.00**									
butterscotch	-0.65	-0.52	-0.61	-0.04	-0.51	0.47	0.01	-0.65	-0.62	-0.48	0.79	**1.00**								
woody	-0.46	-0.24	-0.64	0.04	-0.48	0.45	0.11	-0.69	-0.69	-0.62	0.69	0.80	**1.00**							
spicy	-0.39	-0.15	-0.59	0.01	-0.35	0.47	0.01	-0.67	-0.67	-0.62	0.61	0.73	**0.86**	**1.00**						
sourness	-0.12	-0.31	0.29	-0.22	-0.14	-0.46	-0.35	0.53	0.60	0.61	-0.35	-0.28	-0.44	-0.57	**1.00**					
sweetness	0.21	0.10	0.24	-0.21	0.09	-0.45	0.17	0.35	0.39	0.41	-0.51	-0.41	-0.33	-0.19	-0.06	**1.00**				
overall flavour	0.03	-0.27	0.53	-0.33	-0.07	-0.84	-0.24	**0.86**	**0.91**	**0.88**	-0.73	-0.53	-0.57	-0.56	0.71	0.43	**1.00**			
flavour persistence	0.03	-0.31	0.49	-0.34	-0.05	-0.79	-0.28	**0.86**	**0.90**	**0.89**	-0.68	-0.51	-0.60	-0.61	0.73	0.32	**0.95**	**1.00**		
astringency	0.20	-0.06	0.54	0.16	0.06	-0.20	-0.01	0.28	0.27	0.24	-0.10	-0.31	-0.29	-0.49	0.48	-0.32	0.34	0.40	**1.00**	
bitterness	0.03	0.01	0.33	0.03	-0.25	0.27	0.17	-0.27	-0.28	-0.10	0.25	0.21	0.12	0.01	-0.01	-0.42	-0.29	-0.25	0.27	**1.00**

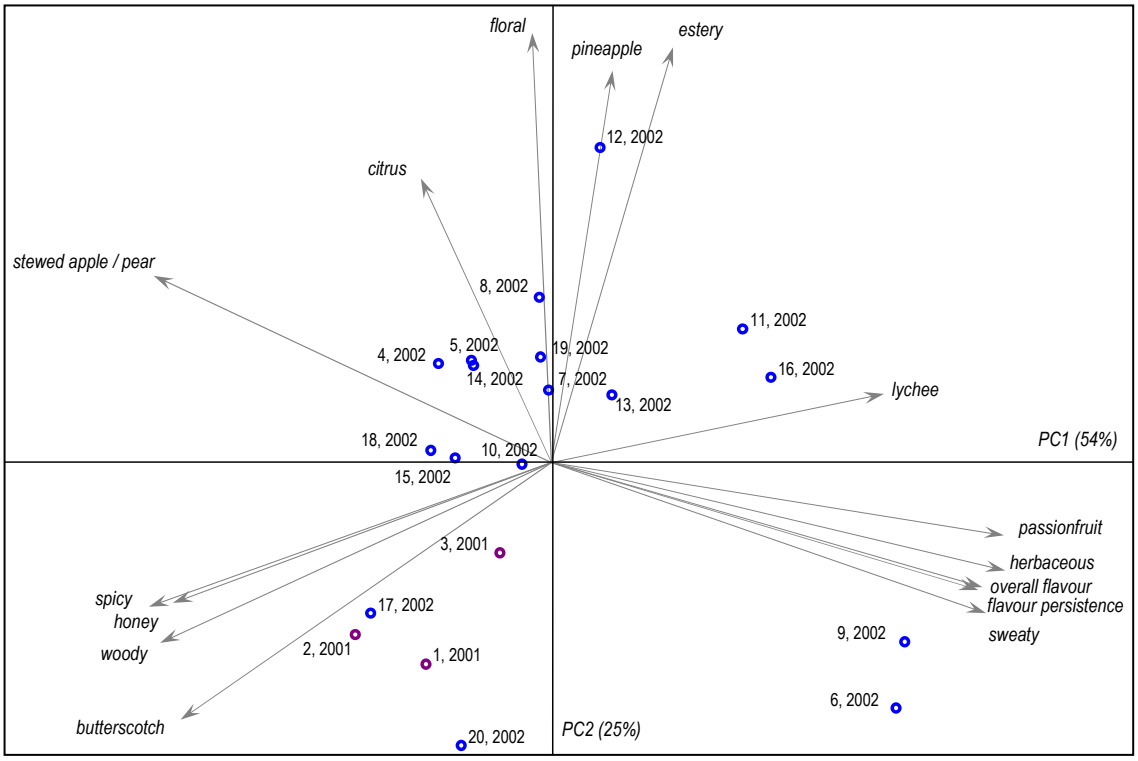
$r \leq -0.85$ and $r \geq 0.85$ are indicated in bold typeface

Although there were many 'fruity' attributes which could be considered similar in nature (e.g. *citrus*, *pineapple*, *stewed apple / pear*, *stone fruit*) these attributes were not highly collinear. This may mean that the panel was well trained to use these attributes, and was not split over a number of attributes when rating the same property. The low variation observed for the scoring of these 'fruity' attributes indicates that it is more likely that a relatively high level of error is associated with the rating of these attributes and that this has resulted in the absence of inter-correlation between these attributes. Although care was taken to reduce the level of noise in this sensory study, through the use of extensive training sessions and replicated assessments, sensory data sets are inherently noisy data sets [124]. Assessment of judge performance also suggested that indeed there may be a high level of random noise associated with the rating of these particular 'fruity' attributes.

The only strong negatively correlated relationship observed in this data set was the negative correlation observed between the aroma attribute *stewed apple / pear* and the attributes *passionfruit*, *sweaty*, *herbaceous*, *overall flavour* and *flavour persistence* ($r < -0.79$).

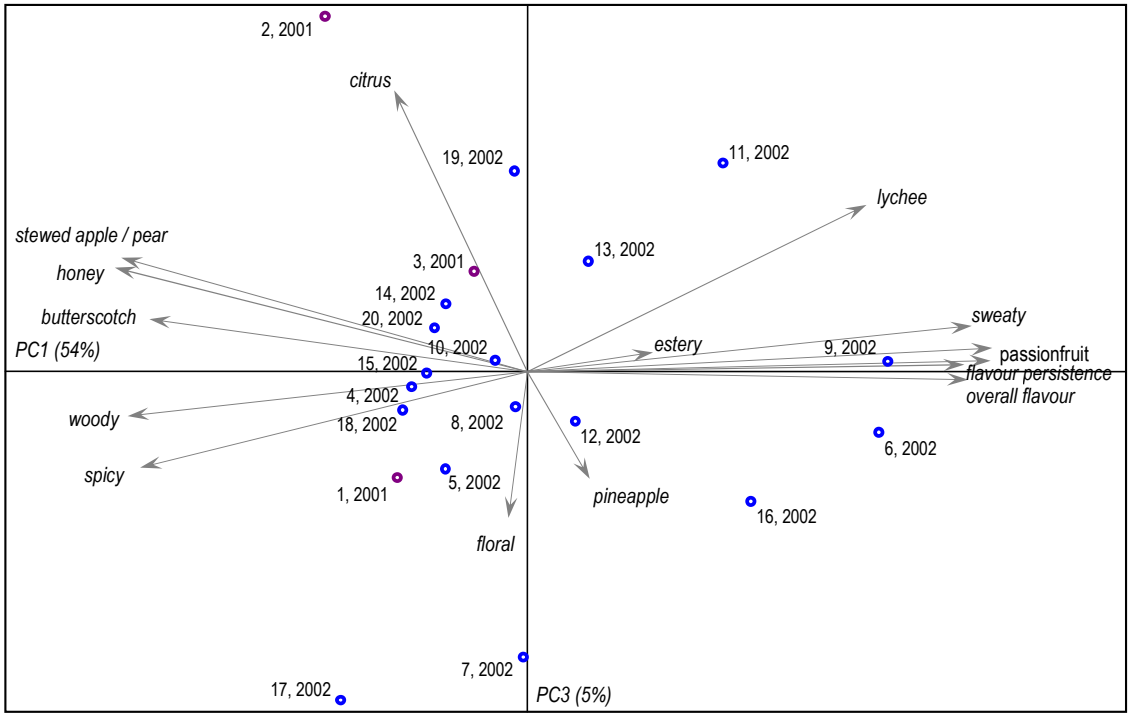
Principal component analysis (PCA) was used to examine any relevant and interpretable structure in the sensory data set [129]. Only the sensory attributes which were statistically different between the wines were included in the PCA. The first three principal components (PCs) explained 84% of variation in the data set. PCA bi-plots are given in Figure 4-1 and Figure 4-2. Wines were differentiated across PC1 as being either relatively high in *lychee*, *passionfruit*, *herbaceous, overall flavour, flavour persistence* and *sweaty* attributes (wines on the right of the plots, Figure 4-1 and Figure 4-2) or being higher in *stewed apple pear*, *honey*, *woody*, *butterscotch* and *spicy* (wines on the left of the plots, Figure 4-1 and Figure 4-2). The three older wines, from the 2001 vintage, typically had higher scores for *spicy*, *honey*, *woody* and *butterscotch* (located in the bottom left of Figure 4-1). The older wines, although rated higher for the *woody* character, were 100% unwooded wine, while wine 14, which contained 5% wooded wine, had a lower score for the *woody* attribute. Wines were differentiated across PC2 according to their intensity of *citrus*, *floral*, *pineapple* and *estery* attributes (wines increasing in intensity of these attributes from bottom to top of plot in Figure 4-1). PC3, which only accounted for 5% of variation, differentiated the wines according to their intensity in the *citrus* property (wines increasing in *citrus* character from bottom to top of Figure 4-2). Wines were not grouped according to retail price, producer, closure type, region or climate (i.e. warm or cool climate).

Figure 4-1 PCA bi-plot of descriptive analysis results for unwooded Chardonnay wines, PC1 versus PC2



For details on the sample codes refer to Table 4-1. Sample scores are calculated from the mean of 20 judges x 3 replicates and attribute loadings (vectors) are shown.

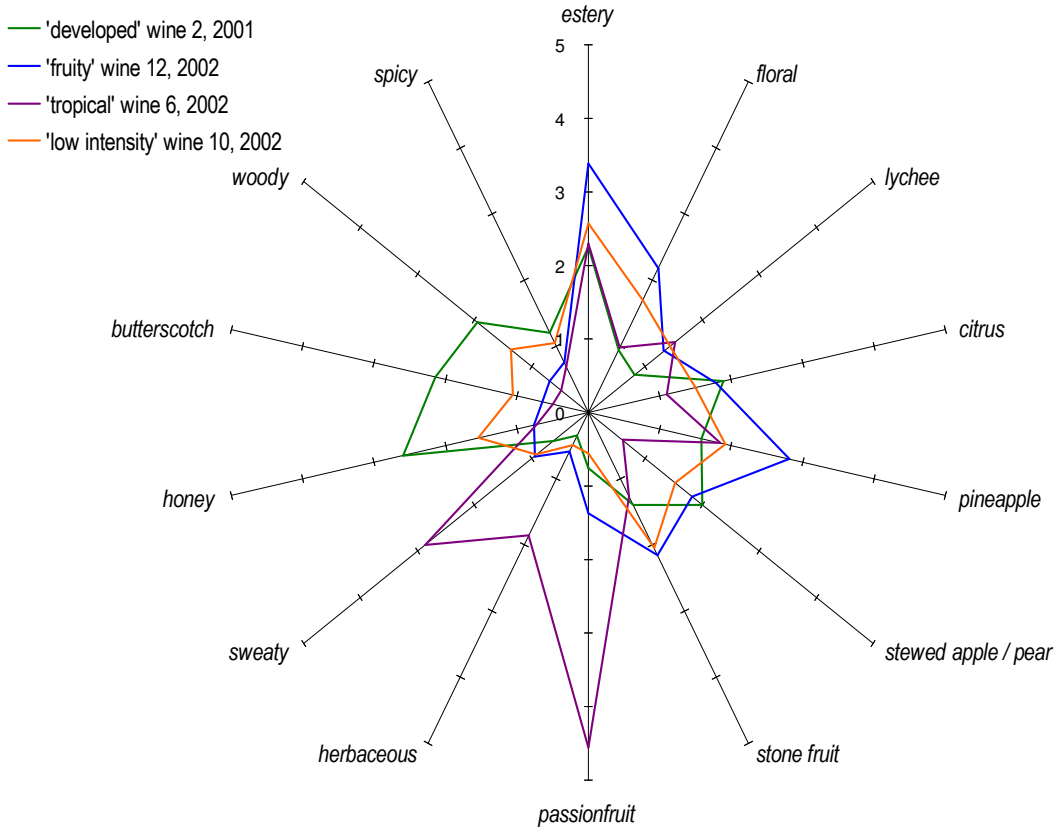
Figure 4-2 PCA bi-plot of descriptive analysis results for unwooded Chardonnay wines, PC1 versus PC3



For details on the sample codes refer to Table 4-1. Sample scores are calculated from the mean of 20 judges x 3 replicates and attribute loadings (vectors) are shown.

Some of the wines were rated relatively low for all aroma attributes, while others were rated relatively high for particular aroma attributes. For example, Figure 4-3 shows a cobweb plot of wines with either high intensity 'developed' aroma (wine 2), high intensity 'tropical' aroma (wine 6) and high intensity 'fruity' characters (wine 12), together with a wine that was low intensity in almost all aroma attributes (wine 10). There were no wines that were scored highly for all attributes.

Figure 4-3 Aroma attribute scores for four unwooded Chardonnay wines



For details on the sample codes refer to Table 4-1. Sample scores are calculated from the mean of 20 judges x 3 replicates.

Overall, the unwooded Chardonnay wines in this study showed a range of different sensory properties, of varying intensities, and the data produced through descriptive analysis was suitable for use, together with compositional data, in multivariate data analysis.

4.2.2 Volatile chemical analysis

The 20 unwooded Chardonnay wines selected for this study were analysed for 59 volatile aroma compounds using the analytical methods described in Chapter 2. Overall, 45 volatile aroma compounds were quantified and a summary of the results including mean, minimum, maximum, standard deviation (SD), coefficient of variation (CV), standard error of the mean (SEM) and the F ratio is given in Table 4-5. A summary of the mean measured concentration of each of the volatile compounds analysed, for all of the unwooded Chardonnay wines in the study, are given in Appendix B.

Table 4-5 Summary of volatile chemical analysis results for the unwooded Chardonnay wines

	mean (µg/L)	minimum (µg/L)	maximum (µg/L)	SD (µg/L)	CV (%)	SEM (µg/L)	F ratio [a]
ethyl acetate	**85185**	**50282**	**212702**	34764	41	7774	one rep
ethyl propanoate	226	130	384	59	26	11	2*
ethyl butanoate	**548**	**309**	**1125**	182	33	32	4**
ethyl 2-methylpropanoate	**82**	**40**	**247**	51	62	8	9**
ethyl 2-methylbutanoate	**10**	**3**	**20**	5	50	0.8	12**
ethyl 3-methylbutanoate	**22**	**10**	**43**	9	41	2	9**
ethyl hexanoate	**1228**	**920**	**1974**	231	19	37	4**
ethyl octanoate	**1492**	**1027**	**2323**	325	22	73	one rep
ethyl decanoate	**570**	**362**	**847**	128	22	29	1[ns]
ethyl dodecanoate	176	60	812	164	93	24	6**
***trans*-ethyl cinnamate**	**3**	0.6	**6**	2	67	0.2	16**
2-methylpropyl acetate	42	3	119	27	64	4	14**
2-methylbutyl acetate	**95**	**13**	**311**	70	74	11	16**
3-methylbutyl acetate	**2430**	**269**	**7266**	1079	44	276	15**
hexyl acetate	213	7	598	138	65	21	18**
2-phenylethyl acetate	212	33	**583**	158	75	26	14**
2-phenylethanol	**18211**	8620	**43921**	8485	47	1448	5**
butanol	934	353	2492	579	62	92	10**
2-methylpropanol	21456	13024	37238	6518	30	1176	3**
2-methylbutanol	21538	12425	34301	6538	30	1251	2*
3-methylbutanol	**136016**	**83686**	**187727**	32087	24	5252	4**
hexanol	2546	939	4814	948	37	165	4**
acetic acid [b]	**368947**	**210000**	**630000**	106453	29	23804	one rep
2-methylpropanoic acid	700	386	1705	313	45	56	4**
3-methylbutanoic acid	337	122	609	127	38	23	3**
hexanoic acid	**5217**	**3926**	**7599**	1012	19	226	one rep
octanoic acid	**7920**	**5387**	**11014**	1516	19	339	one rep
decanoic acid	2304	1413	3120	476	21	106	one rep
linalool	7	0.6	13	3	43	0.50	133**
α-terpineol	10	4	32	6	60	1.0	922**
geraniol	2	nd	4	1	50	0.17	12**
TDN	1	0.3	4	0.9	90	0.14	116**
TPB [c]	0.008	0.003	0.02	0.004	50	0.8	23**
β-damascenone	**2**	**0.8**	**4**	1	50	0.18	9**
guaiacol	0.9	0.2	3	0.8	89	0.13	28**
4-methylguaiacol	0.3	nd	3	0.7	233	0.11	196**
4-ethylguaiacol	0.2	nd	0.7	0.2	100	0.036	4**
4-vinylguaiacol	0.05	0.005	0.1	0.03	60	0.005	16**
4-vinylphenol	0.3	0.02	1	0.3	100	0.04	24**
***cis*-oak lactone**	14	3	**36**	8	57	1.2	102**

	mean (µg/L)	minimum (µg/L)	maximum (µg/L)	SD (µg/L)	CV (%)	SEM (µg/L)	F ratio [a]
vanillin	15	3	81	17	113	2.7	53**
diacetyl [c]	55	nd	**233**	60	109	12	54**
methionol	467	258	**1010**	202	43	32	228**
dimethyl sulfide	**127**	**31**	**350**	74	58	12	197**
carbon disulfide	3	0.7	**17**	4	133	0.6	290**

[a] significance indicated by ** ($p < 0.01$), * ($p < 0.05$), [ns]: not significant, one rep: only one replicate data point was obtained for each wine; [b] acetic acid measured as volatile acidity; [c] analysed in 18 wines only; compound not detected (nd); compound concentrations in bold are above their reported sensory detection threshold listed in Table 1-1, Chapter 1.

All compounds measured were significantly different between the wines (ANOVA, $p < 0.05$) with the exception of ethyl decanoate. For some of the fermentation-derived compounds only one replicate was used and analysis of variance could not be conducted for those volatiles (indicated in by 'one rep' in Table 4-5). The compounds ethyl lactate, propanoic acid, butanoic acid, 2-methylbutanoic acid, nerol, *cis*-rose oxide, β-ionone, 4-ethylphenol, ethanethiol, diethyl sulfide, methyl thioacetate, dimethyl disulfide, ethyl thioacetate and diethyl disulfide were not detected in any of the unwooded Chardonnay wines analysed (below detection limit of analytical method) and have been excluded from the table. The compounds geraniol, 4-methylguaiacol, 4-ethylguaiacol and diacetyl were not detected in some of the wines that were analysed as they were at concentrations below the detection limit of analytical method (indicated by not detected or 'nd' in Table 4-5). The routine chemical analysis for volatile acidity was used for multivariate analysis for the measurement of acetic acid. TPB concentration data was missing for two wines (wines 19 and 20) and diacetyl data missing for two wines (wines 7 and 10). So that these compounds could be included in multivariate analysis for all 20 wines, these wines were given the average concentration (of 18 wines) for these two compounds. This is an acceptable practice when dealing with incomplete data sets in multivariate analysis [152].

The concentration of the volatile compounds measured varied from greater than 100000 µg/L (e.g. ethyl acetate and 3-methylbutanol) to less than 0.01 µg/L (e.g. TPB). Some compounds showed very high variation (CV > 100%) despite being measured at relatively low concentrations, including 4-methylguaiacol, 4-ethylguaiacol, 4-vinylphenol, vanillin, diacetyl and carbon disulfide. Most compounds showed reasonably high variation across the unwooded Chardonnay wines analysed.

Pearson correlations between chemical variables were analysed for the volatile chemical data and a summary of the strongest correlations is tabulated in Figure 4-4.

Figure 4-4 Pearson correlation coefficient matrix (r) of selected unwooded Chardonnay volatile compounds

	ethyl acetate	ethyl 2-mebutanoate	ethyl 3-mebutanoate	2-methylpropyl acetate	2-methylbutyl acetate	3-methylbutyl acetate	hexyl acetate	2-methylpropanol	2-methylbutanol	acetic acid	2-methylpropanoic acid	3-methylbutanoic acid	4-methylguaiacol	*cis*-oak lactone
ethyl acetate	**1.00**													
ethyl 2-mebutanoate	0.30	**1.00**												
ethyl 3-mebutanoate	0.28	**0.94**	**1.00**											
2-methylpropyl acetate	0.18	-0.14	-0.05	**1.00**										
2-methylbutyl acetate	-0.10	-0.16	-0.10	**0.91**	**1.00**									
3-methylbutyl acetate	-0.06	-0.18	-0.09	**0.92**	**0.96**	**1.00**								
hexyl acetate	-0.05	-0.24	-0.16	0.84	**0.94**	**0.93**	**1.00**							
2-methylpropanol	0.30	0.39	0.46	0.78	0.64	0.69	0.54	**1.00**						
2-methylbutanol	0.16	0.30	0.35	0.66	0.65	0.70	0.66	**0.85**	**1.00**					
acetic acid	**0.87**	0.10	0.06	0.15	-0.12	-0.10	-0.07	0.18	0.10	**1.00**				
2-methylpropanoic acid	**0.86**	0.48	0.50	0.49	0.22	0.24	0.19	0.65	0.44	0.65	**1.00**			
3-methylbutanoic acid	0.34	0.61	0.61	0.58	0.54	0.57	0.46	0.87	0.80	0.14	0.62	**1.00**		
4-methylguaiacol	-0.20	0.09	0.21	0.35	0.30	0.24	0.15	0.36	0.21	-0.27	0.15	0.14	**1.00**	
***cis*-oak lactone**	-0.23	0.18	0.27	0.37	0.43	0.30	0.27	0.34	0.28	-0.26	0.12	0.22	**0.87**	**1.00**

$r \leq -0.85$ and $r \geq 0.85$ are indicated in bold typeface

High collinearity was observed between only some of the volatile compounds measured. Specifically, high positive correlations were observed between ethyl 2-methylbutanoate and ethyl 3-methylbutanoate ($r = 0.94$), between the acetates 2-methylpropyl acetate, 2-methylbutyl acetate, 3-methylbutyl acetate and hexyl acetate ($r \geq 0.84$), between ethyl acetate and acetic acid or 2-methylpropanoic acid ($r = 0.86$), and 4-methylguaiacol and *cis*-oak lactone ($r = 0.87$). There were no strong negative correlations ($r > 0.85$) observed with the largest negative correlation being between TPB and hexyl acetate ($r = -0.55$). The lack of strong negative correlation between compounds might be due to the wines being all from young vintages (2001 and 2002). Older wines alter chemically over time due to oxidative and acid hydrolysis reactions. This results in the decrease in concentration of certain compounds (e.g. acetates [64]) and the increase of other compounds (e.g. TDN [199] and dimethyl sulfide [78]), which could result in strong negative correlations between the increasing and decreasing components. No older Chardonnay wines were included in this study. Consequently, this volatile data set does not show strong negative correlations between compounds that might be influenced by age.

To explore the potential sensory role that the volatile compounds measured may play in the aroma of the unwooded Chardonnay wines, odour activity values (OAVs) were calculated for each compound. A summary of these results including each compound's sensory detection threshold (from Table 1-1, Chapter 1), and respective mean, minimum and maximum OAV are given in Table 4-6.

Table 4-6 Odour activity values for each volatile compound measured in the unwooded Chardonnay wines

	literature sensory threshold (µg/L)	mean OAV	minimum OAV	maximum OAV
ethyl acetate	7500 [6]	**11**	**7**	**28**
ethyl propanoate	1840 [4]	0.1	0.1	0.2
ethyl butanoate	20 [6]	**27**	**15**	**56**
ethyl 2-methylpropanoate	15 [6]	**5**	**3**	**16**
ethyl 2-methylbutanoate	1 [6]	**10**	**3**	**20**
ethyl 3-methylbutanoate	3 [6]	**7**	**3**	**14**
ethyl hexanoate	5 [6]	**246**	**184**	**395**
ethyl octanoate	2 [6]	**746**	**514**	**1162**
ethyl decanoate	200 [11]	**3**	**2**	**4**
ethyl dodecanoate	2000 [25]	0.1	0.03	0.4
***trans*-ethyl cinnamate**	1 [6]	**3**	0.6	**6**
2-methylpropyl acetate	1600 [11]	0.03	0.002	0.07
2-methylbutyl acetate	5 [27]	**19**	**3**	**62**
3-methylbutyl acetate	30 [6]	**81**	**9**	**242**
hexyl acetate	670 [4]	0.3	0.01	0.9
2-phenylethyl acetate	250 [6]	0.8	0.1	**2**
2-phenylethanol	10000 [6]	**2**	0.9	**4**
butanol	150000 [4]	0.01	0.002	0.02
2-methylpropanol	40000 [6]	0.5	0.3	0.9
2-methylbutanol	65000 [6]	0.3	0.2	0.5
3-methylbutanol	30000 [6]	**5**	**3**	**6**
hexanol	8000 [6]	0.3	0.1	0.6
acetic acid [a]	200000 [6]	**2**	**1**	**3**
2-methylpropanoic acid	200000 [6]	0.004	0.002	0.009
3-methylbutanoic acid	3000 [6]	0.1	0.04	0.2
hexanoic acid	3000 [6]	**2**	**1**	**3**
octanoic acid	500 [11]	**16**	**11**	**22**
decanoic acid	15000 [6]	0.2	0.09	0.2
linalool	15 [6]	0.5	0.04	0.9
α-terpineol	250 [11]	0.04	0.02	0.1
geraniol	30 [6]	0.07	nd	0.1
TDN	20 [3]	0.05	0.02	0.2
TPB [b]	0.04 [39]	0.2	0.08	0.5
β-damascenone	0.05 [6]	**40**	**16**	**80**

guaiacol	10 [6]	0.09	0.02	0.3
4-methylguaiacol	65 [17]	0.005	nd	0.05
4-ethylguaiacol	70 [17]	0.006	nd	0.02
4-vinylguaiacol	440 [17]	0.005	0.0005	0.01
4-vinylphenol	770 [17]	0.03	0.002	0.1
***cis*-oak lactone**	23 [28]	0.6	0.1	**2**
vanillin	200 [6]	0.08	0.02	0.4
diacetyl [b]	100 [6]	0.6	nd	**2**
methionol	500 [6]	0.9	0.5	**2**
dimethyl sulfide	10 [6]	**13**	**3**	**35**
carbon disulfide	5 [194]	0.6	0.1	**3**

[a] acetic acid measured as volatile acidity; [b] analysed in 18 wines only; compound not detected (nd); sensory detection thresholds were determined in 10% ethanol in water (w/w) [6], 11% ethanol in water (v/v) model wine [11], beer [25], water [27], wine [3, 4, 194], white wine [17, 28, 33, 39].

Of the 45 volatile compounds measured, 16 were always above threshold, a further two were on average above threshold, and an additional five were found above their respective model wine or white wine sensory detection thresholds in at least one of the wines in the study (OAV ≥ 1). The fermentation-derived esters were the most dominant group to be found above threshold, making up 12 of the 16 compounds always measured above threshold. In particular, ethyl hexanoate, ethyl octanoate and 3-methylbutyl acetate were measured in the wines at concentrations more than 100 times their respective sensory threshold concentration. The abundance of esters above sensory threshold in the unwooded Chardonnay wines in the present study is in agreement with other published reports [7, 61].

Compounds with high OAVs (OAV > 5) that are likely to be playing an important role in the aroma of these wines in include ethyl acetate, ethyl butanoate, ethyl 2-methylpropanoate, ethyl 2-methylbutanoate, ethyl 3-methylbutanoate, ethyl hexanoate, ethyl octanoate, *trans*-ethyl cinnamate, 2-methylbutyl acetate, 3-methylbutyl acetate, 3-methylbutanol, octanoic acid, β-damascenone and dimethyl sulfide.

Compounds that were measured around sensory threshold concentration (OAV 0.2 - 5) and might occasionally play an important role in the aroma of some of the wines analysed include ethyl propanoate, ethyl dodecanoate, hexyl acetate, 2-phenylethyl acetate, 2-methylpropanol, 2-methylbutanol, hexanol, 3-methylbutanoic acid, decanoic acid, linalool, TDN, TPB, guaiacol, *cis*-oak lactone, vanillin, diacetyl, methionol and carbon disulfide.

Volatile compounds that are less likely to be influencing the aroma of the unwooded Chardonnay wines with concentrations well below their indicative sensory detection

concentrations (OAV < 0.2) include 2-methylpropyl acetate, butanol, 2-methylpropanoic acid, α-terpineol, geraniol, 4-methylguaiacol, 4-ethylguaiacol, 4-vinylguaiacol and 4-vinylphenol.

None of the monoterpenes measured were found above sensory threshold in the unwooded Chardonnay wines which is in agreement with other studies on Chardonnay wine volatile composition [61, 69, 210]. It is interesting to note that wines with the highest concentrations of linalool and geraniol, including wines 1, 10, 11 and 14, were not the wines that were blended with higher levels of other varieties such as Riesling or Gewürztraminer. The compound β-damascenone was the only norisoprenoid measured in any of the wines above sensory threshold concentration which agrees with published reports [61].

Although the wines were labelled 'unwooded', the oak-derived *cis*-oak lactone was measured at sensorily significant concentrations in some of the wines analysed, in particular wines 2, 14 and 17. As discussed previously, wine 14 contained 5% of a wooded Chardonnay in final blend, so the relatively high *cis*-oak lactone concentration may be expected for that wine. Wine 2 was reported by the winemaker to be 100% unwooded and as the wine was from a relatively small producer in the Hunter Valley, the wine used in the final blend was not sourced from other producers. Consequently the high *cis*-oak lactone content of this wine is surprising. No winemaking details were obtained for wine 17 so it is possible that this wine was not 100% unwooded. It should be noted that some presumably oak-derived compounds (from lignin degradation) have been previously identified in wines that have had no contact with oak-wood [218]. It must be that some of these so-named 'oak-derived' compounds, which were measured in these unwooded Chardonnay wines, were formed from other precursors originating from the grape berry [219].

Diacetyl was measured at sensorily significant concentrations in some of the unwooded chardonnay wines analysed which agrees with reports of diacetyl concentration in Chardonnay by others [203-205]. Diacetyl is formed principally during malolactic fermentation from the metabolism of citric acid [204, 220] and can be influenced by wine contact with yeast lees [221]. Not surprisingly, wines 13 and 15, with among the highest concentrations of diacetyl, were reported to have had lees contact and wine 15 contained 30% MLF wine in the final blend.

The compound dimethyl sulfide was measured at relatively high concentrations in the unwooded Chardonnay wines in the study and was often measured many times above its respective model wine sensory detection threshold concentration. Dimethyl sulfide has been previously reported to be present in Chardonnay wine above its sensory threshold concentration [61].

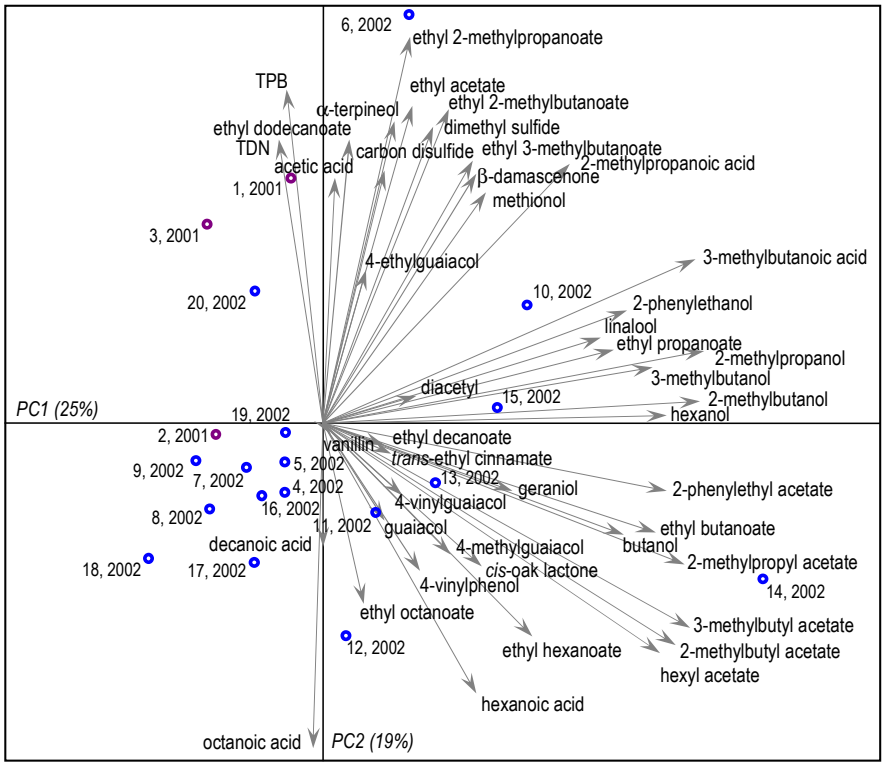
The volatile chemical data were assessed using PCA to examine any relevant and interpretable structure in the data. The first three PCs explained 57% of variation in the data set. PCA bi-plots are given in Figure 4-5 and Figure 4-6. The fourth and fifth PCs explained 11% and 7% respectively, and were also inspected (data not shown).

From visual observations of the PCA, wines were separated across PC1 by having either higher or lower concentration of 2-methylpropanoic acid, 3-methylbutanoic acid, 2-phenylethanol, linalool, ethyl propanoate, 2-methylpropanol, 3-methylbutanol, 2-methylbutanol, hexanol, 2-phenylethylacetate, ethyl butanoate, butanol, 2-methylpropyl acetate, 3-methylbutyl acetate, 2-methylbutyl acetate and hexyl acetate (increasing in concentration left to right of plot, Figure 4-5 and Figure 4-6). Wines were separated across PC2 as having either high concentrations of TDN, TPB, acetic acid, ethyl decanoate, ethyl 2-methylpropanoate, α-terpineol, carbon disulfide, dimethyl sulfide, ethyl 2-methylbutanoate, ethyl 3-methylbutanoate, β-damascenone, methionol, and 2-methylpropanoic acid, or high concentrations of octanoic acid, ethyl octanoate, hexanoic acid, ethyl hexanoate and *cis*-oak lactone. PC3 separated the wines as having either high concentrations of 4-ethylguaiacol, guaiacol, ethyl 3-methylbutanoate, and *cis*-oak lactone, or high concentrations of acetic acid, carbon disulfide, *trans*-ethyl cinnamate, ethyl acetate, 4-vinylphenol, β-damascenone and geraniol.

Obvious groupings of wines were not observed, although several individual wines (wine 2, 6 and 14) were clearly very distinct and separated from the main group of wines. Wine 14 was distinct from the other wines (Figure 4-5 and Figure 4-6) as it had among the highest levels of ethyl esters, acetates, 2-phenylethanol, 2-phenylethyl acetate, and had the highest concentration of *cis*-oak lactone. Wine 6 was distinct from the other wines (Figure 4-5 and Figure 4-6) as it had the highest concentration of dimethyl sulfide, carbon disulfide, ethyl acetate, ethyl decanoate, and ethyl dodecanoate, and had among the highest levels of ethyl propanoate, β-damascenone and TPB.

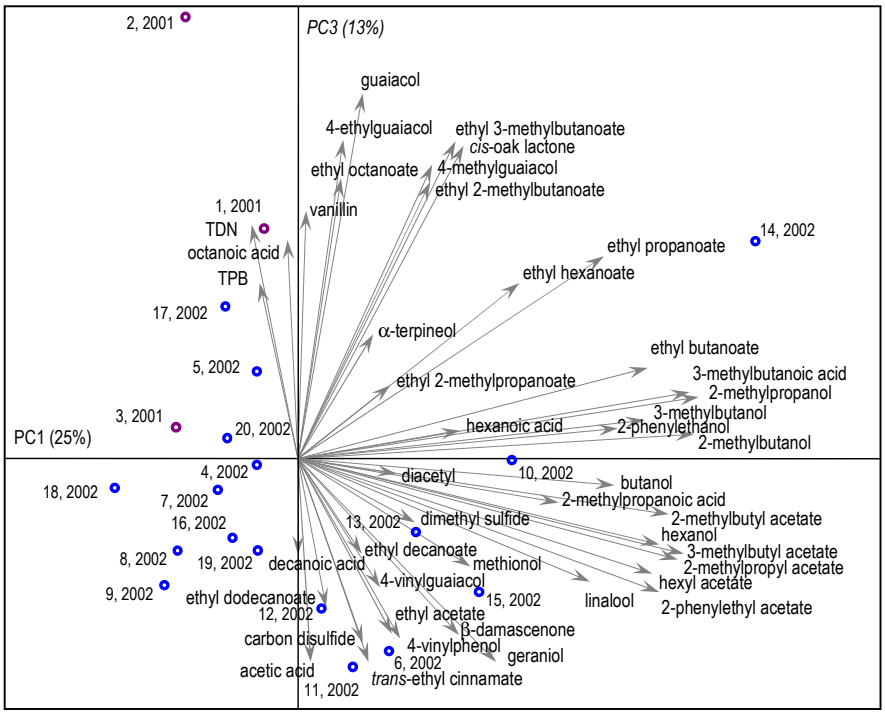
The 2001 wines did not have particularly different volatile profiles to the 2002 wines, although they tended to have slightly higher concentrations of TDN, TPB, vanillin and slightly lower concentrations of the ethyl esters and acetates. Wine 2, 2001, is the exception to this as it had both among the highest and lowest concentrations of various ethyl esters, acetates, and acids as well as among the highest concentration of *cis*-oak lactone. This unique combination of compound concentrations made wine 2, 2001, distinct from the main cluster of wines (Figure 4-6).

Figure 4-5 PCA bi-plot of volatile analytical results for the unwooded Chardonnay wines, PC1 versus PC2



For details on the sample codes refer to Table 4-1, vintages are also indicated. Sample scores are calculated from mean of two replicates and volatile compound loadings (vectors) are shown.

Figure 4-6 PCA bi-plot of volatile analytical results for the unwooded Chardonnay wines, PC1 versus PC3

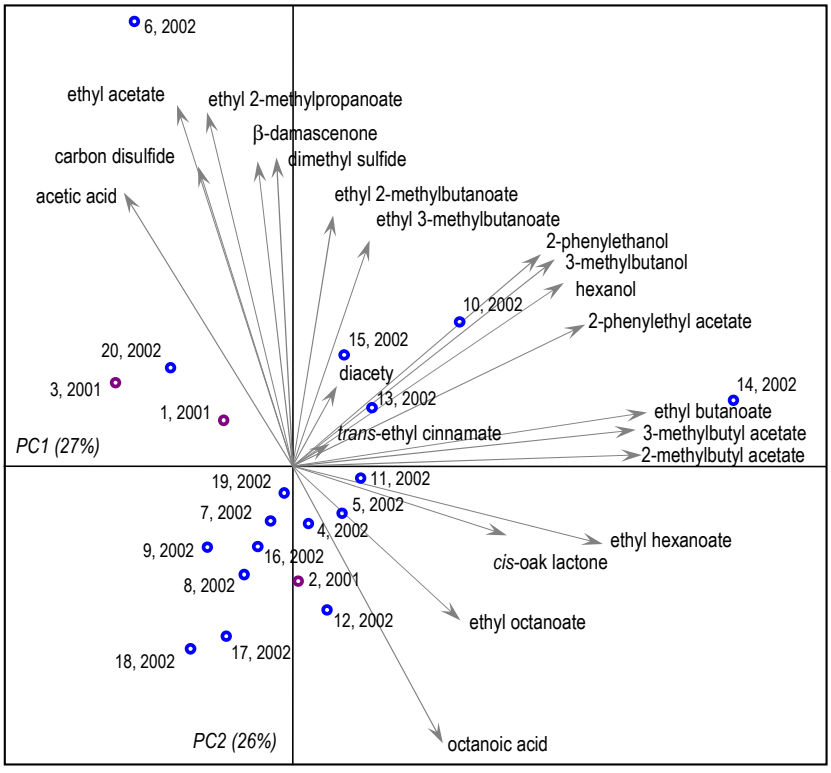


For details on the sample codes refer to Table 4-1, vintages are also indicated. Sample scores are calculated from mean of two replicates and volatile compound loadings (vectors) are shown.

Visually, the PCA of the volatile chemical data set was very different from the PCA of the sensory data. This might indicate that the volatile data set does not contain the most important volatile compounds that explain the variance in the aroma properties of the unwooded Chardonnay wines. Alternatively, it might be that compounds which are not playing a role in the aroma of the wines have high variation and are strongly influencing the volatile chemical data PCA results. To determine if this was the case, PCA was applied only to those compounds that were possibly most likely to have an impact on the aroma of the wines (i.e. compounds with OAV > 1 in at least one of the wines analysed). Under these conditions, the first three PCs explained 67% of variation in the data set. PCA bi-plots are given in Figure 4-7 and Figure 4-8 . From visual observations of the PCA with all compounds with OAVs < 1 excluded, the separation of the wines does not appear to dramatically compared to the PCA of all volatile variables (refer to Figure 4-6).

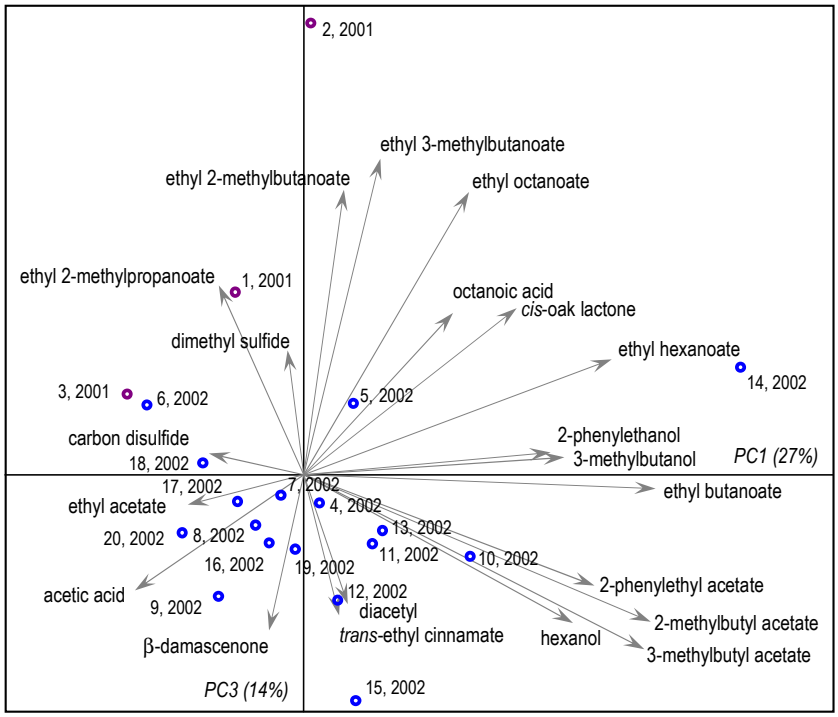
Although the PCA of the volatile chemical data did not appear to match the separation of wines observed in the sensory descriptive analysis, the variation found within the volatile variables measured made this data set suitable for more in-depth investigations using multivariate data analysis.

Figure 4-7 PCA bi-plot of volatile compounds with OAV > 1 for the unwooded Chardonnay wines, PC1 versus PC2



For details on the sample codes refer to Table 4-1, vintages are also indicated. Sample scores are calculated from mean of two replicates and volatile compound loadings (vectors) are shown.

Figure 4-8 PCA bi-plot of volatile compounds with OAV > 1 for the unwooded Chardonnay wines, PC1 versus PC3



For details on the sample codes refer to Table 4-1, vintages are also indicated. Sample scores are calculated from mean of two replicates and volatile compound loadings (vectors) are shown.

4.2.3 Multivariate analysis of sensory and chemical data

Multivariate analysis was performed to compare the unwooded Chardonnay sensory and volatile chemical data sets. Prior to multivariate analysis, the redundancy in the volatile chemical data set was reduced through grouping variables that were collinear, or had similar biochemical origins, or had similar aroma properties or were likely to be acting additively (for more discussion refer to Section 3.2.3, Chapter 3). Each new grouped variable was calculated by dividing the concentration of each compound by its own sensory threshold followed by adding them together [18]. Compounds that were grouped are tabulated in Table 4-7.

PCA bi-plots of the volatile chemical data including the new grouped variables, are shown in Figure 4-9 and Figure 4-10. The first three PCs accounted for 59% of variation in the simplified data set including grouped variables. This was a slight improvement on the 57% of variation explained in the PCA of all the volatile data (refer to Section 4.2.2, Figure 4-5 and Figure 4-6). By visual observation of the PCA, the simplified data set was somewhat different to the full volatile chemical data set. This is probably due to the fact that each compound in the 'grouped' variable has been 'weighted' by its sensory threshold value, resulting in a shift on the influence of certain compounds within a 'grouped' variable. Compounds well below sensory threshold, but with high variation, have in effect been 'tuned

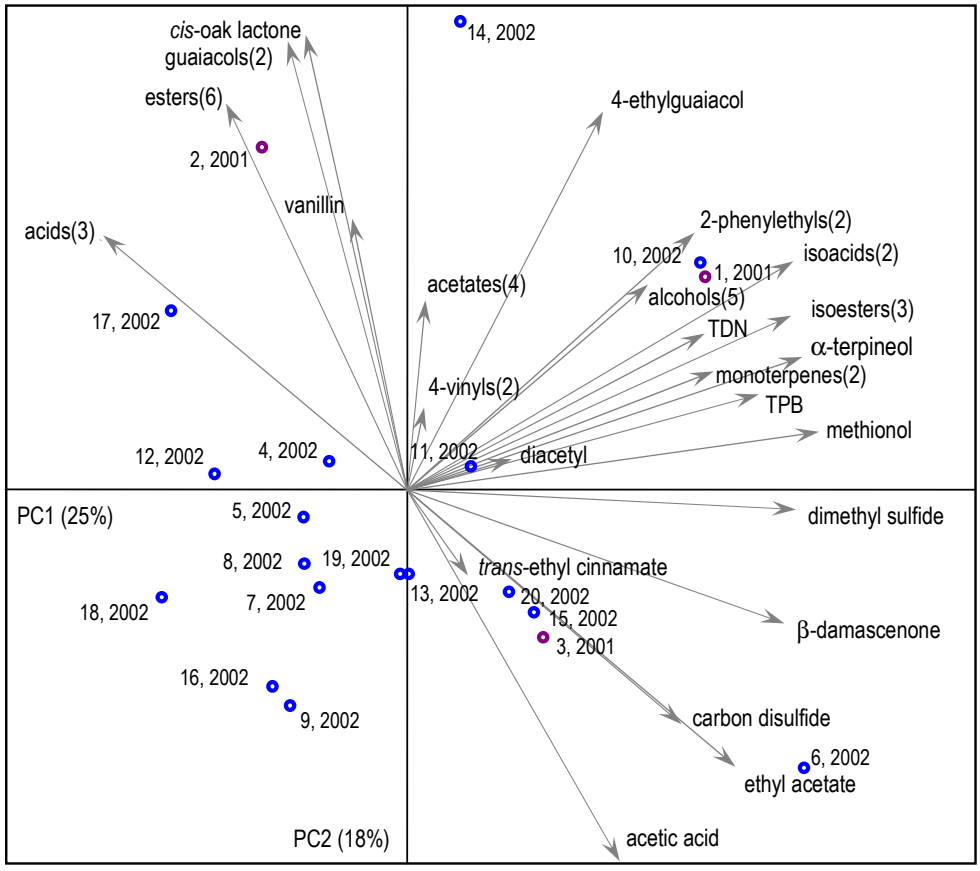
down' in terms of their contribution to explain the variation observed between the wines by PCA. This might result in a PCA plot which reflects variation among the volatile data which is more closely related to the sensory contribution of each compound, or group of compounds, in the wines.

Table 4-7 Volatile chemical variables that were grouped into a single variable

new group (number of compounds)	compounds included in group
isoesters(3)	ethyl 2-ethylpropanoate, ethyl 2-methylbutanoate, ethyl 3-methylbutanoate
esters(6)	ethyl propanoate, ethyl butanoate, ethyl hexanoate, ethyl octanoate, ethyl decanoate, ethyl dodecanoate
acetates(4)	2-methylpropyl acetate, 2-methylbutyl acetate, 3-methylbutyl acetate, hexyl acetate
2-phenylethyls(2)	2-phenylethyl acetate, 2-phenylethanol
alcohols(5)	2-methylpropanol, 2-methylbutanol, 3-methylbutanol, butanol, hexanol
isoacids(2)	2-methylpropanoic acid, 3-methylbutanoic acid
acids(3)	hexanoic acid, octanoic acid, decanoic acid
monoterpenes(2)	linalool, geraniol
guaiacols(2)	guaiacol, 4-methylguaiacol
4-vinyls(2)	4-vinylguaiacol, 4-vinylphenol

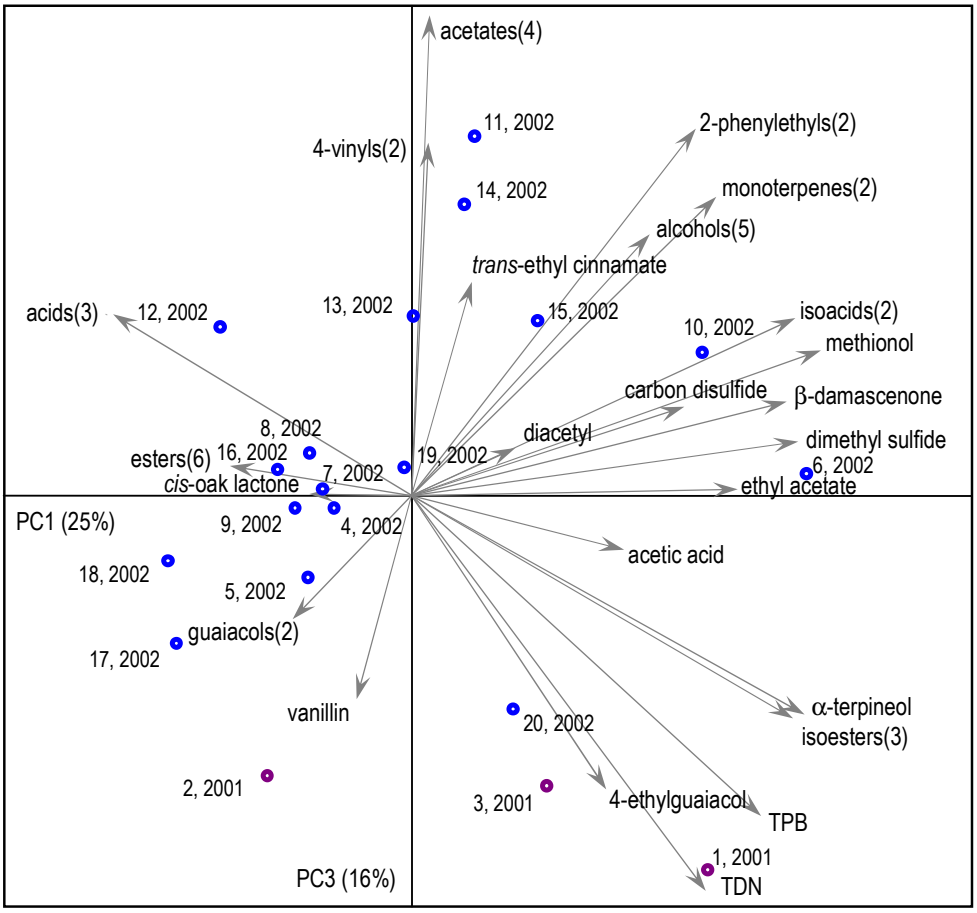
the sensory threshold data used for each compound in this table to calculate the new variables are given in Table 4-6

Figure 4-9 PCA bi-plot of volatile compounds and grouped variables for the unwooded Chardonnay wines, PC1 versus PC2



For details on the sample codes refer to Table 4-1, vintages are also indicated. Sample scores are calculated from mean of two replicates and volatile compound loadings (vectors) are shown.

Figure 4-10 PCA bi-plot of volatile compounds and grouped variables for the unwooded Chardonnay wines, PC1 versus PC3



For details on the sample codes refer to Table 4-1, vintages are also indicated. Sample scores are calculated from mean of two replicates and volatile compound loadings (vectors) are shown.

The new simplified volatile data set, including 'grouped variables', was used for multivariate data analysis as it contained less variables (24 instead of 45), and the risk of modelling redundant information was reduced (for further discussion, refer to Section 3.2.3, Chapter 3).

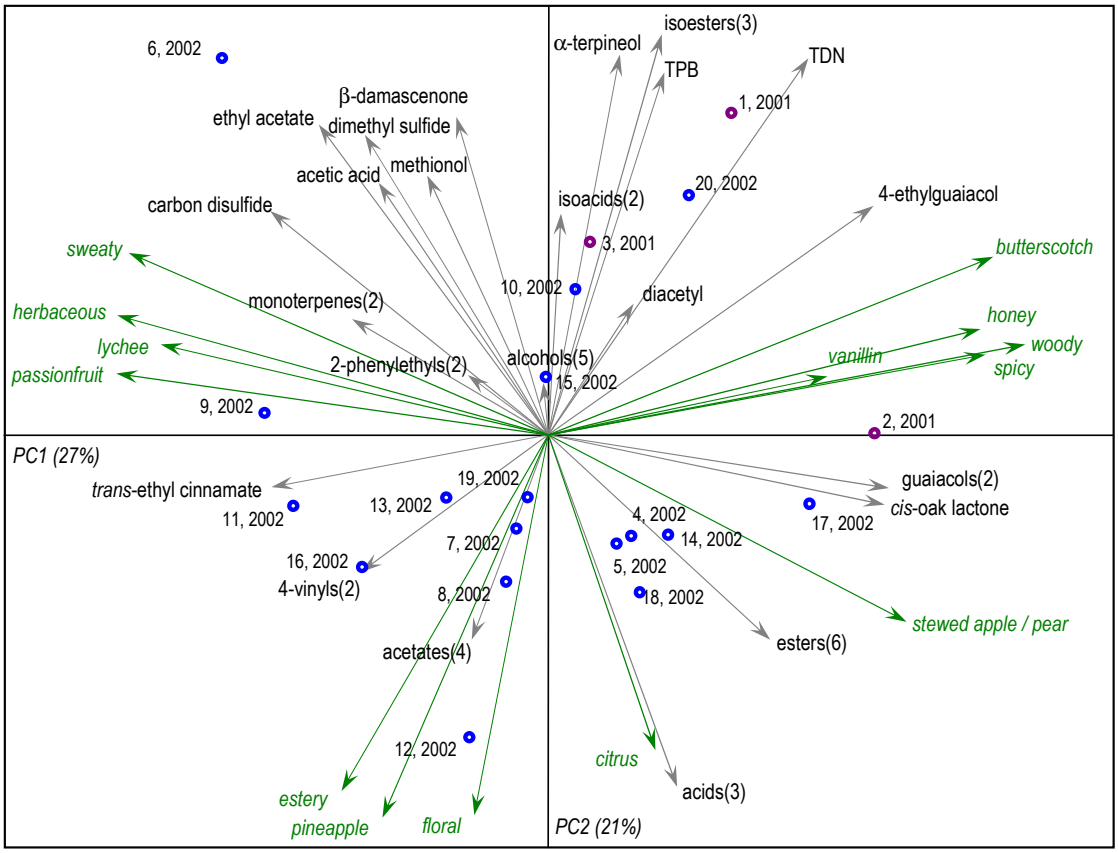
4.2.3.1 Relationships between sensory and compositional data

An explorative investigation of the possible relationships between aroma attributes and the volatile chemical data was carried out using PCA. The flavour and routine chemical data were not included in multivariate data analysis. The PCA bi-plots of the combined sensory and volatile chemical data sets are given in Figure 4-11 and Figure 4-12. By PCA, 62% of variation was explained in the combined data set within the first three PCs.

By visual inspection of the PCA plot, wines that were rated highly for *estery*, *floral* and *pineapple* also had high concentration of the acids(3), acetates(4) and 4-vinyls(2). Wines high in *citrus* properties also had relatively high concentrations of the esters(6), acids(3) and acetates(4). The scoring for the attribute *stewed/apple pear* related to the concentration of

esters(6), acids(3), *cis*-oak lactone and guaiacols(2) whereas the scoring for *lychee* related to the concentration of *trans*-ethyl cinnamate, monoterpenes(2) and carbon disulfide.

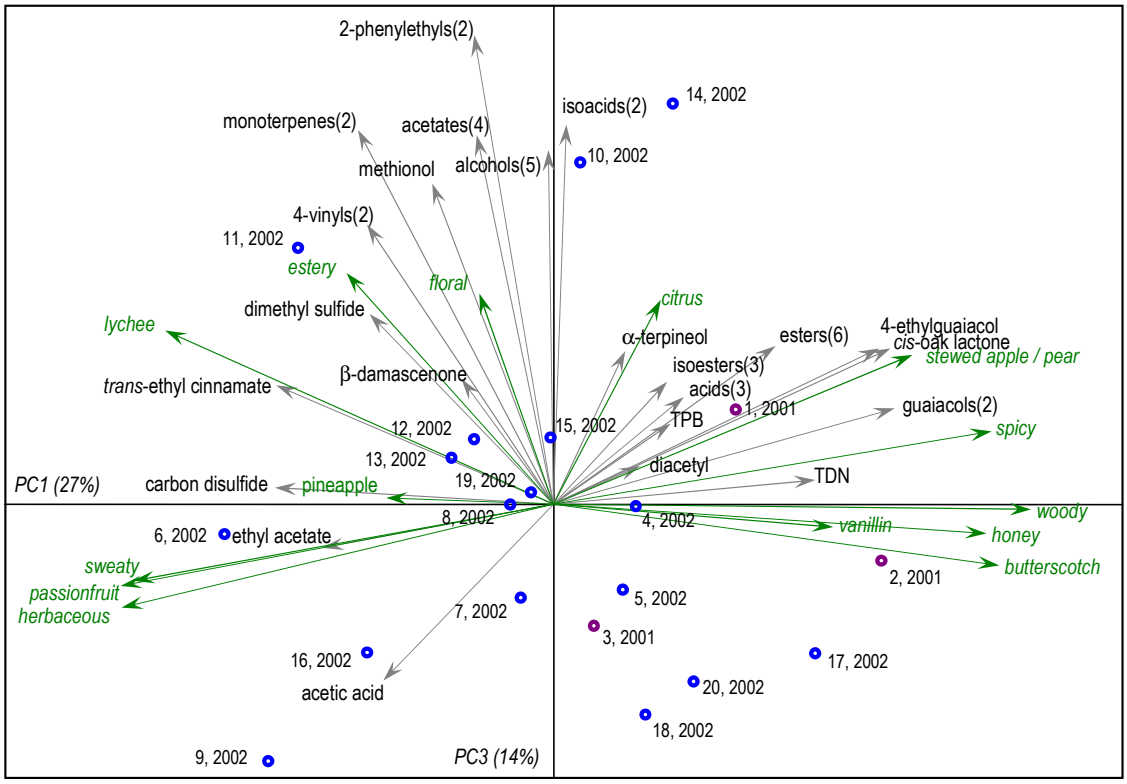
Figure 4-11 PCA bi-plot of sensory and volatile chemical data for the unwooded Chardonnay wines, PC1 versus PC2



For details on the sample codes refer to Table 4-1. Sample scores, volatile compound loadings (black vectors) and sensory attribute loadings (green vectors) are shown.

Wines that were scored highly for *sweaty*, *passionfruit* and *herbaceous* had relatively high concentrations of carbon disulfide, *trans*-ethyl cinnamate, ethyl acetate and acetic acid (measured as volatile acidity). Those wines that were scored higher for *butterscotch*, *honey*, *woody* and *spicy* were also relatively high in 4-ethylguaiacol, vanillin, *cis*-oak lactone, guaiacols(2), TDN and diacetyl concentration.

Figure 4-12 PCA bi-plot of sensory and volatile chemical data for the unwooded Chardonnay wines, PC1 versus PC3



For details on the sample codes refer to Table 4-1. Sample scores, volatile compound loadings (black vectors) and sensory attribute loadings (green vectors) are shown.

4.2.3.2 Prediction of sensory properties using compositional data

Partial least squares (PLS) regression was employed to relate the unwooded Chardonnay wine volatile compositional and sensory data sets with the aim to identify particular compounds that relate to specific aroma attributes. PLS1 was used to develop predictive equations of sensory scores for each aroma property (*y*-variable) using the volatile chemical data (*x*-variables). Three sets of models were developed to explore the ability of the volatile compositional data to explain the variation in the scoring of sensory attributes.

The first set of models were developed from an initial model built with all *x*-variables (24 in total, refer to Appendix II for results of these models) followed by jack-knifing (JK) assisted backward elimination to find the model for each attribute where all unstable *x*-variables were eliminated and only significantly contributing variables were used [157, 158]. The calibration statistics for the first set of models, including coefficient of determination (R^2), root mean square error of cross validation (RMSECV), F value, optimum number of components used in the PLS model (C_{opt}), and the number of *x*-variables used (*x*-var) to predict each attribute, are given in Table 4-8. The *x*-variables identified as significantly contributing to the models, either positively or negatively correlated, are also given in Table 4-8.

Compared to the models built for each aroma attribute using all 24 *x*-variables (refer to Appendix D) the attributes with improved calibration statistics included *citrus*, *stewed apple / pear*, *herbaceous*, *sweaty*, *honey* and *spicy*. Models for all the other attributes gave poorer regression statistics after using JK to eliminate non-contributing *x*-variables. For the *passionfruit* attribute, no compounds were identified as significantly contributing to the model using JK (no model given in Table 4-8).

In general, the volatile chemical data performed quite poorly in these models to predict the sensory attributes of the unwooded Chardonnay wines. Attributes with the best prediction statistics, where more than 40% of variation was accounted for ($R^2 > 0.40$) include *citrus*, *honey*, *butterscotch*, *woody* and *spicy*. None of these models were found to be statistically significant. Some of the compounds identified as being important to the best models could be of some merit. For example, it is feasible that the ethyl esters and fatty acids might be responsible for the *citrus* property of wine. Nevertheless, the poor prediction statistics of the models (low R^2, high RMSECV) demonstrates the limited value of the results from these models.

Table 4-8 PLS model results using jack-knifing for the prediction of unwooded Chardonnay aroma attribute scores

attribute	R^2	RMSECV	F value	C_{opt}	*x*-var	(+) loaded *x*-variable	(-) loaded *x*-variable
estery	0.33	0.30	3^{ns}	1	3	acetates(4), monoterpenes(2), 4-vinyls(2)	
floral	0.27	0.32	2^{ns}	1	3	acetates(4), 4-vinyls(2)	TDN
lychee	0.14	0.35	1^{ns}	1	2		guaiacols(2), *cis*-oak lactone
citrus	0.49	0.20	8^{ns}	1	2	esters(6), acids(3)	
pineapple	0.13	0.33	3^{ns}	1	1		α-terpineol
stewed apple / pear	0.07	0.41	1^{ns}	1	1	esters(6)	
herbaceous	0.05	0.49	0.5^{ns}	1	2		*cis*-oak lactone, guaiacols(2)
sweaty	0.05	0.68	0.4^{ns}	1	2		*cis*-oak lactone, guaiacols(2)
honey	0.42	0.40	6^{ns}	1	2	TDN	*trans*-ethyl cinnamate
butterscotch	0.46	0.42	5^{ns}	1	3	TDN, 4-ethyl guaiacol, *cis*-oak lactone	
woody	0.40	0.43	6^{ns}	1	2	TDN, 4-ethyl guaiacol	
spicy	0.54	0.26	6^{ns}	1	3	guaiacols(2), 4-ethyl guaiacol, *cis*-oak lactone	

^{ns} not significant, * significant ($p < 0.05$), ** significant ($p < 0.01$)

The second set of models, which was based on the JK models (Table 4-8) and an iterative backward elimination process, was employed to find the model that used the least number of *x*-variables (i.e. the simplest model) and had the best prediction statistics [18, 126]. The calibration statistics for the simplified models are given in Table 4-9 together with the identity of the minimum number of *x*-variables which gave the best possible calibration statistics.

The only attributes for which the JK models could be simplified and improved include *estery*, *citrus*, *sweaty*, *butterscotch* and *spicy*. Removing *x*-variables iteratively from the JK models for all other attributes resulted in even poorer calibration statistics (lower R^2 and higher RMSECV). Reasonable models were achieved for *citrus*, *butterscotch* and *spicy*, where greater than 56% of the variation in the scoring of these attributes was accounted for; however, none of these models were found to be statistically significant.

Table 4-9 Simplified PLS model results for the prediction of unwooded Chardonnay aroma attribute scores

attribute	R^2	RMSECV	F value	C_{opt}	*x*-var	(+) loaded *x*-variable	(-) loaded *x*-variable
estery	0.43	0.27	6^{ns}	1	2	acetates(4), 4-vinyls(2)	
citrus	0.56	0.18	23^{ns}	1	1	esters(6)	
sweaty	0.05	0.67	0.9^{ns}	1	1		*cis*-oak lactone
butterscotch	0.59	0.37	12^{ns}	1	2	TDN, *cis*-oak lactone	
spicy	0.62	0.23	14^{ns}	1	2	4-ethylguaiacol, *cis*-oak lactone	

^{ns} not significant, * significant ($p < 0.05$), ** significant ($p < 0.01$)

The results obtained from the models using JK assisted variable selection (in Table 4-8 and Table 4-9) were not able to be interpreted with any degree of confidence. There are several possible reasons for why these results were poor and why it was difficult to extract useful information from the data.

Poor predictions may result if the most important compounds responsible for the aroma of these unwooded Chardonnay wine have not been measured. The compounds wine lactone [6], and the sulfur-containing compounds 4-mercaptohexyl acetate, 3-mercaptohexanol and 3-mercaptohexyl acetate [6, 48, 49] are known to be important contributors to the aroma of white wine. Their absence from this volatile data set might have resulted in its inability to predict the sensory properties of these wines. There may also be other compounds of vital importance to the aroma of Chardonnay which were not targeted, including compounds that have not yet been identified in wine. Poor prediction may also be attributed to inadequacies in the technique used to group volatile chemical variables. Some of the sensory thresholds used in the calculation of grouped variables were not determined in the same matrix. Therefore, this may be skewing the weighting of the compounds in the group so that the new variable does not account for the real sensory activity of that group of compounds in a wine matrix.

Another explanation for the poor prediction is that perhaps the aroma of Chardonnay wine is too complex to be modelled with just a small number of *x*-variables using PLS, and aiming to minimise the number of *x*-variables in a model is not the most appropriate approach to explore the relationships in this data set. Furthermore, the relationship between the volatile

chemical data and the scoring of sensory attributes might not be linear. The PLS method is not ideally suited to be used for prediction when strongly non-linear relationships exist between *y*-variables and *x*-variables without significant modification [222]. Consequently, this technique yields poorly correlated relationships for attributes where non-linearities exist in the data set.

Another possible explanation for the poor prediction results achieved is that the wines themselves may simply be too similar and not show large enough variation in aroma properties. Furthermore, some of the unwooded Chardonnay attributes were rated with relatively poor judge agreement. This could result in sensory descriptive data where the scores for attributes contain a fairly large amount of random noise which might reduce the ability of PLS to effectively predict these attributes. The unwooded Chardonnay wines were all from young vintages (2002 and 2001), and although every attempt was made to select wines with a broad range of sensory properties, the selection was limited by the variation within this style of wine. The unwooded Chardonnay wine style has a relatively limited range of aroma types.

It might be that there is too much random variability associated with the sensory data set and that this noise is resulting in *x*-variables unduly being identified as unstable (by JK) and eliminated from models. This would result in losing useful information that might better predict the sensory attributes and would result in models that were overfitting *x*-variables which were poorly related to the predicted attribute.

To attempt to overcome problems that might be arising through the use of JK assisted variable selection, where *x*-variables might be unduly eliminated, a third set of models was developed that did not use JK in the first instance. From a model built with all 24 *x*-variables (refer to Appendix II), an iterative backward elimination method was used to remove single *x*-variables, one at a time, to find the simplest (fewest number of *x*-variables) and most powerful model [18]. Model selection was based on the criterion of a lower RMSECV, higher R^2, and significant F value [126]. For each attribute, once the simplest and most powerful model had been found, JK was used to highlight the significantly contributing *x*-variables [145]. The calibration statistics for these models are given in Table 4-10 and those *x*-variables that were highlighted as significantly contributing by JK shown are in bold text.

The iterative backward elimination variable selection method resulted in models where the *x*-variables predicted the scoring of sensory attributes with far greater confidence than when using JK in the first instance. The models tended to use more components (C_{opt}) than the previous two sets of models which might indicate that the regressions were overfitting the *x*-

variables to predict the sensory attributes. In the first and second set of models (Table 4-8 and Table 4-9) only one component (C_{opt}) was used in each of the regressions produced, whereas the final set of models (Table 4-10) used typically two or three components up to as many as five, for *spicy* and six for *citrus*. The number of components (C_{opt}) used in the model was controlled by cross validation which is a technique used to avoid overfitting of the regressions [126]. Additionally, careful examination of the residual variation plots showed that these models were not overfitted.

Table 4-10 PLS model results using iterative backward elimination for the prediction of unwooded Chardonnay aroma attribute scores

attribute	R^2	RMSECV	F value [a]	C_{opt}	*x*-var	(+) loaded *x*-variable [b]	(-) loaded *x*-variable [b]
estery	0.75	0.18	4*	2	8	**acetates(4)**, isoacids(2), **4-vinyls(2)**, *trans*-ethyl cinnamate	α-terpineol, *cis*-oak lactone, diacetyl, carbon disulfide
floral	0.50	0.25	3ns	2	5	**acetates(4)**, 2-phenylethyls(2)	**isoesters(3)**, β-damascenone, diacetyl
lychee	0.67	0.22	2ns	2	9	alcohols(5), **monoterpenes(2)**, 4-vinyls(2), **methionol**, dimethyl sulfide, **carbon disulfide**	***cis*-oak lactone**, **vanillin**
citrus	0.66	0.17	3ns	6	8	**esters(6)**, 2-phenylethyls(2), guaiacols(2)	isoesters(3), acetic acid, α-terpineol, TDN, β-damascenone
pineapple	0.52	0.23	3 ns	2	5	**acetates(4)**	**isoesters(3)**, *cis*-oak lactone, **vanillin**, **diacetyl**
stewed apple / pear	0.48	0.29	4 ns	2	4	acetates(4)	ethyl acetate, monoterpenes(2), TDN
passionfruit	0.26	1.30	1 ns	1	5	monoterpenes(2), *trans*-ethyl cinnamate	**TDN**, **4-ethylguaiacol**, ***cis*-oak lactone**
herbaceous	0.38	0.40	2 ns	3	4	monoterpenes(2), **carbon disulfide**	acids(3), TDN
sweaty	0.32	0.56	3 ns	2	3	**carbon disulfide**	acids(3), TDN
honey	0.53	0.35	2ns	2	6	**TDN**, **4-ethylguaiacol**, *cis*-oak lactone, TPB	**monoterpenes(2)**, ***trans*-ethyl cinnamate**
butterscotch	0.85	0.23	4*	3	11	esters(6), α-terpineol, **TDN**, β-damascenone, **guaiacols(2)**, ***cis*-oak lactone**, TPB, **diacetyl**	2-phenylethyls(2), **monoterpenes(2)**, dimethyl sulfide
woody	0.90	0.19	15**	3	7	**α-terpineol**, **TDN**, ***cis*-oak lactone**, vanillin	acetic acid, **monoterpenes(2)**, dimethyl sulfide
spicy	0.81	0.16	5**	5	9	isoesters(3), acids(3), α-terpineol, **TDN**, **β-damascenone**, ***cis*-oak lactone**, **vanillin**	ethyl acetate, **4-vinyls(2)**

[a] ns not significant, * significant ($p < 0.05$), ** significant ($p < 0.01$); [b] *x*-variables in bold were highlighted by as significant.

The much improved results from the third set of models reinforces the view that there is no 'best method' associated with multivariate prediction and that there are no fixed rules with regard to soft modelling techniques [154]. Each data set presents unique challenges and the same multivariate methodologies cannot be applied blindly to all data sets. Although automatic procedures, such as JK, are very valuable, intuition, experience, prior knowledge and understanding of the limitations of the data set, are more important for the analysis of results to achieve useful interpretations [126].

Calculation of RPD values from the SD of the sensory descriptive data (refer to Table 4-2) and the RMSECV showed that models for attributes *estery*, *floral*, *lychee*, *citrus*, *butterscotch*, *woody* and *spicy* had reasonable prediction ability (RPD = 2 - 3), and the models for *pineapple*, *stewed/apple pear*, *passionfruit*, *herbaceous*, *sweaty* and *honey* had poor prediction ability (RPD = 1) [196, 197].

In the third set of models developed, attributes for which very good calibration statistics were achieved and more than 80% of variation was accounted for ($R^2 > 0.80$), include *butterscotch*, *woody* and *spicy*. Good models were achieved ($R^2 > 0.65$) for the attributes *estery*, *lychee* and *citrus*; and reasonable models ($R^2 \geq 0.50$) were found for *floral*, *pineapple* and *honey*. Poor models ($R^2 < 0.50$) were produced for the attributes *stewed apple / pear*, *passionfruit*, *herbaceous* and *sweaty*. The only regressions that were found to be significant according to the F value were for attributes *estery*, *butterscotch*, *woody* and *spicy*. The good results obtained for *butterscotch*, *woody* and *spicy* may be due to the large variation in the scoring of these attributes across the wines (CV > 45%) and the fact that there was good judge agreement in the scoring of these attributes. Interestingly, the attributes *passionfruit*, *herbaceous* and *sweaty* had among the highest variation in scoring (CV > 70%) and were rated with excellent judge agreement, yet the models for these attributes had the poorest prediction statistics. This is evidence that the volatile chemical data set is missing important compounds which are responsible for these aromas in unwooded Chardonnay wine. The other attributes were typically rated with low variation and moderate judge agreement, consequently the poor calibration statistics achieved for the prediction of these attributes can be expected. More extensive panel training, or the use of fewer attributes in descriptive analysis, might have improved the predictive results obtained for these attributes.

Many logical relationships were observed in the models between sensory properties and particular chemical compounds. The attribute *estery* was best predicted using the acetates(4) variable among others. The acetates(4) were measured in the wines far above sensory detection threshold (refer to Table 4-6) and it is likely that they are playing a role in the *estery* character of these wines.

The acetates(4) were also used to build the best prediction for *floral* together with the 2-phenylethyls(2) variable. Although not highlighted as a significantly contributing variable (using JK), the inclusion of the variable 2-phenylethyls(2) gave the best calibration statistics. The two compounds in this variable, 2-phenylethanol and 2-phenylethyl acetate, have distinct ‘floral’ and ‘rose-like’ aromas as individual compounds and were measured in the wines above their respective sensory threshold concentrations (refer to Table 4-6). Consequently,

it is very likely that these compounds are playing an important role in the *floral* aroma of these wines.

The positive contribution of only the acetates(4) and the negative contribution of four other compounds gave the best calibration statistics for *pineapple*. It is likely that the 'fruity' smelling acetates are responsible for the *pineapple* attribute in these wines.

The *citrus* attribute was best predicted with the esters(6) group which is a likely cause and effect relationship. These 'fruity'-smelling esters were measured in the unwooded Chardonnay wines at concentrations many times their respective sensory thresholds and consequently it is very likely that these compounds are playing an important role in the aroma of these wines. Furthermore, the esters have been identified by other studies as contributors to the 'fruity' aroma of white wines [223] and so it is likely that they are related to the *citrus* aroma of these wines.

Interestingly, all three sulfur-containing compounds which were measured in the wines were included in the best prediction model for *lychee* together with the positive contribution of the monoterpenes(2), alcohols(5) and 4-vinyls(2) and the negative contribution of two other compounds. The aroma of *lychee* is considered to be a tropical fruit type of aroma and many sulfur-containing compounds are thought to give rise to tropical aromas in wine [49]. These particular sulfur-containing compounds do not have 'fruity' types of aromas themselves; however, their presence in wine may be a good marker for the presence of other sulfur-containing compounds which might be responsible for the *lychee* character in the wines. Similarly for the attribute *sweaty*, the compound carbon disulfide might be a good marker of other sulfur-containing compounds which could be responsible for the *sweaty* aroma observed in wine.

No compounds were identified by JK as significant in the model for the *stewed apple / pear* attribute. This is not surprising considering the rating of this attribute was fairly noisy (low variation and poor judge agreement).

The attribute *honey* was best predicted using the positive contribution of TDN, 4-ethylguaiacol, *cis*-oak lactone and TPB and the negative contribution of two other compounds. Of these positively contributing compounds only *cis*-oak lactone was measured in the wines above sensory threshold concentration. The low R^2 achieved for the prediction of *honey* indicates that the compound/s responsible for this attribute may not have been measured.

The compounds diacetyl and *cis*-oak lactone were identified, among others, as being important to the prediction model for *butterscotch*. The compound diacetyl, which has a buttery aroma [205], and *cis*-oak lactone, which has a coconut aroma [28], were measured above their respective model wine and white wine sensory thresholds (refer to Table 4-6) and are very likely to be playing a role in the perception of a *butterscotch* aroma in these wines. The oak-derived compounds *cis*-oak lactone and vanillin were identified as important to the prediction of *woody* which is likely to be a causative relationship.

Spicy was best predicted using the positive contribution of TDN, β-damascenone, *cis*-oak lactone, vanillin and the negative contribution of two vinyl compounds. It is possible that *cis*-oak lactone is playing a role in the *spicy* aroma of wine and it is interesting that β-damascenone was also identified as potentially playing a role. The compound β-damascenone was measured in the wine far above its model wine sensory threshold concentration and is possibly playing a very important role in the aroma of these wines as indicated by its presence in some of the models in Table 4-10. *Spicy* and *butterscotch* were the only attributes where β-damascenone was identified as positively contributing to the models of these attributes. For the attributes *floral* and *citrus*, β-damascenone was playing a negative role in the prediction indicating that β-damascenone was masking the perception of these attributes.

It was observed that for the models of some attributes, the relationship between predicted versus measured tended toward a non-linear relationship. For example, the plots of the predicted versus measured scores from the models developed for the *citrus* and *passionfruit* attributes and to a certain degree for the *lychee* attribute, all tended toward a non-linear relationship. This indicates that the relationships between volatile composition and some of the sensory properties of these unwooded Chardonnay wines might not be simple linear relationships. As discussed previously, PLS is a linear method, and does not perform well for data sets where the relationships are strongly non-linear. This may also have contributed to the relatively poor results obtained for the prediction of some of the unwooded Chardonnay wine sensory attributes in this study. Non-linear multivariate methods, such as artificial neural networks (ANN) were not appropriate for use in this study due to the small sample size (n = 20).

Due to the relatively poor performance of the models to predict some of the sensory properties of these wines, it is likely that there are compounds missing from the volatile data set, which could be used to explain some of the aromas perceived in this set of wines. Nevertheless, the results obtained from the PLS models developed for the prediction of unwooded Chardonnay sensory properties have highlighted a number of compounds that

might be of greatest importance to the aroma of this variety and style of wine. These compounds include 2-methylpropyl acetate, 2-methylbutyl acetate, 3-methylbutyl acetate, hexyl acetate, ethyl propanoate, ethyl butanoate, ethyl hexanoate, ethyl octanoate, ethyl decanoate, ethyl dodecanoate, 2-phenylethyl acetate, 2-phenylethanol, *cis*-oak lactone, diacetyl and β-damascenone. The compound dimethyl sulfide was measured well above its reported sensory threshold concentration in the study wines even though it was not found to be particularly important to the models developed. The compounds linalool, geraniol, 4-vinylphenol, 4-vinylguaiacol, 4-ethylguaiacol, 4-methylguaiacol, guaiacol, methionol, TDN and TPB were also occasionally used to build the best predictive models but are less likely to be important to the aroma of these wines as they were measured at typically sub-threshold concentrations in the wines analysed.

4.3 Conclusion

The use of quantitative sensory and accurate, precise chemical analytical methods, together with the application of multivariate techniques, have allowed the identification of the volatile aroma compounds likely to contribute most strongly to the aroma properties of a set of commercial Australian unwooded Chardonnay wines. The volatile compounds identified include mostly yeast fermentation-derived compounds, and a small number of grape-derived norisoprenoids and oak-derived compounds. Further work should be done to identify and measure those compounds which may be useful in explaining the variation of some of the poorly predicted attributes. In particular, the compounds responsible for the *passionfruit*, *herbaceous* and *sweaty* aromas in unwooded chardonnay wine need to be investigated.

For unwooded Chardonnay wine, no single compound appears to have overriding influence on aroma. However, the ethyl esters and acetates do appear to play a very dominant role in the aroma of young unwooded Chardonnay wine.

4.4 Materials and methods

4.4.1 Wines

To select suitable wines for the study, an informal preliminary sensory assessment of a broad range of commercial Australian unwooded Chardonnay wines was conducted. Seventy six unwooded Chardonnay wines were selected by reference to tasting notes from wine show information and current reports on commercial wine in the wine press. The wines were sourced, according to availability, from a range of producers, regions (63% SA, 7% NSW, 4% Tas, 13% Vic, 11% WA, 3% non-regional blend), vintages (1% 1996, 1% 1998, 1% 1999, 14% 2000, 46% 2001, 36% 2002), and retail prices ($4 - $23 / bottle). Wines were presented in coded glasses as sets of four - six wines to a panel of six - nine AWRI staff, with extensive wine tasting experience, over a number of sessions spanning several weeks (20th Sept 2002 – 6th May 2003). Preliminary screening sessions were conducted as described for the Riesling wines (refer to Section 3.4.1, Chapter 3). At the end of the screening process, 20 suitable unwooded Chardonnay wines were chosen that were deemed to encompass the range of sensory characteristics observed across all of the unwooded Chardonnay wines screened for the study, and included wines that had both high and low intensity aromas. Wines that were selected as candidates for the study were without obvious faults such as aldehyde, reduced or ethyl acetate aromas (or other), and did not show aroma and flavour characters associated with yeast lees contact, MLF or oak-wood contact. The wines selected for the study were analysed by the Australian Wine Research Institute's Analytical Services for a number of chemical variables including alcohol, specific gravity, pH, free and total sulfur dioxide, titratable acidity (at pH 8.2), total dry extract, glucose and fructose, and volatile acidity (as acetic acid) [187].

4.4.2 Sensory descriptive analysis

A 20-membered panel of judges was selected, comprising ten male and ten female panellists, aged 21 - 51 years (average age 34 years), all of whom were staff and students of The Australian Wine Research Institute with previous experience in sensory studies. Fifteen members of the Chardonnay sensory descriptive analysis panel also took part in the Riesling study (refer to Chapter 3).

Training sessions were conducted over seven weeks (20th Mar – 8th May 2003) and involved seven discussion sessions, five booth training sessions and seven practice sessions using the computers in the booths. By consensus, 17 aroma terms and six in-mouth flavour terms were selected to rate during the formal sessions (see Table 4-11). Sensory reference standards were developed for each aroma term during the training (Table 4-11). Training

sessions were conducted as described for the Riesling wines (refer to Section 3.4.2, Chapter 3).

Table 4-11 Composition of sensory reference standards

aroma attribute	sensory reference standard composition
estery	2 mL stock estery mix [a] in 100 mL base wine [b]
floral	spot of talc powder (heritage rose) in 100 mL base wine
lychee	4 mL canned lychee syrup (Admiral Lychees in light syrup) in 100 mL base wine
citrus	1 tsp orange marmalade (Rose's Sweet orange marmalade) in 100 mL base wine
pineapple	7 mL canned pineapple juice (Golden Circle brand) in 100 mL base wine
stewed apple / pear	tsp stewed apple (freshly stewed granny smith apple, no skin) + 10 mL canned pear juice + quarter canned pear (SPC halved pears in natural juice) in 100 mL base wine
stone fruit	10 mL canned peach juice (SPC sliced peaches in natural juice)+ one slice canned peach + 10 mL canned apricot nectar (Berri brand) in 100 mL base wine
passionfruit	small piece of fresh passionfruit skin + pulp in glass
herbaceous	cut grass and clover leaves in 100 mL base wine
sweaty	0.2 mL hexanoic acid stock (10 g/L) + 0.1 mL 3-methylbutanoic acid stock (10 g/L) in 100 mL base wine
honey	1 mL honey (Capilano brand) in 100 mL base wine
butterscotch	1 butterscotch (Werthers Original brand) dissolved in 100 mL base wine
woody	French oak chips (large chips medium toast, World Cooperage Company, AC2434) soaked (at least 30 mins) in 100 mL base wine
spicy	2 shakes nutmeg (McKenzie's brand) one shake mixed spice (McKenzie's) in 100 mL base wine
flavour attribute	
sourness	no standard
sweetness	no standard
overall flavour	no standard. The overall intensity of retronasal flavour experienced after spitting
flavour persistence	no standard. The length of time that retronasal flavour persisted.
astringency	no standard. The degree of drying experienced in the mouth after spitting
bitterness	no standard

[a] 0.5 g 2-methylpropyl acetate, 0.09 g ethyl butyrate, 0.2 g ethyl hexanoate, 0.2 g ethyl octanoate, in 100 mL redistilled ethanol; [b] 100 mL of Yalumba Chenin Blanc, 2002, 2 L cask wine (11% alcohol / volume).

Formal rating sessions were held in which judges evaluated the 20 unwooded Chardonnay wines in triplicate (15 sessions, 13th May 03 – 12th June 2003). Formal sessions were conducted as described for the Riesling wines (refer to Section 3.4.2, Chapter 3).

4.4.3 Volatile chemical analysis

After the sensory study was complete for the unwooded Chardonnay wines, two bottles of each wine label were individually divided into glass ampoules (1 x 20 mL, 4 x 50 mL) and a screw cap bottle (1 x 500 mL, sealed with foil), sealed under nitrogen, and stored at -18ºC until chemical analysis could take place. Analytical methods, as described in Chapter 2, were applied to measure a number of volatile compounds in the wines. Time spent in

storage for the wines prior to the application of each analytical method is shown in Table 4-12.

Table 4-12 Storage time for unwooded Chardonnay wines prior to chemical analysis

analytical method	time spent in storage at -18ºC (months)	
fermentation-derived compounds	1 month	(June – July 2003)
diacetyl and *trans*-ethyl cinnamate	10 months	(June – April 2004)
grape- and oak-derived compounds	2 months	(June – August 2003)
4-vinylguaiacol and 4-vinylphenol	3 months	(June – September 2003)
methionol	10 months	(June 2003 – April 2004)
low molecular weight sulfur compounds	14 months	(June 2003 – August 2004)

4.4.4 Statistical and multivariate analysis

The statistical and multivariate data analysis was conducted as described for the Riesling wine data (refer to Section 3.4.4, Chapter 3).

Chapter 5 Comparison of data for Riesling and unwooded Chardonnay

5.1 Introduction

Riesling and Chardonnay are both important commercial Australian white wine varieties which have quite distinct aroma and flavour characteristics presumably due to differing volatile profiles. In this chapter the major differences in the sensory profiles and the volatile composition of the Riesling and unwooded Chardonnay wine in this study will be discussed, and the results from the prediction models developed for each variety (in Chapter 3 and Chapter 4) will be compared.

5.2 Results and discussion

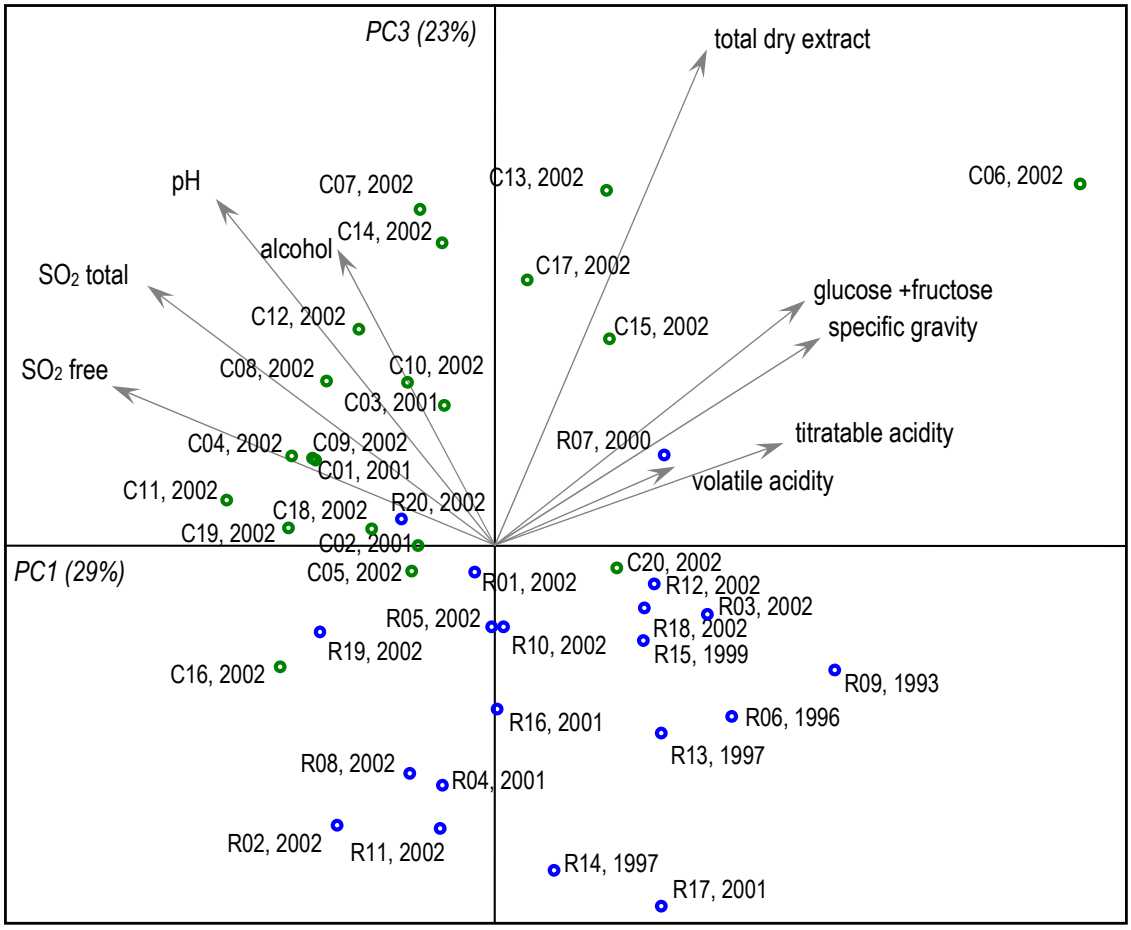
In both the Riesling and unwooded Chardonnay studies, each set of wines was selected to represent the greatest variation in sensory properties within each variety. As a consequence, both sets were limited by the variation available for each commercial Australian variety. A longer and more extensive preliminary screening, involving a larger number of wines, was conducted for the unwooded Chardonnay compared to the Riesling. This was primarily due to the fact that less variation was observed in the unwooded Chardonnay style of wine, and it was more difficult to find suitable wines for the study, which had different types of aromas of varying intensity. Many of the wines tasted in the preliminary screening of the unwooded Chardonnay wine were not suitable for selection due to heavy oak or MLF influence (which was not desired for this study) or showed winemaking faults. Although many older vintage wines of unwooded Chardonnay were tasted, none had suitable properties for the study. In contrast, most of the wines tasted in the preliminary screening for the Riesling study would have been suitable candidates for the study. This made the selection of Riesling wines more straightforward, and choices were based on maximising the variance in aroma properties that were observed across the wines during the preliminary tastings.

5.2.1 Comparison of routine chemical data between varieties

The routine chemical data obtained for the wines selected from both varieties were analysed by PCA. A plot of PC1 versus PC3 is given in Figure 5-1 which shows some separation of the two varieties. In Figure 5-1 the unwooded Chardonnay wines are generally grouped in the top half of the plot while the Riesling wines are generally grouped in the lower half of the plot. PC2 did not separate the two varieties but separated the wines according to alcohol content and level of volatile acidity (plot of PC2 not shown). Some separation was also observed between the young (2002) and the aged Riesling wines (2001 – 1993) as shown in

Figure 5-1 where the aged Riesling wines are grouped to the bottom right of the plot. This separation is most likely influenced by the values for SO_2 free and total which is known to decrease as wine ages.

Figure 5-1 PCA bi-plot of routine chemical data for Riesling and unwooded Chardonnay wines, PC1 versus PC3



For details on the sample codes refer to Chapter 3, Table 3-1 and Chapter 4, Table 4-1, Riesling wines (in blue) are labelled by the sample code prefix R and unwooded Chardonnay wines (in green) by the prefix C. Routine chemical data loadings (vectors) are shown.

By analysis of variance (ANOVA), the unwooded Chardonnay wines were significantly higher in alcohol, pH, SO_2 free, SO_2 total and total dry extract ($p < 0.05$). There was no significant difference between the varieties for the parameters specific gravity, titratable acidity, glucose plus fructose and volatile acidity.

5.2.2 Comparison of sensory descriptive data between varieties

The sensory descriptive analysis was conducted separately for the set of Riesling and set of unwooded Chardonnay wines and the studies were separated by 3 months. For the descriptive studies, there were 15 panellists who served on the panel for both studies (20 judges in unwooded Chardonnay panel, 16 judges in Riesling panel). The differences observed by the comparison of the data collected from each study might be real; however,

they could also be attributed to the fact that the two varieties were analysed by two slightly different panels on two different occasions in the context of two different studies. Nevertheless, it is interesting to compare and contrast the results from both descriptive studies.

The Riesling descriptive study used more terms than for the unwooded Chardonnay study. This is not surprising considering there were both aged and young wines in the Riesling study, each of which required quite different descriptive terms. On the other hand, there were only young wines in the unwooded Chardonnay study. Many common terms were used in each study, including *estery*, *floral* (or *perfumed floral*), *lychee*, *pineapple*, *stewed apple* (*stewed apple / pear*), *stone fruit* (*apricot*), *passionfruit*, *herbaceous*, *honey* and *butterscotch* (*caramel*). A generic *citrus* term was used in the unwooded Chardonnay study whereas individual 'citrus-fruit' terms were used in the Riesling study, including *lemon*, *grapefruit* and *lime*. For the Riesling study an extra 'floral' term was used (*dried rose*) which was appropriate to aid in distinguishing between the different types of 'floral' properties of this typically 'floral' variety. Unique terms used in the Riesling study were *toasty*, *kerosene* and *rubber / plastic*, which were all used to describe the older 'reserve' vintage Riesling wines. Unique terms used in the unwooded Chardonnay study were *sweaty*, *woody* and *spicy*. Wines that were scored highly for the *sweaty* term were also scored highly for *passionfruit* and *herbaceous* aromas. These terms were found to be highly collinear. The *woody* and *spicy* terms were used to describe wines that were probably influenced by oak-derived compounds. As discussed in Chapter 4, some of the so-called unwooded Chardonnay wines contained some low levels of oak-influenced wine in the final blend. This is not common practice for a Riesling style of wine and none of the Riesling wines in this study were influenced by oak. Consequently, the *woody* and *spicy* terms are unique to the unwooded Chardonnay style of wine in this study. The flavour terms used in each study were the same.

The average results (of 20 wines) for the aroma attribute scores from the descriptive studies of the Riesling and unwooded Chardonnay wines are shown in Figure 5-2 and Figure 5-3, respectively. The mean scores for the 2002, 2001 vintages (and 1993 - 2000 vintages for the Rieslings) for each attribute are also shown in these plots.

As shown in Figure 5-2 and Figure 5-3, the sensory differences observed for the Riesling wines between 2002 and 2001 vintages, were larger than for the unwooded Chardonnay wines. The sensory differences between the older 2000 – 1993 vintage Riesling wines and the young Riesling wines were even greater.

Figure 5-2 Aroma sensory descriptive results for Riesling wines

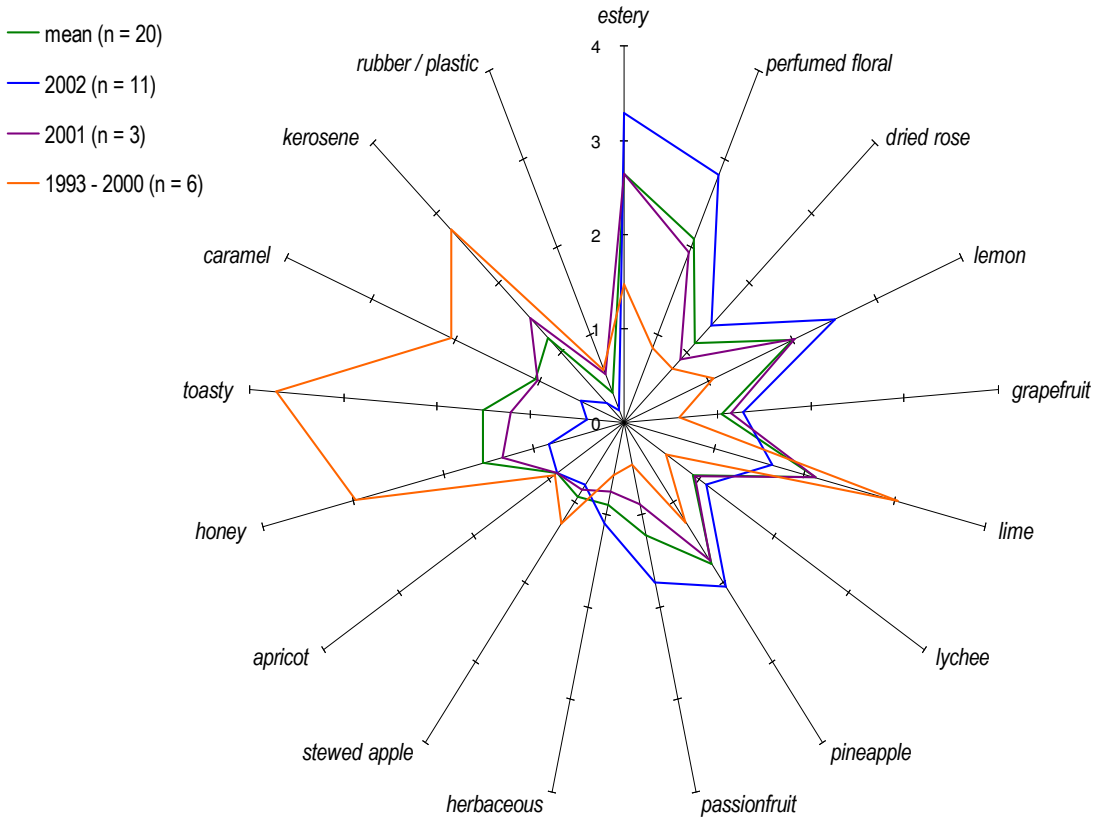
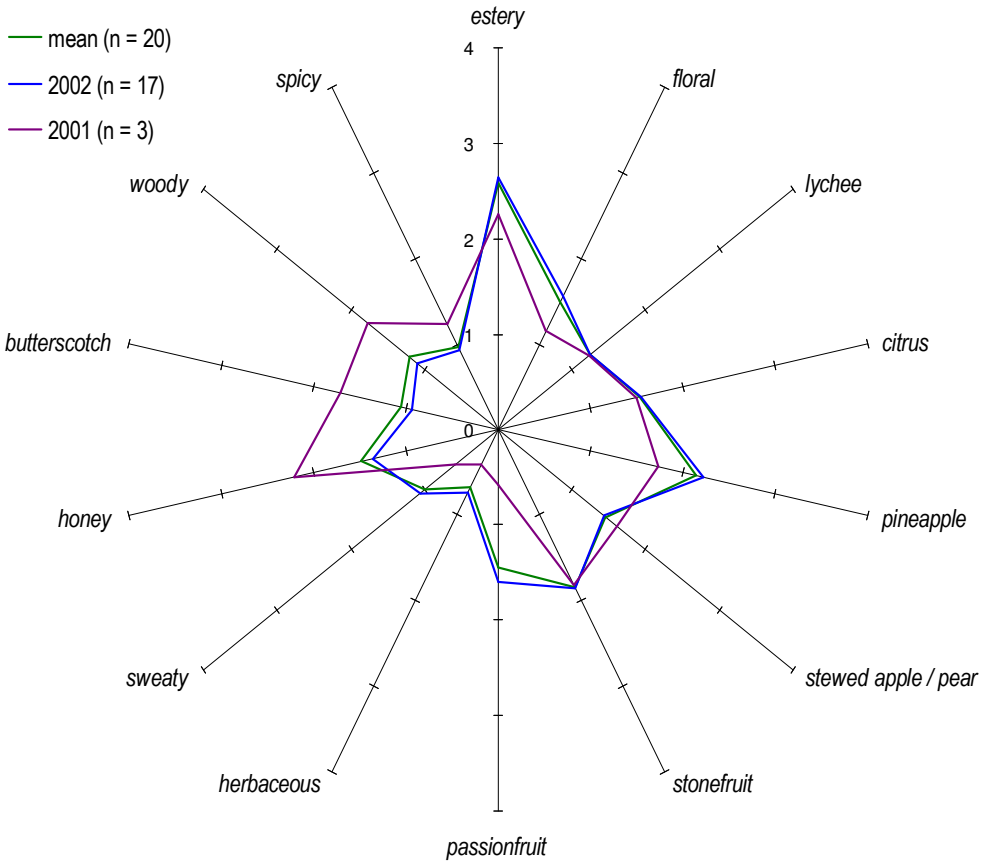


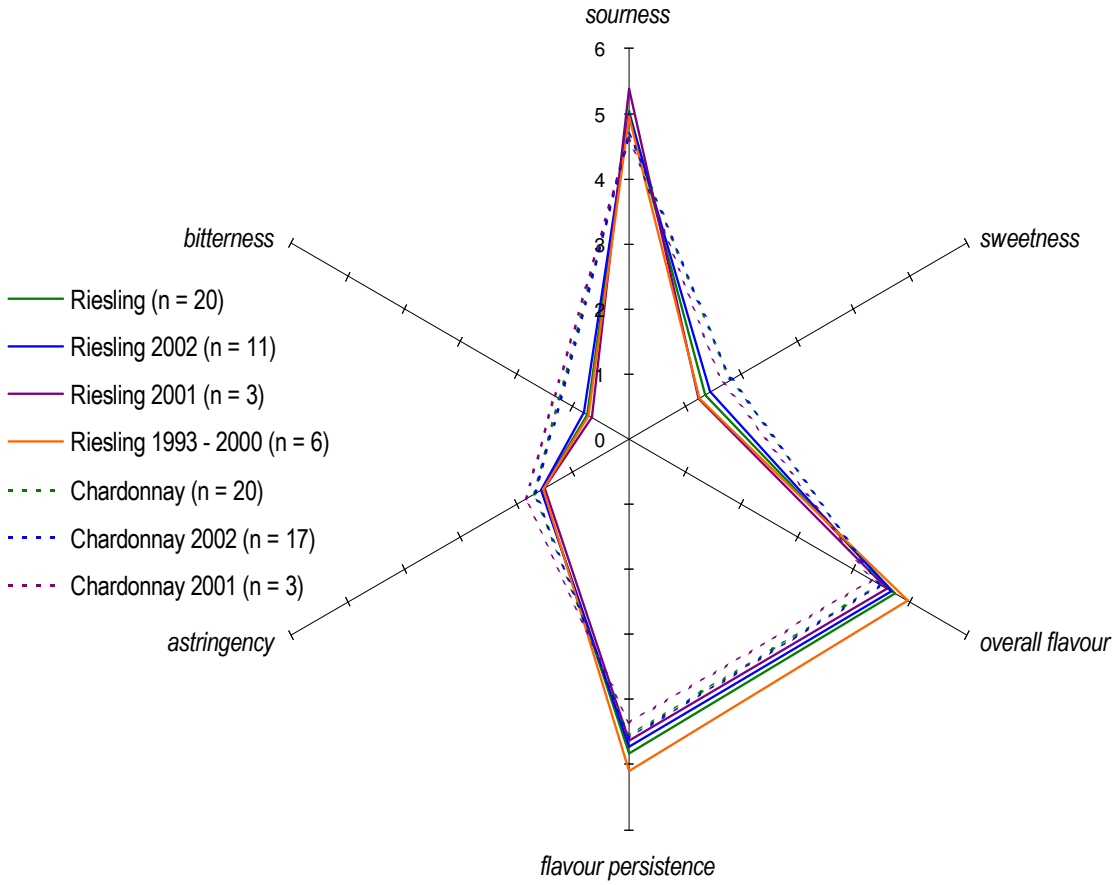
Figure 5-3 Aroma sensory descriptive results for unwooded Chardonnay wines



Generally, the 'floral' and 'fruity' terms used in the Riesling study were rated with higher variation (CV > 30%) compared to similar terms rated in the unwooded Chardonnay study (CV < 30%). The term *passionfruit* was the exception to this, where the rating for *passionfruit* in the unwooded Chardonnay study showed much higher variation (CV = 104%) than in the Riesling study (CV = 84%). Similarly, the scoring of the unwooded Chardonnay *herbaceous* term also had higher variation than in the Riesling study.

The flavour terms were scored by the panel in similar ways for each variety, with the Riesling wines rated with slightly higher variation than the unwooded Chardonnay wines. Figure 5-4 shows the average flavour attribute scores for each variety including the average scores for the 2002 vintage and 2001 vintage (and 2000 – 1993 vintages for the Riesling) wines from each descriptive study. For both varieties, the variation observed between vintages for the flavour attributes was very low compared to the scores for aroma attributes. On average, the unwooded Chardonnay wines were scored slightly higher for *astringency*, *bitterness*, *sweetness* and lower for *sourness*, *overall flavour* and *flavour persistence*.

Figure 5-4 Flavour sensory descriptive results for Riesling and unwooded Chardonnay wines



The results obtained from the Riesling sensory study were generally better suited for multivariate data analysis because the variation in the scoring of the aroma attributes was generally greater than for the unwooded Chardonnay study. Furthermore, judge agreement was higher for the Riesling wine aroma attributes than for the unwooded Chardonnay wine aroma attributes, which is probably a result of the larger variation observed for the Riesling wines.

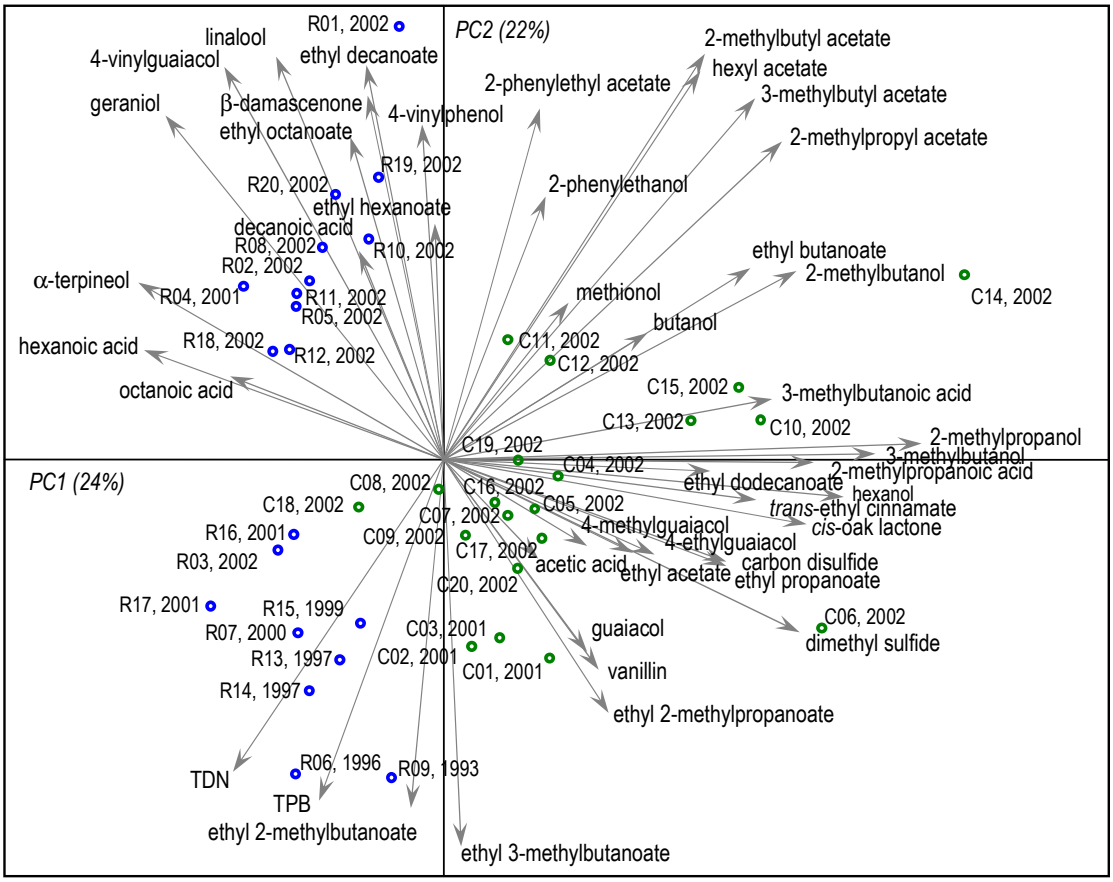
It may have been prudent to include some of the MLF or oak-influenced unwooded Chardonnay wines, from the preliminary screening, in the final set of 20 study wines. This would have resulted in a set of wines with broader sensory variation and the descriptive data obtained from sensory analysis would be more suited for the multivariate data analysis applied. Furthermore, the inclusion of these different styles of wine may have better represented the types of wine commercially available in this style of wine as oaked Chardonnay is the most common use for this grape variety in Australia. Nevertheless, an initial objective of this study was to focus only on those volatile compounds that were derived from the fruit, or from fermentation, so that the requirements for volatile chemical analysis would be simplified.

5.2.3 Comparison of volatile chemical data between varieties

The volatile compounds that were measured in each set of wines were almost identical with the exception of nerol, *cis*-rose oxide and 4-ethylphenol which were not detected in any of the unwooded Chardonnay wines and diacetyl which was not measured in the Riesling wines (due to instrumentation difficulties and time constraints). PCA was performed on the combined volatile data set including all wines, from both varieties, for the 44 compounds that were in common to both analyses. By PCA, 57% of variation was accounted for by the first three PCs. Plots of the PCA are given in Figure 5-5 and Figure 5-6.

Inspection of the PCA plot reveals that the first PC separated the wines according to variety with Riesling wines on the left and unwooded Chardonnay wines generally on the right of the plot (Figure 5-5 and Figure 5-6). This indicates that the most important difference in the volatile chemical data set was due to varietal influences. These differences are likely to be due to genetic and viticultural related differences but could also arise from variance in the winemaking style for these two wine varieties. Although both sets of wines are Australian commercial white wines, they clearly have distinct volatile profiles.

Figure 5-5 PCA bi-plot of volatile compounds measured in the Riesling and unwooded Chardonnay wines, PC1 versus PC2

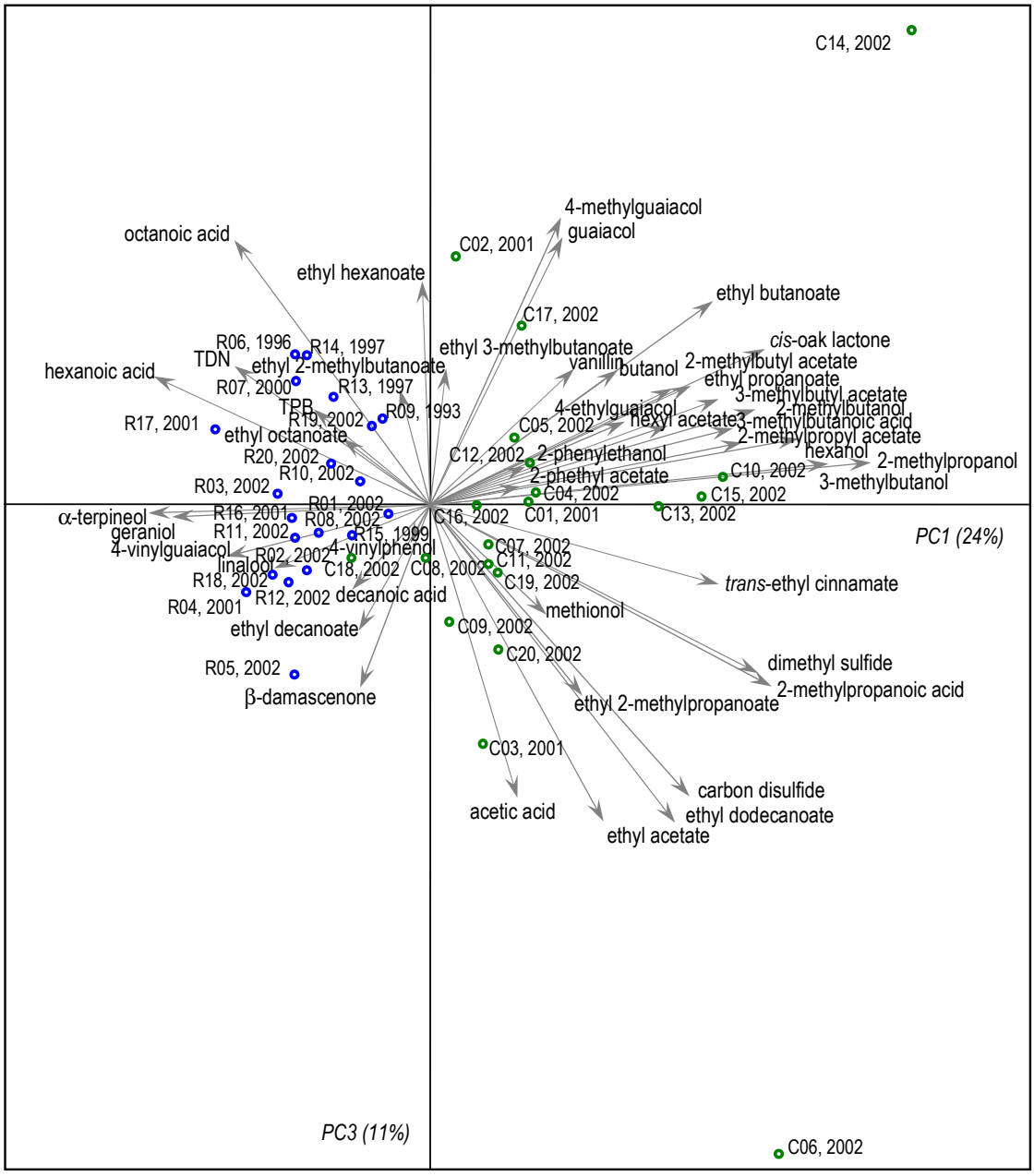


For details on the sample codes refer to Chapter 3, Table 3-1 and Chapter 4, Table 4-1, Riesling wines (in blue) are labelled by the sample code prefix R and unwooded Chardonnay wines (in green) by the prefix C. Volatile compound loadings (vectors) are shown.

The second PC generally separated the wines by age with both the older unwooded Chardonnay wines (2001) and older Riesling wines (2001 – 1993) appearing in the lower part of the plot and the younger wines (2002) in the upper part of the plot (Figure 5-5). It is interesting to note that that similar volatile differences, caused by wine ageing, were common to both varieties. Furthermore, the age-influenced differences can distinguish a wine of only one year additional maturation, as is seen in the case of the 2001 vintage unwooded Chardonnay wines.

PC3 was strongly influenced by unwooded Chardonnay wines 6 and 14 (Figure 5-6). The distinction of these two wines was also observed in the PCA of the 20 unwooded Chardonnay wines (refer to Section 4.2.2, Chapter 4). Overall the wines were not separated in the third PC by viticultural region, retail price, closure type, or other obvious variables.

Figure 5-6 PCA bi-plot of volatile compounds measured in the Riesling and unwooded Chardonnay wines, PC1 versus PC3



For details on the sample codes refer to Chapter 3, Table 3-1 and Chapter 4, Table 4-1, Riesling wines (in blue) are labelled by the sample code prefix R and unwooded Chardonnay wines (in green) by the prefix C. Volatile compound loadings (vectors) are shown.

The volatile data for the 2001 and 2002 vintage wines were also compared between the varieties. The older Riesling wines (2000 and older vintages) excluded from this analysis. Table 5-1 summarises the analytical results for the volatiles quantified for the 2001 and 2002 Riesling wines (n = 14) and the 2001 and 2002 unwooded Chardonnay wines (n = 20), by both concentration and odour activity values (in brackets).

Table 5-1 Comparison of Riesling and unwooded Chardonnay volatile chemical data for 2002 and 2001 vintage wines

	2001 and 2002 Riesling wines (n = 14)			2001 and 2002 Chardonnay wines (n = 20)		
	mean (µg/L)	minimum (µg/L)	maximum (µg/L)	mean (µg/L)	minimum (µg/L)	maximum (µg/L)
ethyl acetate	75109 (**10**)	49073 (**7**)	117130 (**16**)	85185 (**11**)	50282 (**7**)	212702 (**28**)
ethyl propanoate *	182 (0.1)	109 (0.06)	261 (0.1)	226 (0.1)	130 (0.1)	384 (0.2)
ethyl butanoate	469 (**23**)	311 (**16**)	599 (**30**)	548 (**27**)	309 (**15**)	1125 (**56**)
ethyl 2-methylpropanoate	64 (**4**)	44 (**3**)	102 (**7**)	82 (**5**)	40 (**3**)	247 (**16**)
ethyl 2-methylbutanoate	8 (**8**)	4 (**4**)	17 (**17**)	10 (**10**)	3 (**3**)	20 (**20**)
ethyl 3-methylbutanoate	16 (**5**)	8 (**3**)	32 (**11**)	22 (**7**)	10 (**3**)	43 (**14**)
ethyl hexanoate	1356 (**271**)	1159 (**232**)	1704 (**341**)	1228 (**246**)	920 (**184**)	1974 (**395**)
ethyl octanoate *	1940 (**970**)	1266 (**633**)	3219 (**1610**)	1492 (**746**)	1027 (**514**)	2323 (**1162**)
ethyl decanoate *	871 (**4**)	528 (**3**)	1449 (**7**)	570 (**3**)	362 (**2**)	847 (**4**)
ethyl dodecanoate *	81 (0.04)	26 (0.01)	160 (0.08)	176 (0.1)	60 (0.03)	812 (0.4)
trans-ethyl cinnamate *	0.9 (0.9)	0.2 (0.2)	2 (2)	3 (**3**)	0.6 (0.6)	6 (**6**)
2-methylpropyl acetate	33 (0.02)	7 (0.005)	58 (0.04)	42 (0.03)	3 (0.002)	119 (0.07)
2-methylbutyl acetate	90 (**18**)	nd	191 (**38**)	95 (**19**)	13 (**3**)	311 (**62**)
3-methylbutyl acetate	1895 (**63**)	80 (**3**)	3667 (**122**)	2430 (**81**)	269 (**9**)	7266 (**242**)
hexyl acetate	192 (0.3)	1 (0.001)	411 (0.6)	213 (0.3)	7 (0.01)	598 (0.9)
2-phenylethyl acetate	344 (**1**)	9 (0.04)	2059 (**8**)	212 (0.8)	33 (0.1)	583 (**2**)
2-phenylethanol	22639 (**2**)	9175 (**1**)	85393 (**9**)	18211 (**2**)	8620 (0.9)	43921 (**4**)
butanol	856 (0.006)	529 (0.004)	1473 (0.01)	934 (0.01)	353 (0.002)	2492 (0.02)
2-methylpropanol *	12661 (0.3)	9390 (0.2)	18784 (0.5)	21456 (0.5)	13024 (0.3)	37238 (0.9)
2-methylbutanol	18737 (0.3)	12511 (0.2)	30606 (0.5)	21538 (0.3)	12425 (0.2)	34301 (0.5)
3-methylbutanol	95554 (**3**)	66767 (**2**)	105628 (**4**)	136016 (**5**)	83686 (**3**)	187727 (**6**)
hexanol *	1451 (0.2)	904 (0.1)	2254 (0.3)	2546 (0.3)	939 (0.1)	4814 (**1**)
acetic acid [a]	335000 (**2**)	180000 (0.9)	620000 (**3**)	368947 (**2**)	210000 (**1**)	630000 (**3**)
2-methylpropanoic acid *	501 (0.003)	339 (0.002)	771 (0.004)	700 (0.004)	386 (0.002)	1705 (0.009)
3-methylbutanoic acid	295 (0.1)	221 (0.07)	460 (0.2)	337 (0.1)	122 (0.04)	609 (0.2)
hexanoic acid *	8116 (**3**)	5324 (**2**)	10001 (**3**)	5217 (**2**)	3926 (**1**)	7599 (**3**)
octanoic acid *	9074 (**18**)	7578 (**15**)	11230 (**22**)	7920 (**16**)	5387 (**11**)	11014 (**22**)
decanoic acid *	2673 (0.2)	1696 (0.1)	3670 (0.2)	2304 (0.2)	1413 (0.1)	3120 (0.2)
linalool *	64 (**4**)	2 (0.1)	160 (**11**)	7 (0.5)	0.6 (0.04)	13 (0.9)
α-terpineol *	73 (0.3)	39 (0.2)	117 (0.5)	10 (0.04)	4 (0.02)	32 (0.1)
nerol	8 (0.02)	3 (0.01)	26 (0.05)	nd	nd	nd
geraniol *	19 (0.6)	7 (0.2)	36 (**1**)	2 (0.07)	nd	4 (0.1)
cis-rose oxide	0.2 (0.8)	0.06 (0.3)	0.3 (**1**)	nd	nd	nd
TDN *	10 (0.5)	1 (0.07)	56 (**3**)	1 (0.05)	0.3 (0.02)	4 (0.2)
TPB [b]	0.008 (0.2)	0.002 (0.05)	0.03 (0.7)	0.008 (0.2)	0.003 (0.08)	0.02 (0.5)
β-damascenone *	4 (**79**)	2 (**31**)	7 (**132**)	2 (**40**)	0.8 (**16**)	4 (**80**)
guaiacol *	0.3 (0.03)	0.2 (0.02)	0.7 (0.07)	0.9 (0.1)	0.2 (0.02)	3 (0.3)
4-methylguaiacol	0.1 (0.002)	0.03 (0.0004)	0.3 (0.004)	0.3 (0.005)	nd	3 (0.05)
4-ethylphenol	0.2 (0.002)	nd	1 (0.01)	nd	nd	nd
4-ethylguaiacol *	0.04 (0.0006)	nd	0.2 (0.003)	0.2 (0.003)	nd	0.7 (0.01)

	2001 and 2002 Riesling wines (n = 14)			2001 and 2002 Chardonnay wines (n = 20)		
	mean (µg/L)	minimum (µg/L)	maximum (µg/L)	mean (µg/L)	minimum (µg/L)	maximum (µg/L)
4-vinylguaiacol *	0.2 (0.0006)	0.05 (0.0001)	0.5 (0.001)	0.05 (0.0001)	0.005 (0.0001)	0.1 (0.0002)
4-vinylphenol *	0.4 (0.0005)	0.02 (0.00003)	0.9 (0.001)	0.3 (0.0004)	0.02 (0.00003)	1 (0.001)
***cis*-oak lactone** *	0.9 (0.04)	0.02 (0.0009)	4 (0.16)	14 (0.6)	3 (0.1)	36 (**2**)
vanillin *	1 (0.006)	nd	9 (0.04)	15 (0.08)	3 (0.02)	81 (0.4)
diacetyl [b]	m	m	m	55 (0.6)	nd	233 (**2**)
methionol	461 (0.9)	285 (0.6)	1061 (**2**)	467 (0.9)	258 (0.5)	1010 (**2**)
dimethyl sulfide *	15 (**1**)	4 (0.4)	25 (**3**)	127 (**13**)	31 (**3**)	350 (**35**)
carbon disulfide *	0.3 (0.07)	nd	0.6 (0.1)	3 (0.6)	0.7 (0.1)	17 (3)

[a] acetic acid measured as volatile acidity; [b] for Chardonnay wines measurement made in 18 wines; * indicates statistically significant difference between varieties (ANOVA, $p < 0.05$); OAV given in brackets, OAV > 1 in bold typeface; compound not detected (nd), missing data (m).

The unwooded Chardonnay wines were typically higher in concentration of fermentation-derived ethyl esters, acetates and alcohols, with the exception of ethyl hexanoate, ethyl octanoate, ethyl decanoate, 2-phenylethyl acetate and 2-phenylethanol which were higher in the young Riesling wines. The unwooded Chardonnay wines were higher in some fermentation-derived acids, including acetic acid, 2-methylpropanoic acid and 3-methylbutanoic acid, while the Riesling wines were higher in others, including hexanoic acid, octanoic acid and decanoic acid. The unwooded Chardonnay wines had a broader range of concentrations for most of the fermentation-derived volatiles than the Riesling wines with the exception of 3-methylbutyl acetate, acetic acid, hexanoic acid, decanoic acid, 2-phenylethyl acetate, and 2-phenylethanol. This might indicate the strong influence that the fermentation-derived volatiles have on the aroma differences between the unwooded Chardonnay wines. This could also be a reflection of the fact that there are 20 unwooded Chardonnay wines being compared to a pool of only 14 Riesling wines from the 2002 and 2001 vintages.

The Riesling wines were significantly higher in concentration, and had broader concentration ranges, for all of the monoterpenes and the norisoprenoids TDN and β-damascenone (ANOVA, $p < 0.05$). This might indicate that the grape-derived volatiles play a more important role in the aroma differences in Riesling wines than for unwooded Chardonnay.

The unwooded Chardonnay wines were significantly higher in concentration and had broader ranges of concentration for *cis*-oak lactone, vanillin, the guaiacols and phenols. Exceptions to this were 4-vinylguaiacol, which had both higher concentrations and a broader concentration range in the Riesling wines, and 4-vinylphenol, which had quite similar concentrations in both varieties. This highlights the potential importance of compounds more usually associated with oak contact, guaiacols and phenols to the differences in aroma properties of the commercial 'unwooded' Chardonnay wines.

Similar concentrations of methionol were found between the two varieties (of 2002 and 2001 vintage). The compounds dimethyl sulfide and carbon disulfide were higher in concentration in the unwooded Chardonnay wines and had higher standard deviations. It should be noted, however, that the concentration of dimethyl sulfide was much greater, than all of the unwooded Chardonnay wines, in some of the older Riesling wines in the study (for more details refer to Section 3.2.2, Chapter 3). Nevertheless, these results show that these sulfur-containing compounds may be potentially important for the variation in sensory properties of the unwooded Chardonnay wines.

There are a number of compounds that have been reported in the literature to be important contributors to the aroma of white wine but have not been measured in the present work. In particular, 4-mercapto-4-methylpentan-2-one [6, 48, 49], 3-mercaptohexanol [48, 49], 3-mercaptohexyl acetate [48, 49], and wine lactone [6] have all been implicated as very important to the aroma of white wine and were not measured in the study wines. Various other compounds, such as HDMF (3-hydroxy-4,5-dimethyl-2(5H)-furanone) [6, 60], methional [224], and 2-methoxy-3-isobutylpyrazine [43], have also been implicated as important to the aroma of some white wines, but were not measured in the wine in this study because of the absence of suitable analytical methods in this laboratory. Analytical methods for these volatile compounds were also not available to us at the time of writing. It is important to acknowledge the potential deficiencies in the volatile chemical data to allow more realistic interpretation of the results from the prediction of sensory attributes using the volatile chemical data at hand.

5.2.4 Relationships between sensory and wine composition for each variety

5.2.4.1 The relationship between aroma intensity and wine composition

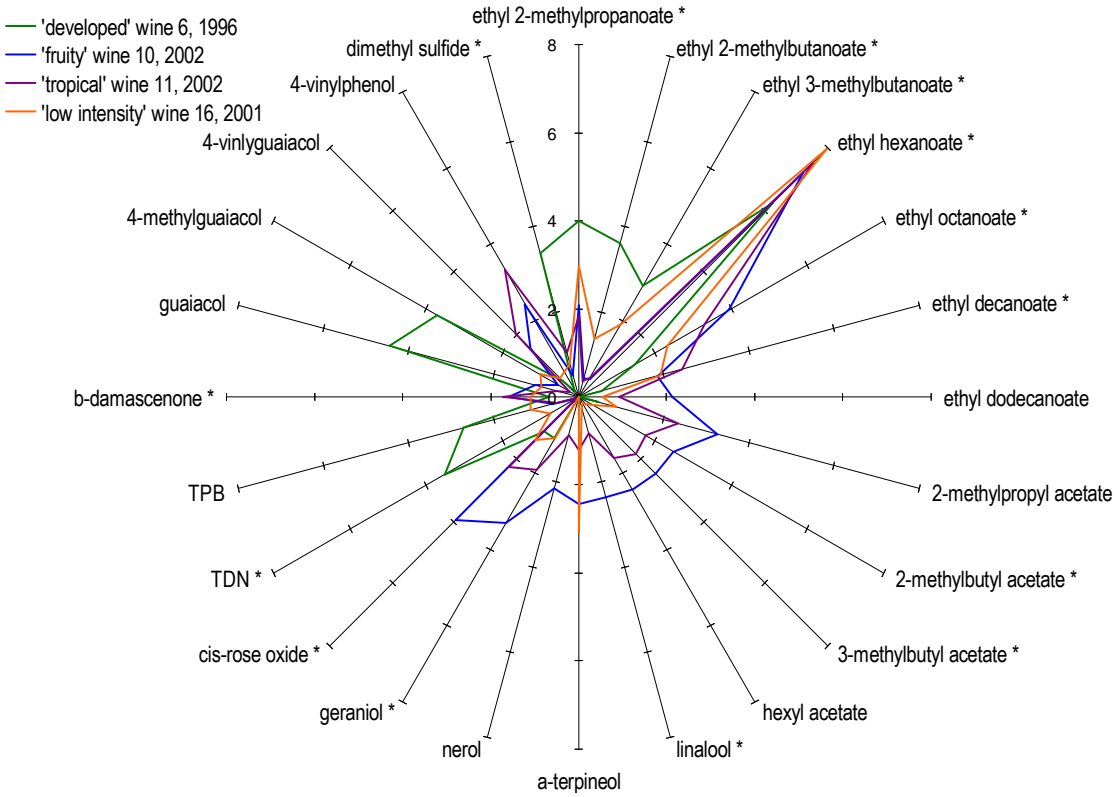
To examine the possible compositional relationship with overall aroma intensity, for the wines in this study, the volatile composition of wines of low and high intensity were compared for each variety. The volatile composition of selected Riesling wines with high 'developed' (wine 6), 'fruity' (wine 10) and 'tropical' (wine 11) aroma intensities, together with a wine of low intensity in all attributes (wine 16), are shown in Figure 5-7. Figure 5-8 shows the volatile composition for examples of unwooded Chardonnay wines with high 'developed' (wine 2), 'tropical' (wine 6) and 'fruity' (wine 12) aroma intensities and a wine of low aroma intensity (wine 10). For each variety, only those compounds which were found to be most important to the PLS models developed for the prediction of sensory attributes are shown.

For both varieties, the lower intensity wines were not unusually high or low in pH, alcohol content, titratable acidity or free and total SO_2 and it is therefore unlikely that these wine

constituents were responsible for masking the perception of aroma for these wines. Wines of low overall aroma intensity did not typically have lower concentrations for all (or most) of the volatile compounds measured and overall did not have generally more sub-threshold concentrations of volatile compounds. Furthermore, low intensity wines were not lower in concentration for all of the most important aroma compounds. For example, one of the lowest aroma intensity unwooded Chardonnay wines (wine 10, Figure 5-8) had the fourth highest concentration (of the 20 unwooded Chardonnay wines analysed) of the compound β-damascenone which was found to be important to the prediction of a number of sensory properties for this variety.

As shown in Figure 5-7, a Riesling wine of low overall aroma intensity (wine 16) had among the highest concentration of ethyl hexanoate, ethyl decanoate, and α-terpineol. Wine 16 had lower concentrations of ethyl octanoate and ethyl dodecanoate, all of the acetates, linalool, geraniol, *cis*-rose oxide, β-damascenone, 4-vinylphenol and 4-vinylguaiacol compared to the 'fruity' and 'tropical' wines (wine 10 and 11). Compared to the high intensity 'developed' Riesling wine (wine 6), the low intensity wine (wine 16) had lower concentrations of TDN, TPB, guaiacol, 4-methylguaiacol, dimethyl sulfide, ethyl 2-methylpropanoate, ethyl 3-methylbutanoate and ethyl 2-methylbutanoate. In general, the low intensity Riesling wine was neither high in concentration for all of those compounds important to the aroma of a high intensity 'fruity' or 'tropical' wine or high in concentration for all of those compounds important to the aroma of a high intensity 'developed' Riesling wine. Instead, wine 16 appeared to be high in only a small number of compounds that were either important to the aroma of a 'fruity' wine (e.g. ethyl hexanoate) or compounds that were important to the aroma of a 'developed' Riesling wine (e.g. ethyl 2-methylpropanoate). It might be that this wine, from the 2001 vintage (1.5 years old), is in transition between being a wine with 'fruity' or 'tropical' characters and a wine that has more 'developed' characters. These results indicate (Figure 5-7) that wine 16 might have decreased in concentration for those compounds important to an intensely 'fruity' wine, and may be just starting to increase in concentration for the compounds important to an intensely 'developed' wine.

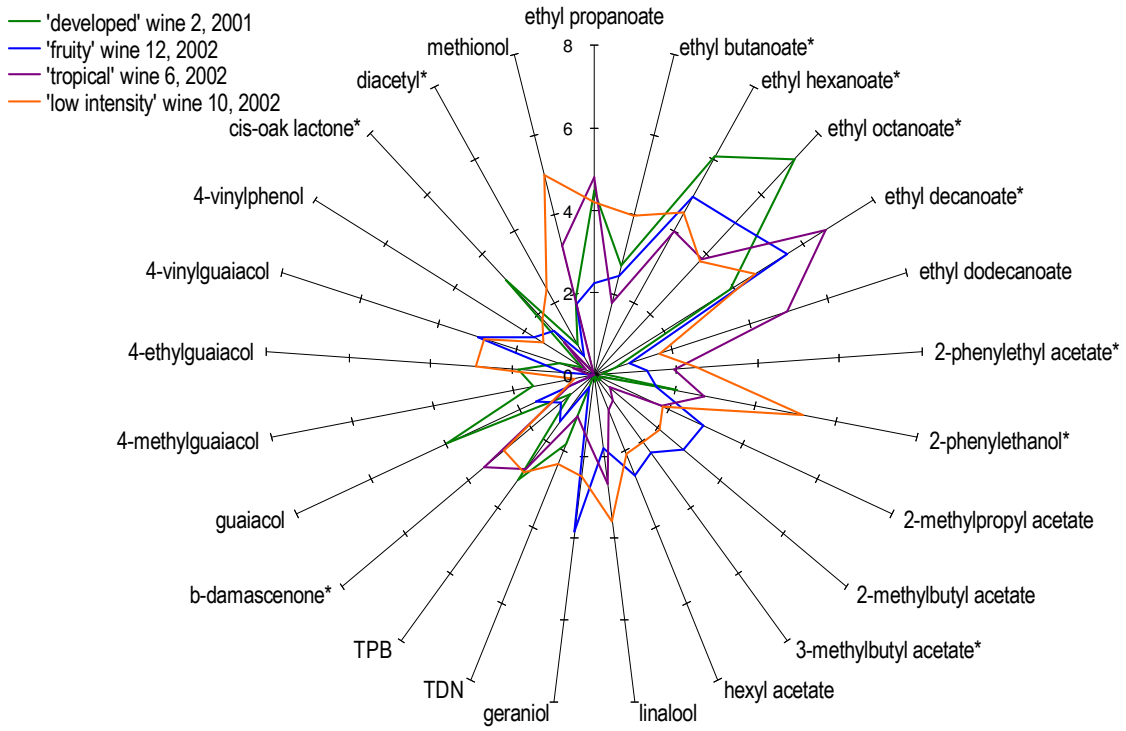
Figure 5-7 Volatile composition of selected Riesling wines with varying aroma intensities



For details on the sample codes refer to Chapter 3, Table 3-1. Compositional data are autoscaled (1/standard deviation); * indicates compounds measured above sensory threshold in at least one of the Riesling wines analysed.

For the unwooded Chardonnay wines (Figure 5-8), the low aroma intensity wine (wine 10) was higher in concentration of ethyl butanoate, 2-phenylethanol, linalool, TDN, 4-ethylguaiacol, diacetyl and methionol, and lower only in ethyl octanoate, than the high aroma intensity wines (wines 2, 6 and 12). Unlike the Riesling wines, no obvious pattern was observed from the compositional data that differentiated the low intensity wine from the higher intensity wines. This is further evidence that there may be volatile compounds important to the aroma of these unwooded Chardonnay wines that were not measured in this study. Alternatively, the aroma intensity of unwooded Chardonnay wine might arise from a complex balance between numerous volatile aroma compounds, rather than a generally low concentration of a number of key volatile compounds.

Figure 5-8 Volatile composition of selected unwooded Chardonnay wines with varying aroma intensities



For details on the sample codes refer to Chapter 4, Table 4-1; compositional data are autoscaled (1/standard deviation); * indicates compounds measured above sensory threshold in at least one of the unwooded Chardonnay wines analysed.

5.2.4.2 Prediction of sensory properties using compositional data

The objective of the PLS models developed for each variety (in Chapter 3 and Chapter 4) was to use this multivariate method to explore the possible relationships between wine composition and the sensory perception of wine and not to develop predictive equations suitable for routine application.

The methodology used for multivariate analysis in Chapter 3 (Section 3.2.3.2) and Chapter 4 (Section 4.2.3.2) was slightly different for each variety. Although the jack-knifing (JK) technique of variable selection worked quite well for the Riesling wine data, it did not perform so well for the unwooded Chardonnay wine data where an iterative backward elimination process was used to enable reasonable interpretations to be made. As discussed in Section 5.2.2 the variation in sensory properties between the unwooded Chardonnay wines was small and attributes were typically rated with poor judge agreement in comparison to the Riesling wines. This might have resulted in the JK technique to unduly identify *x*-variables as unstable in the unwooded Chardonnay data set, leading to elimination of potentially meaningful information. The higher variation and better judge agreement observed in the Riesling sensory data was aided by the fact that older 'reserve' vintage wines were also included in the Riesling set which increased the sensory differences observed between the wines.

The calibration statistics for the final set of PLS models developed for the Riesling aroma attributes ranged in R^2 (0.29 – 0.98), RMSEP (0.17 – 0.87) and used 2 – 7 *x*-variables. The optimal number of components used for all of the Riesling models was one (C_{opt}) (refer to Section 3.2.3.2, Chapter 3). Of the 16 aroma attributes modelled with PLS for Riesling, 13 were found to have significant models (F value, $p < 0.05$). In comparison, the calibration statistics of the models for the unwooded Chardonnay aroma attributes ranged in R^2 (0.26 – 0.90), RMSEP (0.16 – 1.30) and used 3 – 11 *x*-variables and between 1 and 6 components (C_{opt}) (refer to Section 4.2.3.2, Chapter 4). Of the 13 aroma attributes modelled for the unwooded Chardonnay wines, only four were found to have significant models. Overall the models for the Riesling wine attributes performed much better in multivariate data analysis than for the unwooded Chardonnay wines. For this reason, more confident interpretations can be made for the Riesling wines than for the unwooded Chardonnay wines.

A summary of the volatile compounds (including grouped variables) that were identified in the final PLS models developed to predict the aroma properties of each variety are tabulated in Table 5-2. The compounds listed are in order of frequency of use for both positively or negatively loaded compounds in the PLS model. For more information on the compounds included in the grouped variables, or the PLS models themselves, refer to Chapter 3 and Chapter 4.

For the sake of this comparison, the grouped variables that were used in the models are given in Table 5-2, rather than a list of individual compound's names. This is because the importance of some compounds may be exaggerated due to their involvement in a grouped variable. For example, in the unwooded Chardonnay wines the compounds ethyl propanoate and ethyl dodecanoate were always measured below sensory threshold, yet they were included in the esters(6) variable for multivariate analysis. Their weight on the combined variable was minimal, but as the variable itself was found to be important to the models these individual compounds were also highlighted as important. It may be that individually, ethyl propanoate or ethyl dodecanoate are not particularly important to the aroma of the unwooded Chardonnay wines and that their exclusion from a reconstructed aroma 'model' may make little or no difference to the aroma of that 'model' system. Alternatively, it is possible that these esters, in combination with the other ethyl esters, may be additive in generating aroma in the wine. The importance of these individual esters can only be confirmed through sensory reconstruction experiments.

All of the compounds that were used in the PLS models for the Riesling wine were also used in the models for the unwooded Chardonnay wine with the exception of *cis*-rose oxide (not detected in unwooded Chardonnay). This might indicate that generally the same compounds

are responsible for the aroma of each variety, and that the variation in concentration of those compounds gives rise to the characteristic aroma nuances of each variety. Additional compounds were also used in the PLS models for the unwooded Chardonnay aroma attributes. These included the fermentation-derived isoacids(2), acids(3), *trans*-ethyl cinnamate and 2-phenylethyls(2), oak-derived *cis*-oak lactone, vanillin, 4-ethylguaiacol, MLF-derived diacetyl and the sulfur-containing methionol and carbon disulfide. Some of these compounds were below sensory threshold and may not be indicators of causative relationships. Others are unique to the style of wine and might be important to the characteristic aromas of unwooded Chardonnay wine.

Table 5-2 Volatile compounds used in the final PLS models for each variety

Riesling wine		unwooded Chardonnay wine	
positively loaded compounds	negatively loaded compounds	positively loaded compounds [b]	negatively loaded compounds [b]
monoterpenes(3)	**isoesters(3)**	**acetates(4)***	TDN*
guaiacols(2)	**TPB**	TDN*	***cis*-oak lactone***
TPB (*and* ***TDN***) [a]	**esters(5)**	***cis*-oak lactone***	monoterpenes(2)*
acetates(4)	4-vinyls(2)	**carbon disulfide***	**diacetyl***
esters(5)	**TDN**	α-terpineol*	**isoesters(3)***
isoesters(3)	guaiacols(2)	4-vinyls(2)*	α-terpineol
β-damascenone	**monoterpenes(3)**	***trans*-ethyl cinnamate**	**β-damascenone**
dimethyl sulfide	**β-damascenone**	**2-phenylethyls(2)**	vanillin*
***cis*-rose oxide**	nerol	**esters(6)***	**ethyl acetate**
α-terpineol	**acetates(4)**	guaiacols(2)*	**acids(3)**
4-vinyls(2)		TPB	**dimethyl sulfide**
		β-damascenone*	**carbon disulfide**
		vanillin*	**acetic acid**
		isoacids(2)	4-ethylguaiacol*
		monoterpenes(2)*	***trans*-ethyl cinnamate***
		methionol*	**2-phenylethyls(2)**
		dimethyl sulfide	4-vinyls(2)*
		4-ethylguaiacol*	
		diacetyl*	
		isoesters(3)	
		acids(3)	

[a] as discussed in Chapter 3, TDN and TPB were highly collinear and were relatively interchangeable in the PLS models so neither could be disqualified from being important (refer to Section 3.2.3.2, Chapter 3); [b] compounds labelled with * were identified as significant by JK; bold typeface indicates compounds, or variables that contained compounds, that were above sensory threshold in at least one of the wines analysed.

The most commonly used positively loaded group for the Riesling wines was the monoterpenes(3) and for the unwooded Chardonnay wines the acetates(4). This indicates that the grape-derived monoterpenes are of greater importance to the aroma of Riesling wine whereas the fermentation-derived esters are of greater importance to unwooded Chardonnay wine aroma. The acetates(4) were also of high importance to the models developed for the Riesling aroma attributes. On the other hand, the monoterpenes(2) were far less frequently used in the unwooded Chardonnay PLS models and as they were generally measured below

sensory threshold concentration, it is likely that that they are not of particular importance to the aroma of this variety.

For both varieties the more 'developed' attributes (e.g. *honey*, *caramel*, *toasty*, *kerosene*, *woody*, *spicy*) had better calibration statistics than the 'fresh', 'fruity' and 'floral' attributes (e.g. *estery*, *floral*, *lemon*, *citrus*, *pineapple*, *lychee*). This result might suggest that panellists were able to clearly differentiate between 'developed' attributes, but were not able to clearly distinguish between the numerous 'fresh', 'fruity' and 'floral' attributes that were rated in the descriptive studies. Fewer 'fruity' attributes might have improved the sensory results and hence the power of the models. Alternatively, it might be that the compounds responsible for the 'developed' characters in wine have a more straightforward relationship with the aroma nuances they contribute to, and are not easily influenced by masking effects of other compounds. Although PLS models developed for the 'developed' attributes often used a larger number of *x*-variables, these *x*-variables were usually single aroma compounds, rather than grouped variables containing numerous volatile compound concentrations as for the 'fresh', 'fruity' and 'floral' attributes. Furthermore it is possible that the additive contribution of the acetates, esters and monoterpenes is far more complex than could be simplified in a single representative 'grouped variable', and the calculation of the 'grouped variable' might have contributed to the reduced predictive ability of the models developed for the 'fresh', 'fruity' and 'floral' attributes.

Some similar compounds were found to be important to comparable aroma properties between the two varieties. The *estery* attribute in both the Riesling and unwooded Chardonnay wines used the positive contribution of the acetates (3-methylpropyl acetate, 2-methylbutyl acetate, 3-methylbutyl acetate and hexyl acetate) among other different compounds. The *floral* (or *perfumed floral* for the Riesling wines) was also predicted using the positive contribution of these acetates and the negative (for unwooded Chardonnay) and positive (for Riesling) contribution of β-damascenone in both varieties. Interestingly, β-damascenone was used for the prediction of the *floral* attributes in an opposite manner between the two varieties which indicates that this compound may be playing a different role in the aroma of Riesling and unwooded Chardonnay wine. The *lychee* attribute was predicted in both varieties using the positive contribution of the monoterpenes (geraniol and linalool) and the *honey* attribute was predicted in both varieties using the positive contribution of TPB. The *caramel* (or *butterscotch* for the unwooded Chardonnay) was predicted by both varieties using the positive contribution of TPB and the guaiacols (4-methylguaiacol and guaiacol) and the negative (for Riesling) and positive (for unwooded Chardonnay) contribution of the esters (ethyl butanoate, ethyl hexanoate, ethyl decanoate, ethyl octanoate and ethyl dodecanoate). The similarity in the variables loaded in the prediction of

comparable attributes indicates that some compounds may be responsible for similar aroma nuances in different wine varieties. This result also is good evidence that the PLS models might be identifying some real cause and effect relationships.

For both varieties, the collinear attributes *passionfruit* and *herbaceous* (and *sweaty* for the unwooded Chardonnay wines) were very poorly predicted using the volatile chemical data and it is likely that the compounds responsible for these attributes have not been measured in the study wines. At low concentration, the sulfur-containing compounds 4-mercapto-4-methylpentan-2-one, 3-mercaptohexanol, 3-mercaptohexyl acetate are thought to contribute 'tropical', 'passionfruit' and 'grapefruit' aromas to wine at low concentrations [49]. It is highly likely that the absence of compositional data for these compounds has resulted in poor predictions for the *passionfruit* attribute. This result highlights the need to measure these compounds in the wines in this study and indicates that these sulfur-containing compounds are likely to be crucial contributors to the aroma differences observed between the wines for both the Riesling and unwooded Chardonnay wine data sets. Additionally, the poor predictions, resulting from missing compositional data, demonstrate that the models developed in this study are not overfitting the chemical data (i.e. forcing models to be built from redundant information). The poor predictions also indicate that the predictions developed for the other attributes rated do actually rely on volatile compounds that have a causative relationships with the sensory attributes.

Several factors could improve the PLS models generated for the two studies, including a greater number of wine samples, fewer 'similar' sensory attributes used in the descriptive study, more training for the sensory panel, and the inclusion of data for important compounds missing from the volatile data set. Most importantly, the interpretation of models would be tremendously improved if the prediction ability of the models could be tested by an independent validation set. Alternatively, sensory reconstruction experiments involving the important volatile compounds identified in this study could support and improve the interpretations made from the prediction models. Due to time constraints, the analysis (sensory and volatile) of a validation set of wines could not be included in this study, and sensory reconstitution studies were also not conducted. These studies will be the topic of on-going research.

5.3 Conclusion

Fermentation-derived compounds were relatively high in concentration and had a large influence on the sensory properties of unwooded Chardonnay. On the other hand, the grape-derived compounds were relatively high in concentration and were of greater importance to the aroma attributes of Riesling wine which agrees with published reports [70].

Fermentation-derived compounds were also important to the prediction of Riesling wine aroma, but perhaps secondary after the grape-derived monoterpenes. The sulfur-containing compounds measured in this study were found to be of greater importance to the variation in the aroma properties of unwooded Chardonnay wines than for the Riesling wines, with the exception of dimethyl sulfide, which was found to be of importance to the prediction of the 'developed' aromas of Riesling wine.

It is obvious from this study that the compounds responsible for the *passionfruit* and *herbaceous* aromas in both varieties have not been measured. These compounds are likely to be the same for both varieties and probably include the sulfur-containing compounds (e.g. 4-mercapto-4-methylpentan-2-one, 3-mercaptohexanol, 3-mercaptohexyl acetate). Measurement of these compounds is expected to improve the prediction of these attributes, especially considering they were attributes scored by the sensory panel with high variation between different wines and with excellent panel agreement.

Overall, many of the same compounds were used to explain the aroma of both varieties, indicating that the aroma of different white wine varieties is partly due to the same volatile compounds but present at different concentrations. Additional compounds were also implicated as being important to the aroma of unwooded Chardonnay that did not appear to be important to the aroma of Riesling wine. These included compounds specific to that style of wine, for example oak-derived and MLF-derived compounds.

This study has increased our knowledge about those compounds that are likely to be playing an important role in the characteristic aroma of both Riesling and unwooded Chardonnay wine. Nevertheless, these results need to be tested to confirm causative relationships between volatile aroma compounds and the perception of specific aroma nuances in wine.

Chapter 6 Prediction of wine sensory properties using rapid instrumentation

6.1 Introduction

To enable the wine industry to rapidly respond to the changing demands of both consumers and the market, it is important to have a quantitative means for assessing sensory properties. Methods that can be used for wine quality assessment include objective measurements (e.g. analysis of volatile compounds) or more subjective measurements (e.g. sensory analysis), which can provide reliable information about the quality of the wine. However, many of these methods are unsuitable to be used or adopted by the wine industry for rapid analysis of wine quality. For example, analysis of volatile compounds in wine to assess wine aroma by gas chromatography-mass spectrometry (GC-MS) involves expensive instrumentation and time consuming sample preparation using solvents. Sensory analysis using a trained panel is often used in assessing wine quality characteristics, however, this method is also time consuming and expensive.

Rapid screening techniques to determine quality characteristics of foods and beverages are of great interest to the food industry. These techniques are relatively inexpensive, easy to operate, often require little or no sample preparation, can be used in-line or at-line to give results quickly. Two techniques which are both commonly used in the food industry for rapid analysis are the electronic nose (Enose), and visible (VIS) and near infrared (NIR) spectroscopy.

The Enose, and more recently the electronic tongue, were developed to characterise complex food or beverage samples in the hope that they might replace sensory analysis using human subjects for routine assessment. Broadly, an Enose usually involves gas sensors or detectors while an electronic tongue consists of liquid sensors. There are many different types of Enose instruments available on the market including Enoses based on metal oxide sensors, conducting polymer sensors, quartz crystal membrane sensors, or mass spectrometers [225-227]. It is not the objective of this chapter to give an exhaustive compilation of these different types of Enose sensors.

Enose technology is used to measure the headspace of a food or beverage sample to obtain a ‘fingerprint’ measurement of the volatiles in the headspace. The headspace ‘fingerprint’ contains information directly or indirectly related to the volatile compounds which may be responsible for the aroma sensory properties of that sample. In recent years, a number of food and beverage studies have been published that demonstrate the relationship between

Enose measurements and the rating of sensory properties by sensory panellists. For example, relationships have been found between Enose and the sensory properties of tomatoes (e.g. for *sourness*, *grassy/green* flavour) [228], for yerba mate (i.e. *Ilex paraguariensis* infusion) [229] and for apple juices [230]. A benefit of mass spectrometry based (MS) Enose over other Enose sensors is that it detects mass fragments formed during ionisation of volatile compounds. Some of these volatiles can be directly responsible for the sensory differences between samples and measuring the mass fragments of these compounds can provide some understanding of the chemical basis for sensory differentiation [231]. Furthermore, MS Enose is based on the very wellknown and commonly used technology of mass spectrometry and the stability, sensitivity and reproducibility of this technique has long been established [232].

Spectroscopy is becoming a more attractive analytical technique for measuring quality parameters in food and beverages with decreasing instrument prices and improved equipment and data analysis techniques [233]. The main advantages of using spectroscopic techniques are rapid sample data acquisition, the possibility of simultaneous determination of several quality parameters and the ability to replace expensive and time consuming reference techniques such as chemical and sensory analysis [234-236]. Among spectroscopic techniques, near infrared (NIR) spectroscopy has been used as a method to predict quality determining parameters of different foods and agricultural products due to the speed of analysis, minimal sample preparation and low cost [237, 238]. Most of the established NIR methods involve the development of calibrations for the quantitative prediction of food components such as protein, moisture and fat. In general terms, NIR technology assesses organic chemical structures containing O-H, N-H and C-H bonds through the absorption of energy in the NIR region of the spectrum [237, 238]. The NIR spectrum of any organic material can give a global signature or 'fingerprint' of composition which can be used to elucidate particular compositional characteristics in the food matrix not easily detected by targeted chemical analysis [239, 240]. This opens the possibility of using NIR spectra to determine attributes of foods such as quality scores or even sensory characteristics [237, 240].

Enose and NIR, together with multivariate data analysis techniques, have been used to predict various sensory properties of foods and beverages. Some examples are given in Table 6-1. Very few examples were found in the literature that use either Enose or NIR to predict the aroma properties of wine (e.g. [241]) and no published studies could be found where the combination of these two techniques (Enose and NIR) was used to predict sensory properties of foods or beverages.

The matrix of wine is made up of a complex mixture of chemicals including water, alcohol, phenolic compounds, organic acids, volatile aroma compounds and sugars, all of which can contribute to the sensory characteristics of a wine [242, 243]. Sensory properties of wine rarely arise from just one or two of these components but from numerous components in the wine matrix [80]. The components responsible for wine aroma and flavour are not only involved in complex interactions with each other, resulting in masking and additive effects, but also are involved in complex interactions with non-flavour active components in the wine matrix (e.g. water, alcohol, non-volatile compounds). Due to these complex interactions, it might not be simple to obtain a robust prediction of the sensory properties of a wine through the measurement of just a small number of wine components without taking into account the matrix of wine as a whole.

Table 6-1 Multivariate methods used to predict sensory properties of foods and beverages using VIS-NIR or Enose

instrumental method	multivariate method	matrix	prediction
Enose	PCA	cod roe [244]	aroma and flavour properties (e.g. *egg flavour*, *seaweed odour*, *metallic flavour*, *sweet taste*)
Enose	PLS	soy sauce [245]	aroma properties (e.g. *alcoholic*, *fishy*)
Enose	PLS	Italian red wine [241]	aroma and flavour properties (e.g. *ruby red*, *spiced clove*, *jam*, *astringency*, *persistence*)
Enose	ANOVA-PLS	pork meat [246]	aroma and flavour properties (e.g. *sweet*, *cardboard*, *green*, *bitter*, *livery*)
MS Enose	PCA	cheddar cheese [231]	aroma and flavour properties (e.g. *nutty*, *fruit*, *brothy*, *egg-like*, *catty*)
NIR	PLS	beef meat [247]	sensory properties (e.g. *tenderness*, *juiciness*, *flavour*, *texture*, *chewiness*, *acceptability*)
NIR	PCR	beef meat [248]	sensory properties (e.g. *hardness*, *tenderness*)
NIR	PLS	different fish species [249]	sensory properties (e.g. *odour*, *appearance*, *taste* and *texture*)
NIR	PLS	sausages [250]	aroma and flavour properties (e.g. *odour*, *flavour intensity*, *juiciness*, *off-flavour*, *flavour of smoke*)
NIR	PLS	coffee [251]	aroma and flavour properties (e.g. *acidity*, *body*, *bitterness*, *aftertaste*)
NIR	PLS	cheese [252]	consistency (e.g. *springy*, *sticky*, *soluble*, *hard*) and flavour properties (e.g. *cheesy*, *acid*, *sweet*, *unclean*)
NIR	PLS	apple varieties [253]	sensory properties (e.g. *roughness*, *crunchiness*, *mealiness*, *sweet taste*, *sour taste*)
VIS-NIR	PLS	red and fortified wines [254]	overall 'quality grade'

PCA: principal component analysis; PCR: principal component regression; PLS: partial least squares regression; ANOVA: analysis of variance

The use of spectroscopic techniques such as VIS-NIR allow for a measurement of the whole wine matrix to be made, while MS Enose allows a measurement of the 'fingerprint' of the volatile headspace of wine to be made. In combination, these complementary techniques (MS Enose and VIS-NIR) might provide a powerful tool to predict the sensory properties of wine.

In this chapter the potential of using visible (VIS) and near infrared (NIR) spectroscopy and mass spectrometry (MS) based electronic nose (Enose), both as individual techniques and in combination, to predict sensory attribute scores in Riesling and unwooded Chardonnay wine is explored.

6.2 Results and discussion

Riesling and unwooded Chardonnay wine (2 varieties x 20 wine labels x 3 replicates) that had been analysed by sensory descriptive analysis (refer to Section 3.4.2, Chapter 3 and Section 4.4.2, Chapter 4) were analysed by MS Enose (*m/z* 50 – 180) and scanned by VIS-NIR (400 – 2500 nm).

6.2.1 Mass spectrometry based electronic nose

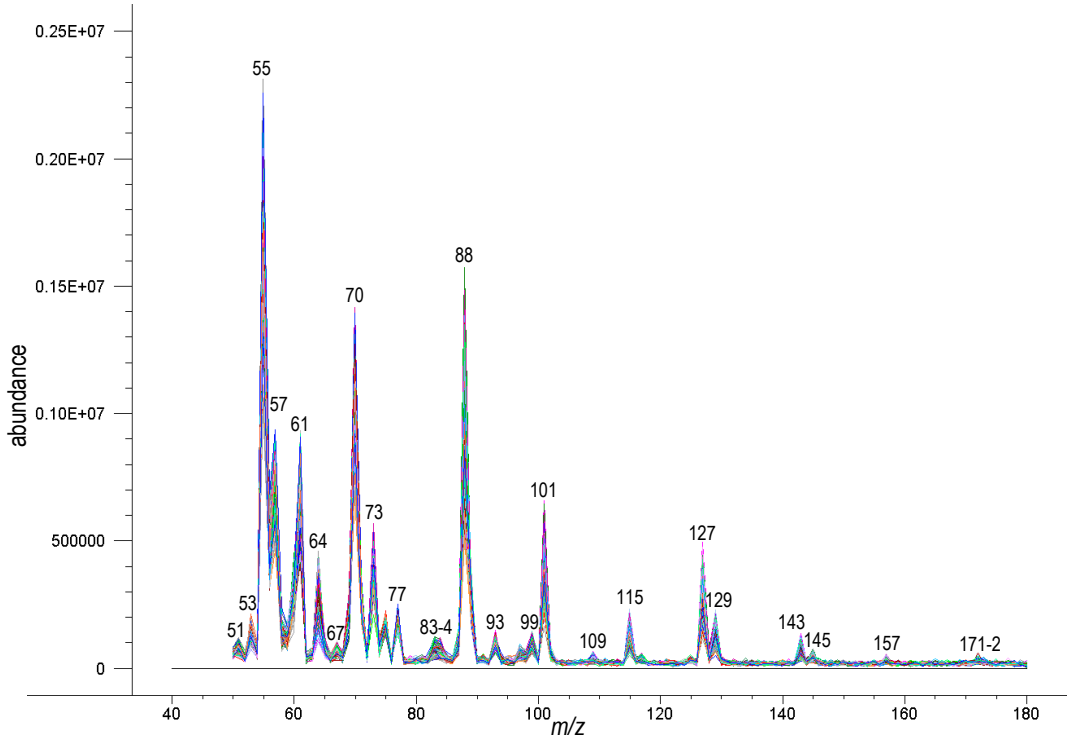
A major difficulty of using MS Enose for wine analysis is that ethanol can be preferentially detected by the MS which reduces the abundance of ions that are fragments of aroma volatiles [227]. Furthermore, ethanol acts as a co-solvent in the wine matrix and so the activity coefficient of the hydrophobic aroma compounds is lowered, resulting in a decreased partitioning into the headspace of the sample [201]. These problems can lead to misleading results with the electronic nose. To minimise the effect of ethanol in this study, a solvent delay was used in the MS Enose method to avoid the initial saturation of the MS with ethanol. Additionally, the ions scanned by MS were above *m/z* 50 so that the ions corresponding to ethanol (*m/z* 46, 31, 29, 17) were not recorded.

The raw spectral data for the MS Enose data for each wine, in triplicate analyses, are shown in Figure 6-1 (2 varieties x 20 wine labels x 3 replicates). Visually, the raw MS Enose spectral data sets for each wine are very similar (Figure 6-1). The major peaks (labelled in Figure 6-1) correspond to *m/z* 55, 57, 70, 73, 88, 99, 101, 115, 129 and 172 and are thought to be fragments originating from various fermentation-derived ethyl esters, acetates, alcohols and fatty acids. By analysis of variance, a total of 63 of the 131 ions scanned by MS Enose were statistically different between the wines (ANOVA, $p < 0.05$). These ions included *m/z* 51, 53, 55-67, 69-71, 73-75, 77, 79, 81-85, 87-91, 93-103, 108, 109, 115, 117, 119, 121, 123, 125, 127-129, 138, 143, 145, 154, 157, 163, 170 and 172.

The MS Enose spectra for the group of Riesling wines (n = 60) and the unwooded Chardonnay wines (n = 60) were compared. The abundance of a total of 46 ions were found to be significantly different between the two wine varieties (ANOVA, $p < 0.05$). The unwooded Chardonnay wines were significantly higher in the abundance of *m/z* 58 and 161. The Riesling wines were significantly higher in abundance of *m/z* 60, 61, 64, 65, 67, 73 - 75, 79, 81 - 84, 87 - 89, 91, 95 - 99, 101, 102, 109, 115 - 117, 119, 121, 125, 127 - 131, 138, 139, 143 - 145, 157, 172 and 177. This result suggests that the total concentration of

different molecules in the headspace of the Riesling wines was greater than for the unwooded Chardonnay wines. This might indicate that the overall aroma intensity of the Riesling wines was greater than for the unwooded Chardonnay wines.

Figure 6-1 Electronic nose mass spectra for Riesling and unwooded Chardonnay wines



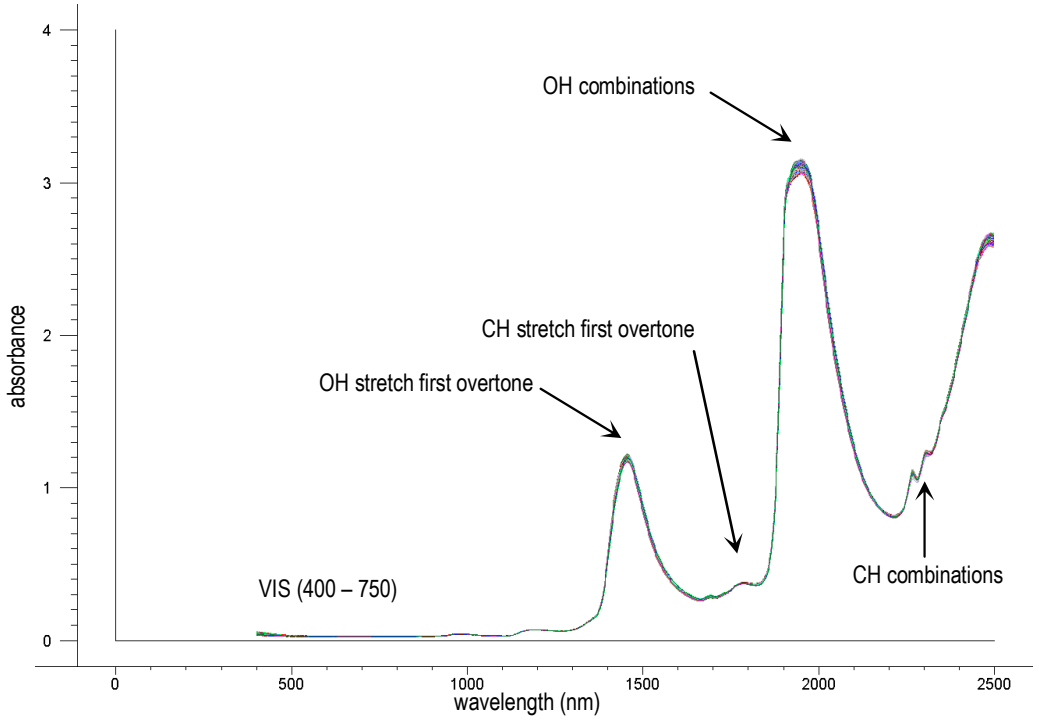
Raw replicate data shown for 2 varieties x 20 wine labels x 3 replicates (n = 120)

Although the peaks observed in MS Enose may relate to specific volatile aroma compounds in the sample, the intention of this study was to use the whole 'fingerprint' spectrum to predict sensory properties of wine, and not to identify individual peaks related to sensory properties or volatile composition.

6.2.2 Visible and near infrared spectroscopy

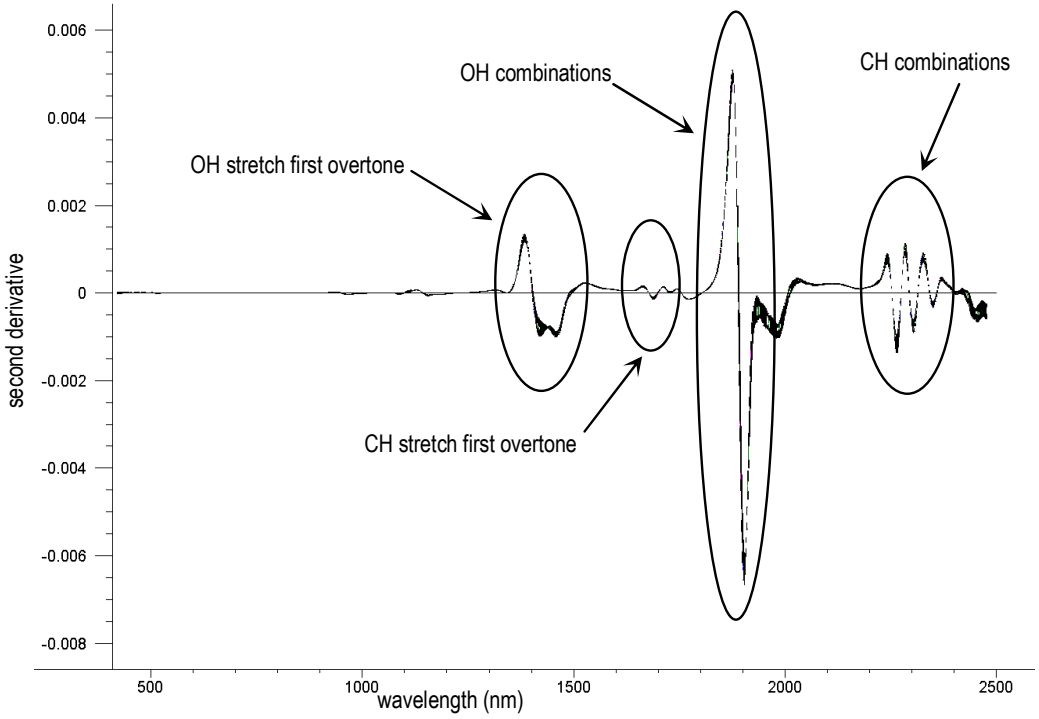
The raw spectral data from the VIS-NIR (400 – 2500 nm) for each wine analysed were visually very similar (shown in Figure 6-2) which is typical of VIS-NIR data for wine [254, 255]. The second derivative of the VIS and NIR spectra for each wine are shown in Figure 6-3.

Figure 6-2 Riesling and unwooded Chardonnay raw VIS-NIR data (400 - 2500 nm)



Raw baseline corrected VIS-NIR data shown (40 x 3 replicates)

Figure 6-3 Second derivative Riesling and unwooded Chardonnay VIS-NIR data (400-2500 nm)



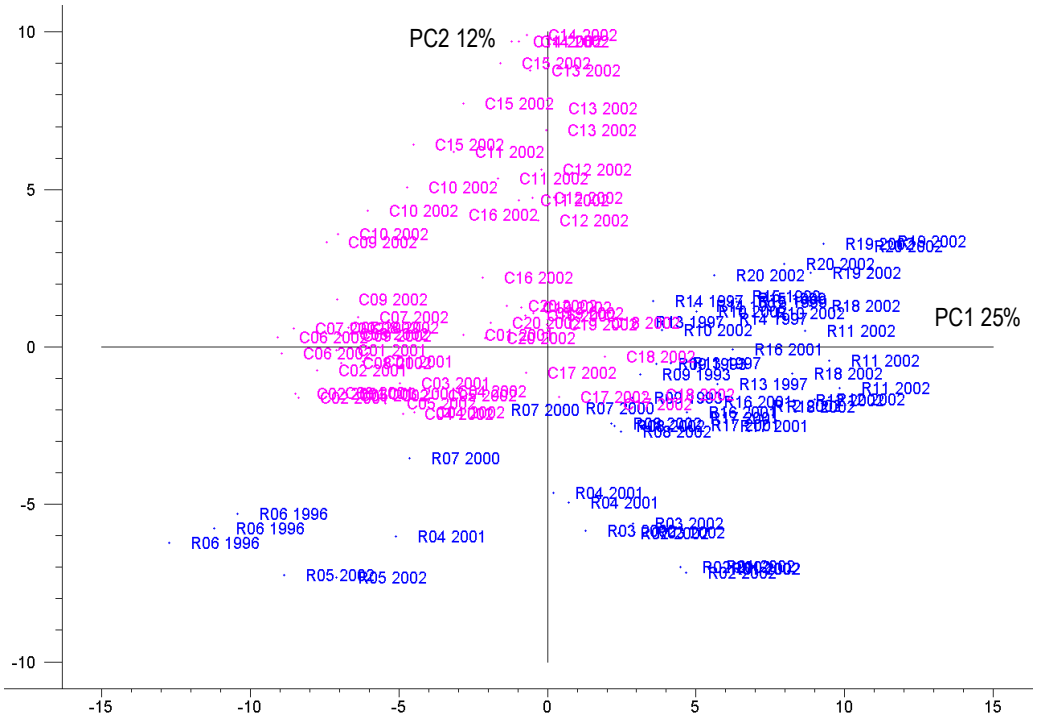
Second derivative VIS-NIR data shown (40 x 3 replicates)

The major features in the raw spectra of the wine samples are two broad bands at 1400 and 1900 nm related to OH first and second overtones, which are associated mainly with water and ethanol [255-257]. In general, no obvious spectral variations either between samples, varieties, viticultural origin or vintage were apparent despite the known variation within the samples for each variety (from sensory and chemical analysis). Small differences between second derivative spectra of different wines were observed between 1300 – 1400 nm (OH stretch first overtone, associated with water and ethanol), 1600 – 1700 nm (CH stretch first overtone, related to sugars), 2000 – 2100 nm (OH combination) and 2200 – 2400 nm (CH combination tones) [255, 257]. For the purposes of this study, the whole VIS-NIR spectrum was used in multivariate analysis even though the NIR region between 1900 – 2000 nm is considered out of scale.

6.2.3 Comparison of MS Enose and VIS-NIR

The smoothed and normalised MS Enose data (*m/z* 50 - 180) and the VIS-NIR raw spectra and second derivative were analysed by principal component analysis (PCA) to examine any relevant and interpretable structure in the data set and to look for outliers. The first three PCs account for 44% of the variation in the Enose data set (Figure 6-4), and 67% in the second derivative VIS-NIR data set (Figure 6-5).

Figure 6-4 PCA scores of Riesling and unwooded Chardonnay by MS Enose

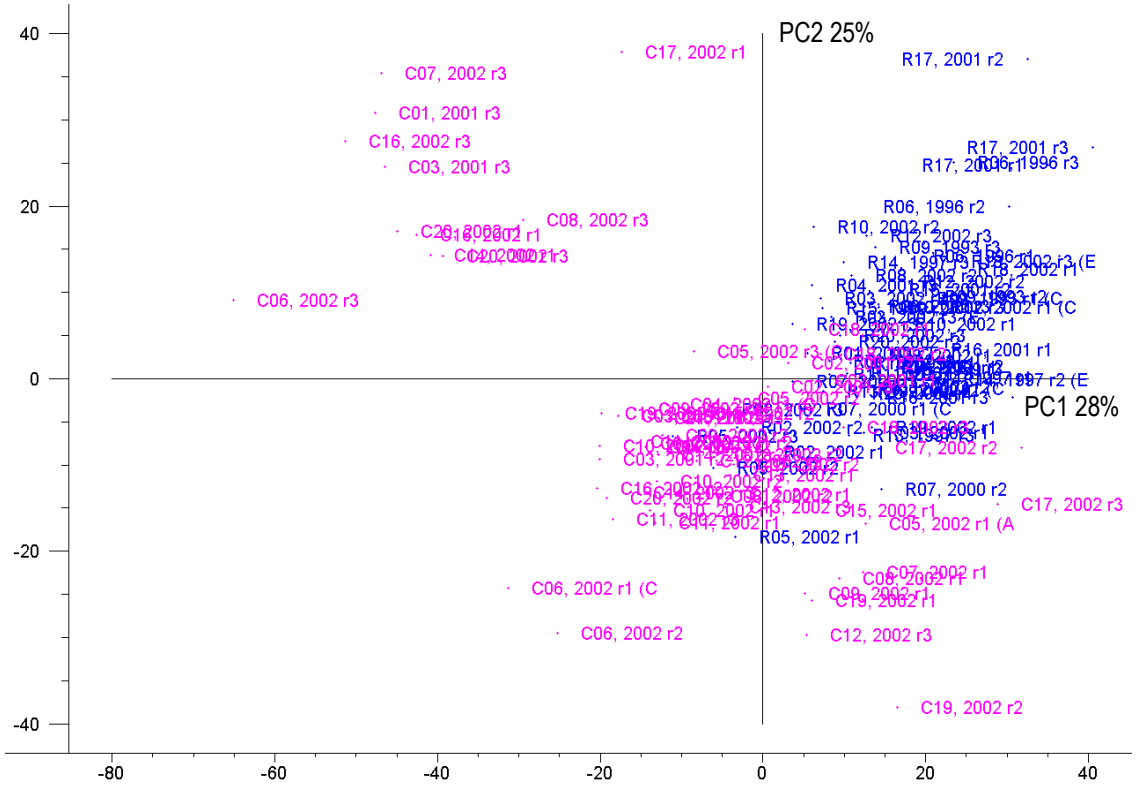


Riesling in blue, unwooded Chardonnay in pink; smoothed and normalised replicate data shown (40 x 3 replicates), This PCA was performed on the smoothed and normalised MS Enose data, autoscaled 1/SD.

Visual observations of the PCA scoreplots for both MS Enose and VIS-NIR data showed that the wines separated into two clusters, corresponding to the two varieties of wine. The discrimination observed for the wine varieties using VIS-NIR was explored further using this data set together with additional samples from the preliminary screening of each variety. Through the use of discriminant PLS regression, 100% of the Riesling and up to 96% of the Chardonnay wines were correctly classified according to their variety (data not shown, refer to publication of this data [255]).

The MS Enose PCA plot showed that the three replicates of each wine label were usually grouped close together which demonstrates good repeatability in the analysis. The MS Enose replicate data was obtained from three wine samples of just one bottle which might reduce the variation observed between replicates. On the other hand, the PCA plot of the VIS-NIR data showed that replicate samples were not always located close to each other. This might be explained by the fact that the replicate data in the VIS-NIR data set was obtained from different bottles of the same label of wine which were scanned on different days over a number of months (after each sensory session). Consequently, some bottle to bottle variation could be responsible for the separation of some replicates.

Figure 6-5 PCA plot of Riesling and unwooded Chardonnay second derivative VIS-NIR data (400 - 2500 nm)



PCA was performed on the baseline corrected second derivative VIS-NIR data autoscaled by 1/standard deviation; Riesling in blue, unwooded Chardonnay in pink; replicate data shown (40 x 3 replicates)

PLS calibration models were developed for each of the sensory properties from the descriptive studies of each variety using either the MS Enose spectra (*m/z* 50 - 180), or the second derivative of the VIS-NIR spectra (400 - 2500 nm). Both the spectral data (MS Enose and VIS-NIR) and the sensory scores were autoscaled (1/standard deviation) prior to developing the PLS models. The calibration coefficient (R_{cal}), the root mean square error in cross validation (RMSECV) and the optimal number of components used (C_{opt}) for each model are given in Table 6-2.

Good prediction statistics were achieved for particular attributes using either VIS-NIR or MS Enose. In general, the Riesling wine sensory attributes were better predicted than the unwooded Chardonnay sensory attributes which is a reflection of the variability and accuracy of the sensory descriptive data for each variety (refer to Chapter 5). For some attributes, namely Riesling attributes *overall flavour* and *flavour persistence* and unwooded Chardonnay attributes *estery* and *pineapple*, meaningful predictions could not be obtained using VIS-NIR or MS Enose.

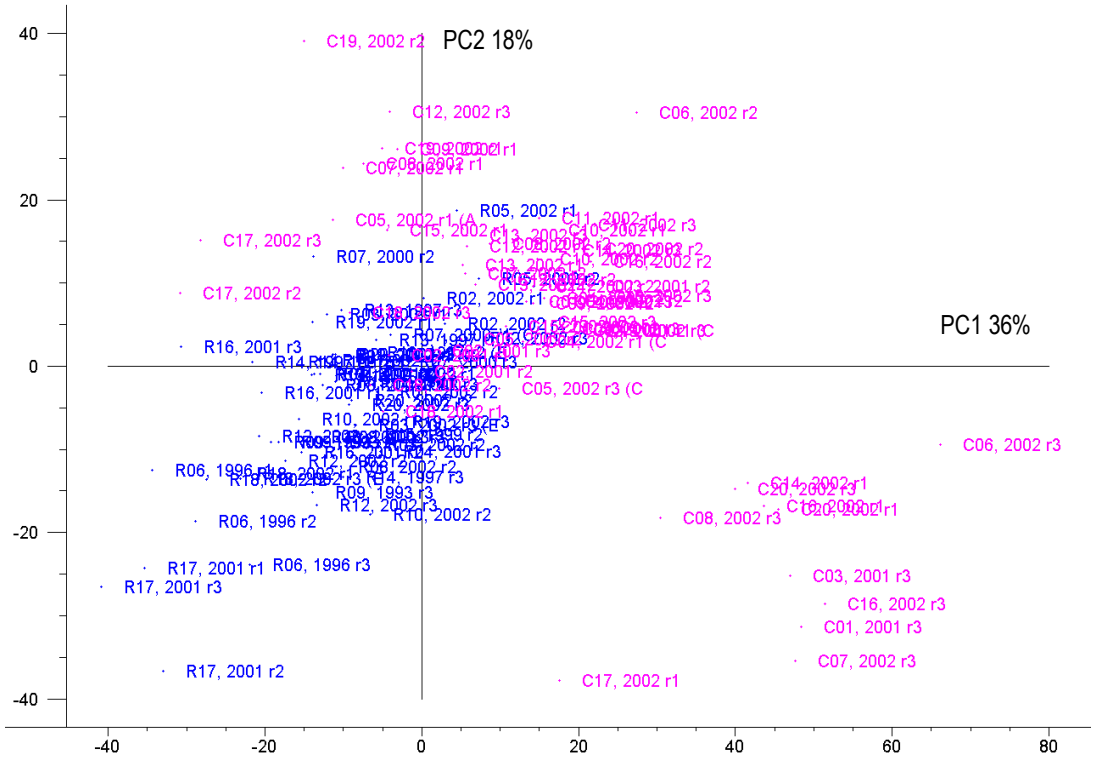
For the models obtained using only VIS-NIR, excellent predictions were achieved, where more than 70% of variation was explained ($R_{cal} > 0.84$), for the Riesling attributes *honey*, *toasty*, *caramel* and *kerosene*. Good models, where more than 50% of variation was accounted for ($R_{cal} > 0.7$), were achieved for the Riesling attributes *estery* and *lime*. Good prediction models were also developed using only MS Enose for the Riesling attributes *perfumed floral*, *passionfruit*, *herbaceous*, *honey* and *toasty*, where more than 50% of variation was accounted for ($R_{cal} > 0.7$). None of the unwooded Chardonnay sensory attributes were satisfactorily predicted using MS Enose or VIS-NIR for this data set.

For both varieties, the more developed characters (e.g. *honey*, *toasty*, *caramel*, *woody*, *spicy*) were generally better predicted by both the MS Enose and VIS-NIR as individual techniques. For the other attributes, the prediction of wine sensory properties between the two methods appeared to be somewhat complementary. For example, the *estery*, *dried rose*, *lime* and *pineapple* attributes were better predicted using VIS-NIR, whereas the *passionfruit* and *herbaceous* attributes were better predicted by MS Enose.

6.2.4 Combined MS Enose and VIS-NIR

The smoothed and normalised MS Enose data and the second derivative of the VIS-NIR data were combined and the new data set analysed by PCA. A PCA plot of PC1 versus PC2 is given in Figure 6-6. The first three PCs explain 60% of variation in the combined data set. The variation observed in the PCA scoreplot was more diverse for the unwooded Chardonnay wines than for the Riesling wines.

Figure 6-6 PCA plot of Riesling and unwooded Chardonnay VIS-NIR data (400 - 2500 nm) and MS Enose data (*m/z* 50 - 180)



PCA was performed on the combined second derivative VIS-NIR and the smoothed and normalised MS Enose data autoscaled by 1/SD; Riesling in blue, unwooded Chardonnay in pink; replicate data shown (40 x 3 replicates)

6.2.5 Prediction of sensory properties using combined MS Enose and VIS-NIR spectral data

PLS calibration models were developed for each of the sensory properties of each variety using a combination of both the second derivative of the VIS-NIR and MS Enose spectra. The combined data set (Enose plus VIS-NIR) was modified using two routines prior to multivariate data analysis. Either each source of data was considered as separate blocks (MS Enose and second derivative of the VIS-NIR) or treated as one block as described in Section 6.4.3. The PLS results from the data treated as separate blocks of data gave slightly better statistics in calibration (results shown in Table 6-2). The PLS results using the data set modified using the first routine, including calibration coefficient (R_{cal}), the root mean square error in cross validation (RMSECV) and the optimal number of components used (C_{opt}) for each model, are given in Table 6-2.

The combination of MS Enose and VIS-NIR produced the best predictive models for most of the sensory attributes in comparison to the results obtained from either technique separately. Nevertheless, some attributes were better predicted using either VIS-NIR or MS Enose alone. Of the three sets of models developed, MS Enose data alone produced the best prediction statistic for Riesling attributes *grapefruit*, *lychee*, *passionfruit*, and *herbaceous,* and unwooded Chardonnay attributes *estery*, *pineapple*, *honey*, *butterscotch*, *spicy*, and *flavour*

persistence. VIS-NIR data alone produced the best prediction statistics for Riesling attributes *dried rose*, *honey*, *caramel*, and *overall flavour*. The combination of the two techniques gave the best calibration statistics for Riesling attributes *estery*, *perfumed floral*, *lemon*, *pineapple*, *stewed apple*, *toasty*, *kerosene*, *rubber / plastic* and *sweetness*, and unwooded Chardonnay attributes *lychee*, *herbaceous*, *sweaty*, *woody* and *overall flavour*. Attributes for which only very poor calibration statistics were obtained ($R_{cal} < 0.35$) by both models include Riesling flavour attributes *sourness*, *sweetness*, *flavour persistence* and unwooded Chardonnay aroma attributes *floral*, *citrus* and *stewed apple / pear*.

It is interesting to note that the best prediction statistics were found for the same or similar attributes rated for both varieties (e.g. *honey*) using different techniques. This might be due to the panel rating these similar attributes, in both descriptive studies, with high agreement and with high variation, which would allow more robust prediction equations to be developed.

The optimal number of components (C_{opt}) used in the PLS models varied within all sets of models and ranged from one to ten. The models developed for unwooded Chardonnay sensory attributes generally used fewer components than models developed for Riesling sensory properties and gave much poorer calibration results. None of the methods (i.e. VIS-NIR, MS Enose or the combination of the two) used to develop models were observed to use fewer or a greater number of optimal components.

The RMSECV (and the SEP) is, in effect, a summation of the error of the instrumental method (e.g. MS Enose and VIS-NIR), the error of the reference method (e.g. sensory descriptive data) and the random noise generated in the model. Given the large amount or random noise associated with descriptive analysis of wine using human subjects, the SD values were not expected to be greater than the RMSECV (or SEP). In these models, the RMSECV values obtained (Table 6-2) were very similar to the SD obtained by the sensory panel for each attribute (refer to Table 3-2, Section 3.2.1, Chapter 3, and Table 4-2, Section 4.2.1, Chapter 4). This result suggests that the error in the models might be entirely derived from the error in the reference method (sensory descriptive analysis), and that very little error is associated with the instrumental methods. This observation suggests that the calibration could be useful for routine practical applications. Sørensen and Jepsen [252] developed NIR calibration models to predict sensory properties in cheese (i.e. for cheesy, acid, and sweet flavour properties) and obtained similar results, and suggested that the use of average values from a sensory panel, as was the case in this study, was the cause of the similarities between the SD and SEP values. Similar results have also been reported by other authors when sensory properties such as tenderness, juiciness, flavour, firmness and chewiness were predicted by NIR in beef meat [247].

Table 6-2 Prediction of wine sensory attributes by rapid instrumental techniques using PLS1

	VIS-NIR			MS Enose			VIS-NIR and MS Enose		
attribute	R_{cal}	RMSECV	C_{opt}	R_{cal}	RMSEP	C_{opt}	R_{cal}	RMSECV	C_{opt}
Riesling									
estery	0.72	0.72	5	0.67	0.76	2	**0.81**	**0.60**	**5**
perfumed floral	0.61	0.89	4	0.73	0.77	8	**0.83**	**0.63**	**6**
dried rose	**0.65**	**0.39**	**9**	0.33	0.47	1	**0.64**	**0.38**	10
lemon	0.67	0.57	9	0.67	0.55	2	**0.77**	**0.48**	**3**
grapefruit	0.59	0.38	4	**0.62**	**0.38**	**9**	0.59	0.39	5
lime	**0.76**	**0.53**	**5**	0.47	0.71	1	0.68	0.62	3
lychee	0.40	0.43	3	0.45	0.41	2	0.43	0.44	3
pineapple	0.62	0.43	4	0.39	0.49	1	**0.68**	**0.39**	**4**
passionfruit	0.38	1.02	3	**0.73**	**0.74**	**4**	0.56	0.91	4
herbaceous	0.18	0.63	1	**0.72**	**0.44**	**3**	0.53	0.57	3
stewed apple	0.39	0.37	4	0.42	0.34	1	**0.50**	**0.32**	**3**
honey	**0.90**	**0.48**	**6**	0.78	0.69	7	**0.89**	**0.49**	6
toasty	0.86	0.85	6	0.73	1.13	2	**0.91**	**0.67**	**5**
caramel	**0.86**	**0.40**	**6**	0.66	0.59	2	0.86	0.41	5
kerosene	0.85	0.67	5	0.66	0.95	2	**0.86**	**0.65**	**4**
rubber / plastic	0.58	0.34	6	0.54	0.35	2	**0.61**	**0.34**	**6**
sourness	0.05	0.38	1	0.03	0.39	1	0.09	0.37	1
sweetness	0.14	0.28	1	0.38	0.27	2	0.45	0.27	4
overall flavour	0.42	0.34	4	0.15	0.37	1	-	-	-
flavour persistence	-	-	-	0.10	0.44	1	-	-	-
unwooded Chardonnay									
estery	-	-	-	0.43	0.42	2	0.08	0.49	1
floral	0.02	0.54	1	0.23	0.52	1	0.11	0.53	1
lychee	0.52	0.40	2	0.27	0.46	1	**0.60**	**0.37**	**2**
citrus	0.18	0.34	1	0.13	0.35	1	0.24	0.33	1
pineapple	-	-	-	0.42	0.45	4	-	-	-
stewed apple / pear	0.25	0.47	1	0.26	0.47	1	0.27	0.48	1
passionfruit	0.35	1.34	1	0.12	1.53	1	0.14	0.45	2
herbaceous	0.39	0.47	2	0.30	0.48	1	0.48	0.46	2
sweaty	0.48	0.59	2	0.43	0.61	2	**0.57**	**0.60**	**7**
honey	0.36	0.52	1	**0.61**	**0.45**	**2**	0.52	0.50	4
butterscotch	0.19	0.64	1	**0.62**	**0.49**	**2**	0.46	0.57	5
woody	0.33	0.56	1	0.58	0.49	2	**0.67**	**0.45**	**10**
spicy	0.36	0.42	2	**0.57**	**0.39**	**6**	0.37	0.41	1
overall flavour	0.53	0.29	2	0.25	0.34	1	**0.58**	**0.30**	**2**
flavour persistence	0.38	0.38	1	**0.50**	**0.37**	**5**	0.43	0.38	1

VIS-NIR (400 - 2500 nm); MS Enose (*m/z* 50 - 180); R_{cal} : correlation coefficient in calibration; RMSECV : root mean square standard error in cross validation; C_{opt} : optimal number of components used in the PLS model; The best model for each sensory property (where $R_{cal} > 0.5$) is in bold typeface

The predictive ability of the models were tested by building a PLS calibration with replicates one and two for each wine label and using the data from replicate three as a separate validation set. Table 6-3 shows the predictive accuracy (i.e. validation) of the calibration models developed including the calibration coefficient (R_{val}), the standard error of prediction (SEP), the optimal number of components used (C_{opt}), slope and bias for each model.

The SEP values obtained from the validation models developed were close to the SD obtained for each sensory attribute during the descriptive studies, again indicating that the calibrations based on these instrumental techniques may be suitable for practical application. In these predictions, the SEP values for each attribute were slightly higher than each attribute's SD. For example, the higher SEP obtained for the model predicting the Riesling attribute *kerosene* (SEP = 0.72), was matched by a higher SD (1.27) and the lower SEP obtained for *caramel* (SEP = 0.38), was matched by a lower SD (0.77) [197].

Table 6-3 Validation statistics for prediction of sensory properties of white wine

Riesling	VIS-NIR and MS Enose					unwooded Chardonnay	VIS-NIR and MS Enose				
attribute	R_{val}	SEP	C_{opt}	Slope	Bias	attribute	R_{val}	SEP	C_{opt}	Slope	Bias
estery	**0.80**	**0.69**	**4**	**0.54**	**-0.31**	***estery***	-	-	1	-	-
perfumed floral	**0.79**	**0.74**	**5**	**0.55**	**0.054**	***floral***	0.08	0.53	1	0.052	-0.15
dried rose	**0.55**	**0.43**	**1**	**0.31**	**0.07**	***lychee***	**0.60**	**0.42**	**3**	**0.38**	**-0.075**
lemon	**0.80**	**0.40**	**4**	**0.67**	**-0.26**	***citrus***	0.46	0.36	1	0.19	-0.14
grapefruit	**0.67**	**0.32**	**4**	**0.57**	**-0.0066**	***pineapple***	0.29	0.46	1	0.14	-0.094
lime	**0.71**	**0.61**	**3**	**0.41**	**0.14**	***stewed apple / pear***	0.43	0.50	1	0.94	-0.23
lychee	**0.50**	**0.48**	**1**	**0.20**	**-0.13**	***passionfruit***	0.47	1.33	1	0.27	0.11
pineapple	**0.73**	**0.36**	**3**	**0.50**	**-0.32**	***herbaceous***	**0.51**	**0.47**	**1**	**0.48**	**-0.057**
passionfruit	0.46	1.0	1	0.17	-0.04	***sweaty***	**0.77**	**0.46**	**5**	**0.67**	**-0.15**
herbaceous	**0.54**	**0.53**	**1**	**0.20**	**-0.063**	***honey***	0.49	0.46	1	0.32	-0.030
stewed apple	**0.47**	**0.35**	**1**	**0.22**	**-0.035**	***butterscotch***	0.31	0.53	1	0.16	0.15
honey	**0.93**	**0.45**	**5**	**0.71**	**0.073**	***woody***	**0.66**	**0.54**	**7**	**0.66**	**0.14**
toasty	**0.92**	**0.70**	**5**	**0.73**	**-0.026**	***spicy***	0.32	0.46	1	0.11	0.46
caramel	**0.92**	**0.38**	**5**	**0.68**	**-0.079**	***overall flavour***	**0.71**	**0.22**	**1**	**0.68**	**-0.033**
kerosene	**0.86**	**0.72**	**4**	**0.64**	**0.24**	***flavour persistence***	0.31	0.31	1	0.47	-0.041
rubber / plastic	0.21	0.46	1	0.071	-0.064						
sourness	0.19	0.37	1	0.073	0.069						
sweetness	0.23	0.28	1	0.089	-0.033						
overall flavour	0.058	0.42	2	0.023	0.42						
flavour persistence	-	-	1	-	-						

VIS-NIR (400 - 2500 nm); MS Enose (*m/z* 50 - 180); R_{val} : correlation coefficient in validation; SEP : standard error of prediction; C_{opt} : optimal number of components used in the PLS model; Models where where $R_{val} > 0.5$ are in bold typeface

The validation results show that Riesling wine attributes *estery*, *perfumed floral*, *dried rose*, *lemon*, *grapefruit*, *lime*, *lychee*, *pineapple*, *herbaceous*, *stewed apple*, *honey*, *toasty*, *caramel*

and *kerosene*, and unwooded Chardonnay wine attributes *lychee*, *herbaceous*, *sweaty*, *woody* and *overall flavour* were well predicted ($R_{val} > 0.5$) using the combination of MS Enose and VIS-NIR data. The results for the Riesling wines show that all Riesling aroma attributes were adequately predicted, demonstrating that this technique could potentially be well suited to objectively and rapidly predict the aroma properties of this variety. On the other hand, the prediction of the unwooded Chardonnay attributes was not adequate (where $R_{val} < 0.05$) for most of the sensory attributes. This is interesting considering the data for the unwooded Chardonnay wines for both MS Enose and VIS-NIR showed greater variation than for the Riesling wines. It might be that the reduced variation in the unwooded Chardonnay sensory data, compared to the Riesling sensory data, is limiting the ability of combined MS Enose and VIS-NIR to make sensible predictions of sensory attribute scores.

The results from this study demonstrate that mathematical relationships can be established between rapid instrumental analytical data (i.e. MS Enose and VIS-NIR) and the sensory properties of wine. It could be that the spectral 'fingerprint' is, in effect, directly measuring the chemical compounds responsible for the sensory properties of wine. Alternatively, the methods of VIS-NIR and MS Enose spectroscopy might be indirectly explaining the variations in the aroma characteristics of the white wines analysed.

It has been reported by other authors that the predictive information related to sensory properties and VIS-NIR spectra did not seem to be related to a specific chemical moiety in the sample [248, 258], and it was not clear which particular spectral information was related to a specific sensory property. It is known [125, 259] that correlations between NIR spectroscopy and sensory properties might be caused by collinearity between compositional variables, between wavelengths or between other sensory properties. It is also well known that many sensory properties are not strictly associated with an identifiable chemical entity in the VIS-NIR region, requiring the use of a large number of seemingly redundant wavelengths to develop calibration models for the prediction of sensory property score.

On the other hand, MS Enose has been used to measure specific volatile compounds in the headspace of foods and beverages. For example, MS Enose has been used to measure of the volatile compound TCA, a known off-flavour in wine when present, at high concentrations [260]. Nevertheless, MS Enose did not appear to be a superior predictive tool when compared with VIS-NIR for the prediction of wine sensory properties. This is interesting considering that MS Enose measures the ions of headspace volatiles which may be directly responsible for specific sensory properties of the wines. It is well known that Enose is a non-selective technique, that is, it detects the major volatiles of the headspace regardless of whether those volatiles are actively contributing to the aroma of the wine or not [227]. The

volatile compounds responsible for the characteristic aromas of wine are often not quantitatively the major volatile component in the headspace of wine; rather they are usually minor constituents present at μg or ng/L levels (e.g. sulfur-containing wine volatiles [47]). Consequently, those volatile compounds at higher concentrations in the headspace, which are typically not significant to the aroma of the wines, may be dominating the spectral data obtained from MS Enose and contributing noise to the regression models developed. Furthermore, the MS Enose method is not likely to be sensitive enough to detect all of the most important volatile aroma compounds which are present at trace levels [227] which would further reduce the spectra's ability to predict the sensory properties of the wines.

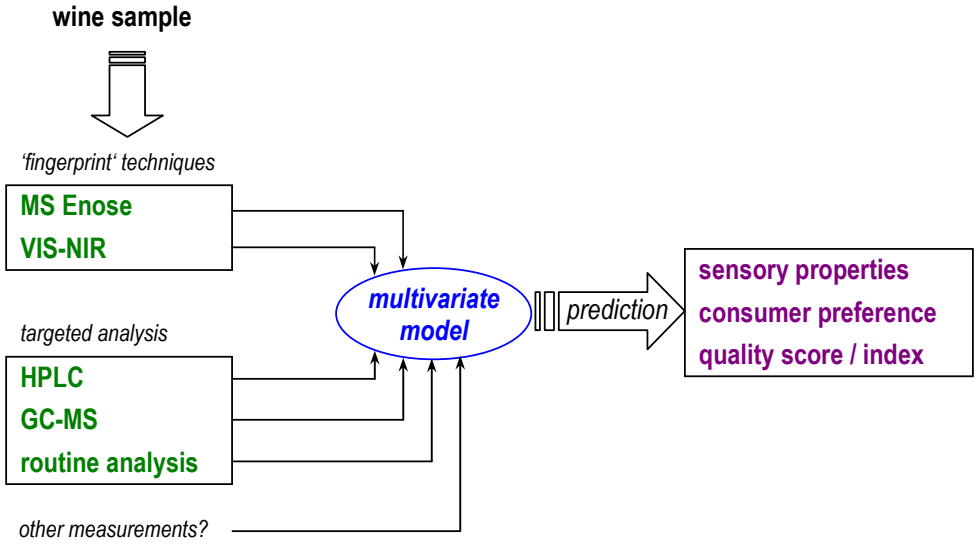
The combination of the two techniques gave the best prediction statistics. This supports the concept that no single volatile or group of volatiles is independently responsible for the aroma of wine. It is the combination of volatile compounds, their relationships with each other and their relationship with other compounds in the wine matrix, which gives rise to the perceived sensory characteristics of wine. In this respect, the 'fingerprint' measurement of a wine by VIS-NIR and MS Enose techniques, could more robustly account for these complex interactions than targeted chemical analysis of individual aroma volatiles.

This study has demonstrated the potential of combining different instrumental techniques to predict sensory characteristics of wine. No other reports were found in the literature using combinative approaches to predict the sensory properties of wine, however, it has been reported that combining complimentary instrumental techniques in other food industries can provide better prediction of sensory properties than the use of a single instrumental technique. For example, the combination of colour and texture measurements and electronic nose, provided a better prediction for fish quality [261] than single instrumental measurements on their own. It is likely that by combining additional measurements (e.g. from other types of Enose sensors or other measurements of wine composition) the calibrations could be improved, particularly for those properties that were not well predicted by either VIS-NIR or MS Enose. In the future, it might be possible to obtain a fast and accurate prediction of wine aroma by combining multiple rapid instrumental techniques. Additionally, the inclusion of targeted analyses of small numbers of volatile compounds or other compositional parameters could complement rapid analytical techniques in predicting wine sensory properties (see Figure 6-7). This concept could be extended to predicting not only sensory properties of wines, but also other quality determining factors such as consumer preference.

This strategy could be used by the wine industry for rapid screening of wines to give an estimation of the sensory properties of wine or determination of approximate quality

category. Additionally, this technique could be used to rank wines according to their sensory properties or to determine how similar, or different, the aroma of particular wines may be. In research, this technique might be suitable for rapid screening of large numbers of wines to determine a suitable subset of wines that have representative aromas for further sensory analysis. This might reduce the time and cost of sensory analysis.

Figure 6-7 Potential of using multiple analytical techniques to predict important wine properties



6.3 Conclusion

This preliminary study has demonstrated that the combination of VIS-NIR and MS Enose has good potential to rapidly and objectively predict a number of sensory properties for white wines. These two techniques were found to be complementary in some prediction models developed as they supplied independent 'fingerprint' information about the samples. Most of the VIS-NIR calibrations developed accounted for more than 50% of the variation ($R_{cal} > 0.7$) and the Riesling sensory properties were much better predicted than the unwooded Chardonnay sensory properties (also refer to discussion in Chapter 5). In order to develop a robust combined VIS-NIR and MS Enose method to determine specific sensory properties, it is imperative to obtain more knowledge about the chemical basis for the relationships described.

Due to the limited number of samples and wine types used in the present study the results must be interpreted with caution. Furthermore, the predictive ability of the PLS calibration models developed need to be evaluated with a new and independent set of samples. More wine varieties and a wider range of sensory properties (aroma and flavour descriptors) should be analysed and used to validate the method, before the technique could be adopted by the wine industry.

6.4 Materials and Methods

6.4.1 Mass spectrometry based electronic nose

Ampoules of unwooded Chardonnay and Riesling wines were thawed for MS Enose analysis which was conducted on the 20th and 24th August 2004 (after storage under N_2 at -18°C as detailed in Section 3.4.3, Table 3-13 and Section 4.4.3, Table 4-12). For each wine, 5 mL of wine was accurately measured into a 10 mL SPME vial in triplicate. Blank vials and vials containing model wine (12% ethanol in water v/v) were also prepared for analysis in duplicate. Samples were analysed with an Hewlett Packard Chemical Sensor (HP4440) equipped with an Hewlett Packard headspace sampler (HP7694, Model G 1290A). Each vial was equilibrated at 75°C for 20 minutes. The headspace volatiles were then transferred to the MS with a 4.2 minute headspace cycle time, 0.5 minute injection time, 0.02 minute loop equilibration time, 0.15 minute loop filling time and a 0.3 minute pressurising time. To prevent condensation, the temperatures of the transport line and carrier line were maintained at 90°C and 95°C, respectively. Helium gas (Air Liquide or BOC gases, ultra high purity) was used as the carrier at a pressure of 4.2 psi and vial pressurisation of 14 psi. Positive ion electron impact spectra at 70 eV were recorded in the range *m/z* 50.0 to 180.0 at a rate of 9.69 scans/second. The mass spectrometer total run time was 0.75 minute with a 0.45 minute solvent delay. The total run time for each sample was approximately 25 minutes.

6.4.2 Near Infrared spectroscopy

All Riesling and unwooded Chardonnay wines were scanned throughout the respective sensory studies by NIR on the same day of opening the bottle. Samples taken from the freshly opened bottles of wine were scanned in transmission mode (400 – 2500 nm) using a scanning monochromator *FOSS NIRSystems6500* (FOSS NIRSystems, Silver Spring, MD, USA). Spectral data were collected using *Vision* software (version 1.0, FOSS NIRSystems, Silver Spring, USA). Samples were scanned in a rectangular cuvette in a 1 mm path length and equilibrated at 33°C over 3 min before scanning. Spectral data were stored as logarithm of the reciprocal of transmittance (log (1/T)) at two nm intervals. The spectrum of each sample was the average of 32 successive scans (1050 data points). NIR from scanning monochromators provide an estimate of the continuous spectrum, composed of many overlapping absorption bands. The bands are defined by three criteria: location, height and width. The height of an absorption band is measured at its peak. The band location measured as the wavelength of its peak. Band width is measured as the width of the peak at half of the peak height [262]. The instruments were allowed to warm up before scanning any sample. Diagnostic tests were performed to verify that the instrument was functioning correctly according to the manufacture's standards. Firstly, the photometric repeatability or noise level of the instrument was ascertained. This test is accomplished by scanning an

internal reference (ceramic disk). This sequence is repeated and three complete scans were displayed by the computer. Secondly, the wavelength accuracy was verified by scanning an internal polystyrene standard paddle which is supplied by the instrument manufacturer and housed within the case of the instrument. This involved locating the major polystyrene peaks and comparing these with the known locations. The third test was to check the instrument response, which gives a measure of the absolute reflectance from the ceramic tile [263].

6.4.3 Multivariate data analysis

Spectra were transported to *The Unscrambler* software (version 7.8, CAMO ASA, Oslo, Norway) for chemometric analysis. The data tables were structured so that the wines were in rows and the variables in columns (VIS-NIR, MS Enose, sensory attribute scores).

The sensory data, including aroma and flavour properties, from the Riesling (Section 3.2.1, Chapter 3) and unwooded Chardonnay (Section 4.2.1, Chapter 4) descriptive analysis studies was used for multivariate data analysis. In each descriptive study, three replicates were obtained, for each wine, for each sensory attribute. Every session the wines were scanned by VIS-NIR so that every replicate bottle had a matching VIS-NIR spectrum. For this reason, the sensory data were averaged only over the number of judges in each descriptive study to give three replicate samples of the same wine label. These replicates were matched with their corresponding VIS-NIR data for multivariate data analysis. Consequently, there were 60 samples (3 replicates x 20 bottles) for each variety. The three replicates obtained from the MS Enose were from a single bottle of each label of wine and did not match the exact bottles used for each replicate during the sensory study. Nevertheless these replicates were used as the three replicates for multivariate data analysis.

Prior to multivariate data analysis, MS Enose data (*m/z* 50 - 180) were transposed, smoothed (moving average, 7 segments [264]) and normalized (mean normalisation) and then transposed again.

The second derivative of the VIS-NIR spectral data (400 - 2500 nm) was used as a mathematical treatment to correct for baseline effects and to separate overlapping peaks [265] and it was performed using Savitzky-Golay transformation and smoothing (10 point and 2^{nd} order filtering).

The combined data set (Enose plus VIS-NIR) was modified using two routines prior to multivariate data analysis. The first routine considered each source of data as separate blocks (MS Enose and second derivative of the VIS-NIR) and each block was pre-treated as

described above (i.e. for smoothing and normalisation). The second routine used considered both sources of spectra as one block. The pre-treatment of the combined raw data (MS Enose and VIS-NIR) was first modified by calculating the logarithm (log 10), followed by smoothing (moving average, 7 segments [264]) and normalisation (mean normalisation).

Principal component analysis (PCA) was performed before partial least squares regression (PLS1) models were developed. PCA was used to examine any relevant and interpretable structure in the data as well as outlier detection [129]. Two outlier samples, which were very different from their respective other two replicates, were removed from both the MS Enose and VIS-NIR data sets, prior to the development of PLS models. Calibration models for the prediction of sensory properties (aroma and flavour) using VIS-NIR and MS Enose spectra were developed using PLS1 regression with full cross validation. The optimum number of terms in the PLS calibration models was determined as indicated by the lowest number of factors that gave the closest to minimum value of the PRESS (prediction residual error sum of squares) function in cross validation [126] in order to avoid overfitting of the models. Both the scores for the sensory properties and the spectra were autoscaled using the (1/STD) option included in *The Unscrambler* software before PLS1 calibration models were developed [125, 129, 201]. Statistics calculated for the calibrations included the coefficient of correlation in calibration (R_{cal}) and the root mean square standard error of cross validation (RMSECV). Note that *The Unscrambler* software uses the abbreviation RMSEP when referring to the RMSECV.

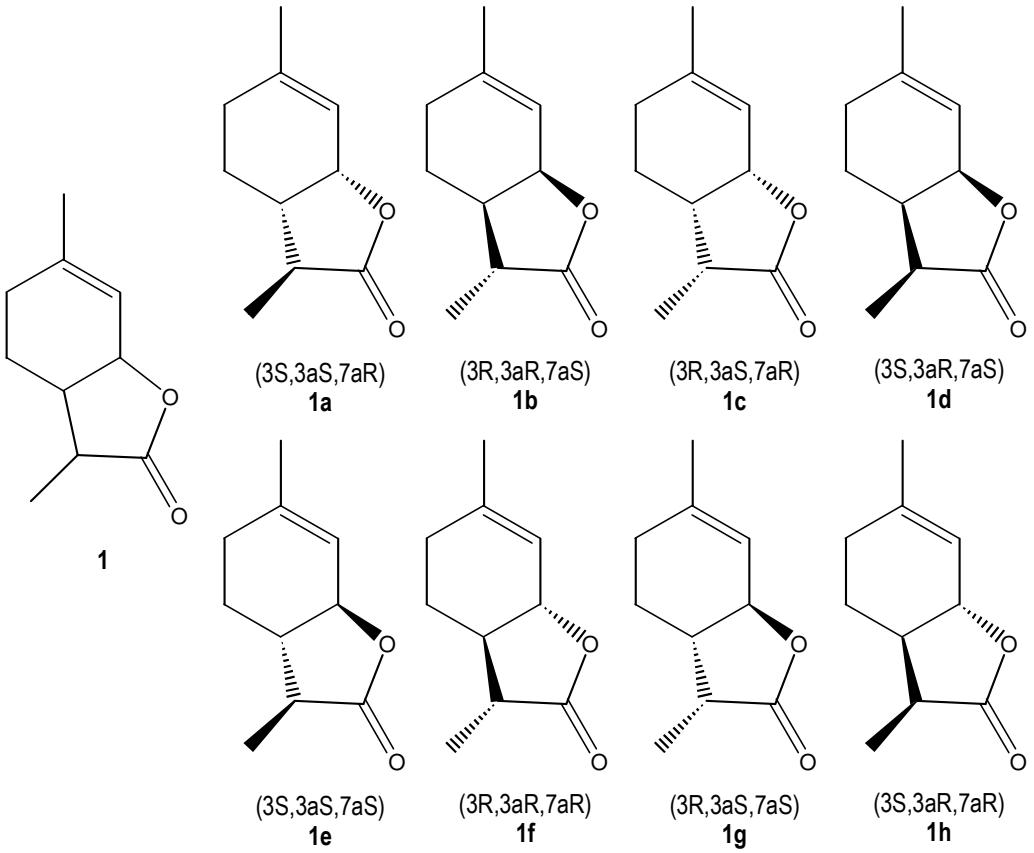
For validation of the models, the samples, of each variety were split into two groups for calibration and validation. The calibration set of samples consisted of the first and second replicates for each wine label (n = 40) while the validation set consisted of the third replicate (n = 20). The prediction accuracy of the models was tested on the validation set using the standard error of prediction (SEP) and the correlation coefficient in validation (R_{val}) [126, 129].

Chapter 7 Study of wine lactone

7.1 Introduction

The lactone, 3a,4,5,7a-tetrahydro-3,6-dimethylbenzofuran-2(3H)-one (**1**, Figure 7-1), was first identified as a constituent of Koala urine by Southwell in 1975 [266]. More than twenty years later this lactone (**1**) was found as a volatile constituent of white wine and was implicated as a potentially important contributor to the aroma of white wine [6, 74]. In 1996, Guth demonstrated that of the eight possible stereo-isomers of this so-called 'wine lactone' (**1**) only one isomer, *3S,3aS,7aR* (**1a**, Figure 7-1), was present in two young white wines [267]. Interestingly, the aroma threshold of this particular isomer (**1a**) is the lowest of all the eight isomers of wine lactone (**1a**-**1h**) at 1 x 10^{-5} ng/L in air [267] and 10 ng/L in model wine [6].

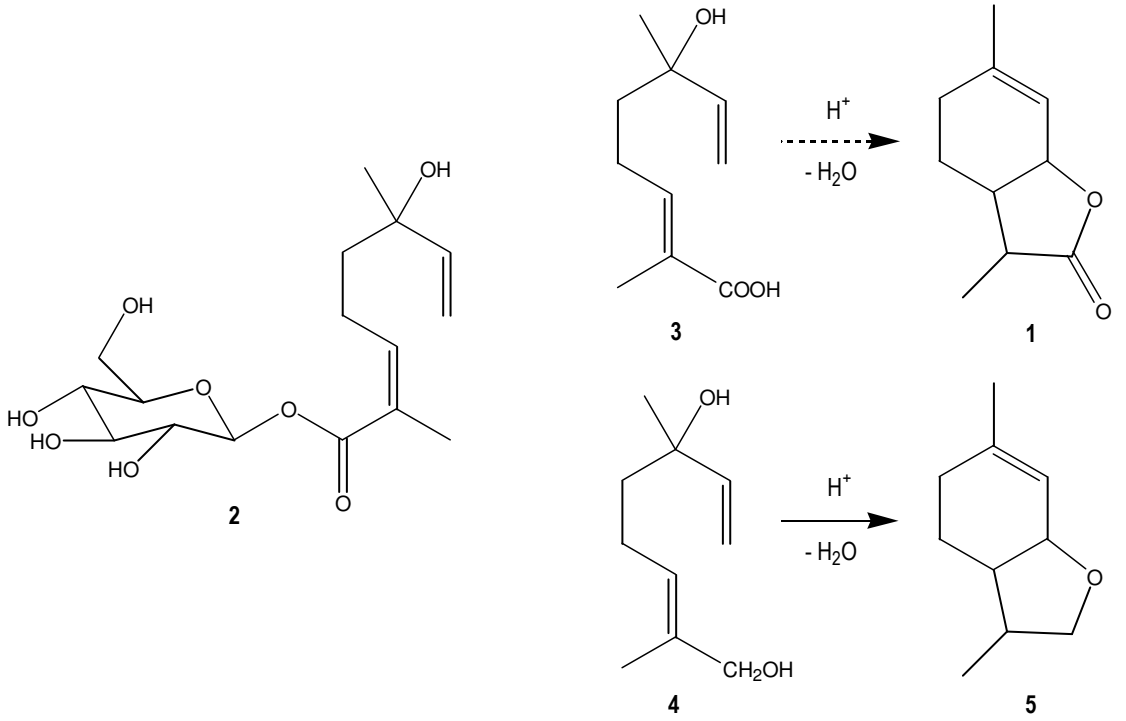
Figure 7-1 Stereoisomers of wine lactone



Some volatile compounds formed from the acid-catalysed degradation of odourless glycoconjugates, which are present in wine, are known for their important contribution to the aroma of wine [75, 268, 269]. Winterhalter et al isolated the glucose ester of (E)-2,6-dimethyl-6-hydroxyocta-2,7-dienoic acid (**2**, Scheme 4) by multilayer coil countercurrent chromatography (MLCCC) from a commercial 1992 vintage Riesling wine and proposed that

(**2**) was a possible precursor for wine lactone [270]. Although this was the first time that this glucose ester (**2**) had been identified as a wine component, glycoconjugates of its reduced form (i.e. the monoterpene diol (**4**), Scheme 4) have been previously identified as wine constituents [268]. In 1988, Strauss et al reported that the monoterpene diol (**4**) under acidic conditions converted into several products including the bicyclic ether (**5**, Scheme 4) [268]. Winterhalter et al suggested that the monoterpenoid acid (**3**) could be expected to form wine lactone (**1**) in an analogous fashion (Scheme 4) [270].

Scheme 4



In 1998, Bonnlander et al tested this hypothesis, by subjecting the synthesised monoterpenoid acid (**3**, Scheme 4) to thermal treatments at pH 3.2, 2.5 and 2.0 respectively. In all cases, wine lactone (**1**) was reported as a major conversion product of the monoterpenoid acid (**3**). However, the absolute stereochemistry of the product was not determined [185, 268]. The possible conversion of the glucose ester to wine lactone was also not tested at that time.

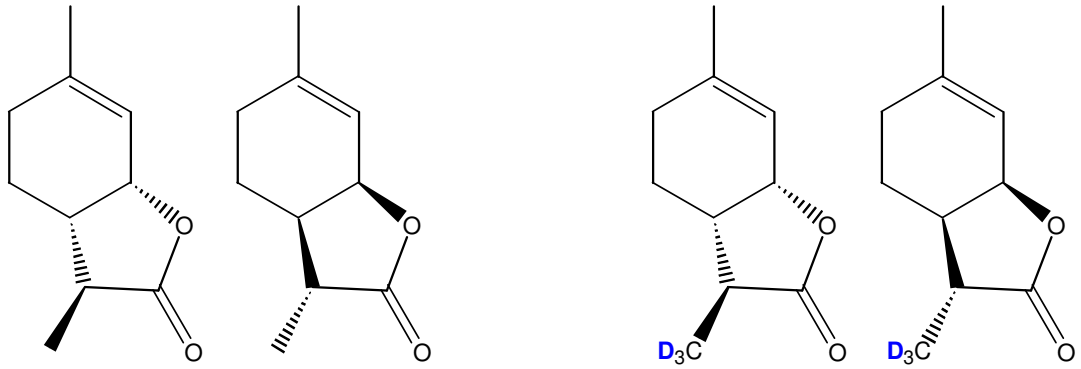
The monoterpenoid acid (**3**) has also been observed as a natural grape hydrolysate constituent. It was first tentatively identified in glycoside hydrolysates of Semillon grape juice by Sefton et al, 1996 [72] and again in an enzyme hydrolysate of Merlot grape juice [73]. The monoterpenoid acid (**3**) could be derived from additional sources, other than the simple glucose ester (**2**), and might independently play a role in wine lactone formation.

The aim of this study was to quantitatively and qualitatively investigate the formation of wine lactone from both the glucose ester (**2**) and the monoterpenoid acid (**3**), through hydrolytic studies and subsequent chiral analysis of the reaction products.

7.2 Results and discussion

Unlabelled enantiomerically pure (3S,3aS,7aR)-tetrahydro-3,6-dimethylbenzofuran-2(3H)-one (**1a**), the racemate (**1a**/**1b**) and deuterium labelled racemic d_3-3S,3aS,7aR and d_3-3R,3aR,7aS-tetrahydro-3,6-dimethylbenzofuran-2(3H)-one (**6a**/**6b**) were synthesised for use in analytical method development (Figure 7-2). Both unlabelled racemic (**1a**/**1b**) and the enantiomerically pure (**1a**) wine lactone were required for chiral analysis. The synthetic methodologies used by Guth (1996) were adopted and modified for the preparation of these compounds [267].

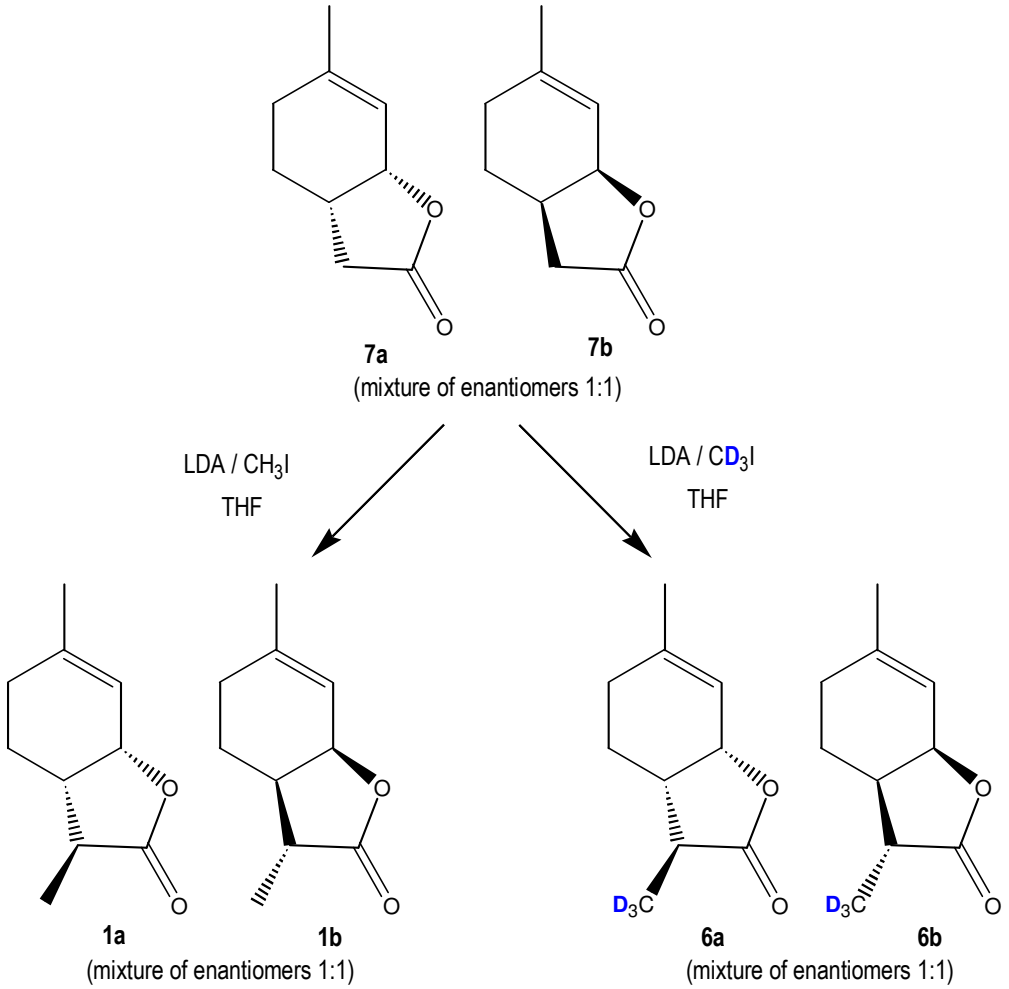
Figure 7-2 Deuterium labelled and unlabelled wine lactone



7.2.1 Synthesis of racemic wine lactone (1a/1b and 6a/6b)

Scheme 5 shows the strategy for the synthesis of racemic labelled and unlabelled wine lactone (**1a**/**1b** and **6a**/**6b**). Racemic wine lactone (**1a**/**1b**) was prepared following Guth's published procedure [267] starting from commercially available isoprene. The deuterium labelled wine lactone (**6a**/**6b**) was prepared in a similar manner, by substitution of iodomethane with d_3-iodomethane to introduce three deuterium atoms into the compound. The identity of the synthetic material was confirmed by comparison of spectral data to published spectra (NMR and MS).

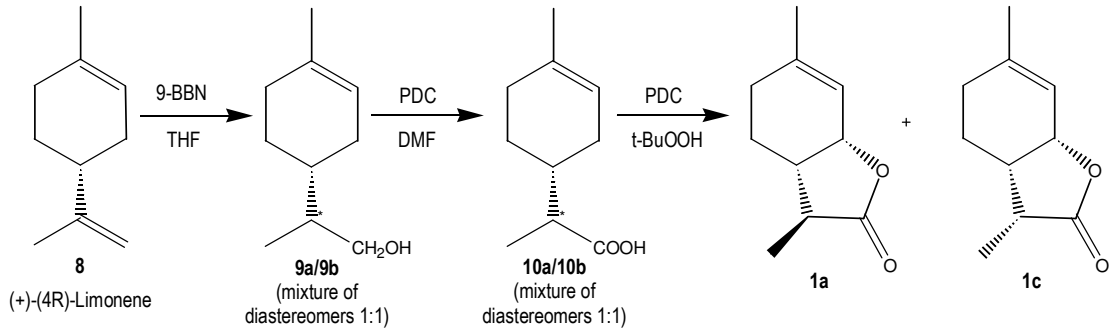
Scheme 5



7.2.2 Synthesis of enantiomerically pure wine lactone (1a)

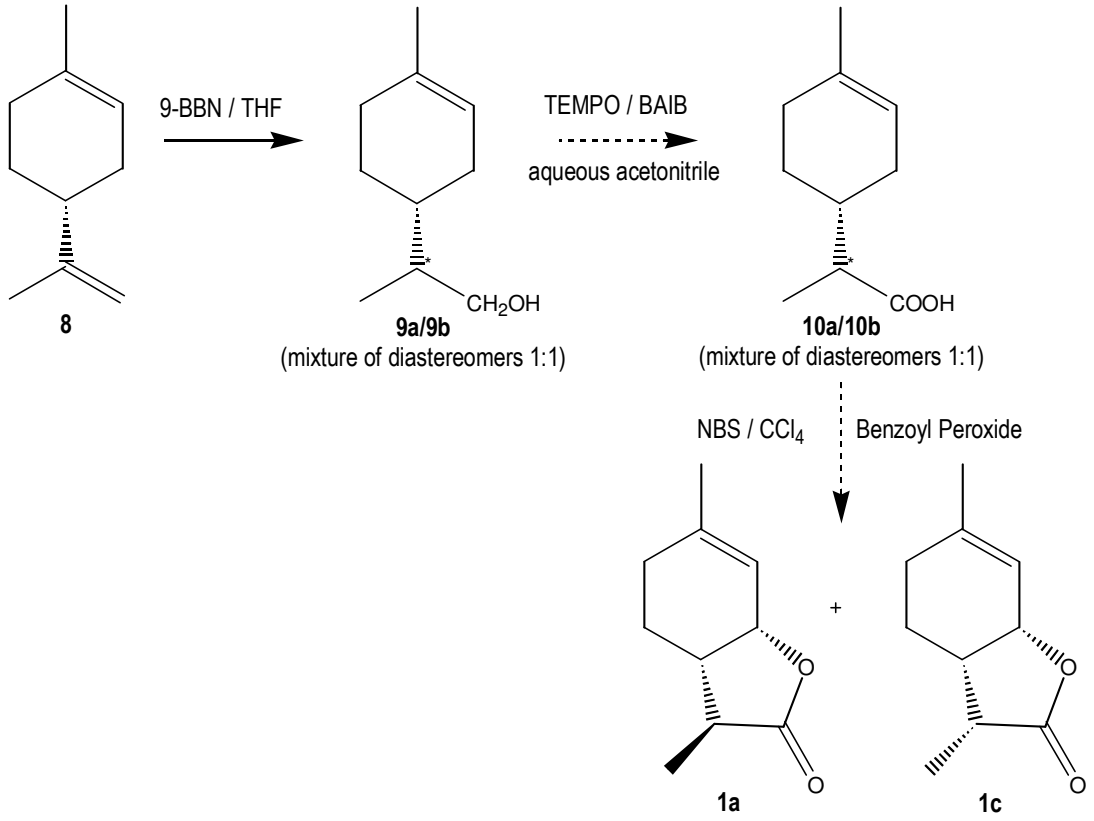
The synthesis of enantiomerically pure wine lactone (**1a**) has been published by Guth as depicted in Scheme 6. According to this synthetic route, a three step reaction from commercially available (+)-(4R)-limonene afforded diastereomers of wine lactone **1a** and **1c**, which were separated by silica gel column chromatography.

Scheme 6



Guth's synthetic scheme involved a problematic and low-yielding step involving allylic oxidation of the acid **10a**/**10b** with pyridinium dichromate (PDC) and tertiary butyl peroxide (t-BuOOH) to give diastereomeric wine lactone (**1a**/**1c**) [267]. The yield for this reaction was not reported by Guth, and in this study was at best found to be around 5%. Also, the work up for this reaction was particularly difficult as the reaction mixture turned to a solid from which it was almost impossible to extract the reaction products. As an alternative to oxidation with PDC, we envisaged that the enantioselective synthesis could be performed using alternative reagents (Scheme 7) to those used by Guth. It was envisaged that the oxidation of the diastereoisomeric alcohols (**9a**/**9b**) to the corresponding acids (**10a**/**10b**) could be performed using the free radical TEMPO and bis-acetoxyiodobenzene (BAIB) in aqueous acetonitrile similar to the conditions used by Raunkjaer et al, 2001 [271]. The subsequent treatment of the acid (**10a**/**10b**) with N-bromosucinnimide (NBS) would result in allylic bromination and spontaneous lactonisation through the intra-molecular elimination of bromine could potentially generate diastereomeric wine lactone (**1a**/**1c**). Both of these steps would avoid the use of pyridinium dichromate (PDC) and could potentially increase the overall yield of the reaction.

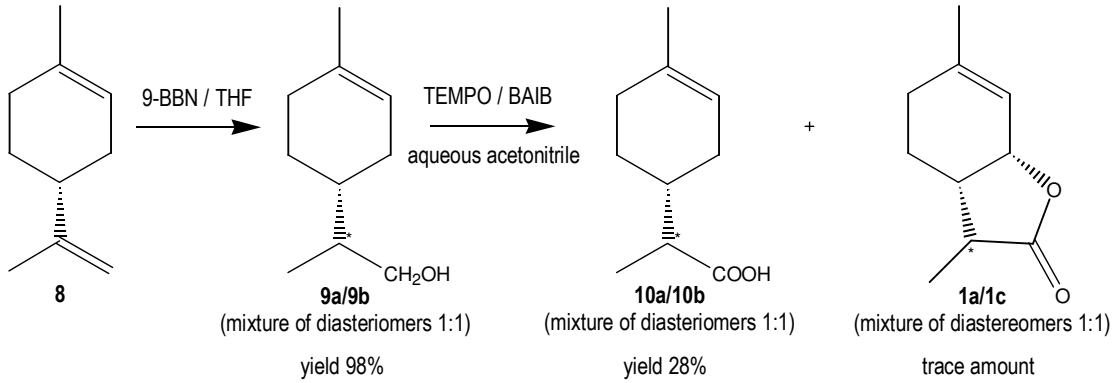
Scheme 7



The regioselective hydroboration of (+)-(4R)-limonene (**8**) with 9-BBN produced the alcohol (2RS)-2-((1R)-4-methylcyclohex-3-enyl)propanol (**9a**/**9b**) in excellent yield (98% compared to

65% reported by Guth [267]) (Scheme 8). The free radical oxidation of the alcohol (**9a**/**9b**) to form the acid (**10a**/**10b**) was a more convenient but lower yielding reaction than the PDC oxidation used by Guth (28% compared to 39% [267]). During the free radical reaction a number of other by-products were also formed. Importantly, it was observed that small quantities of diastereomeric wine lactone (**1a** and **1c**) were also produced directly (Scheme 8).

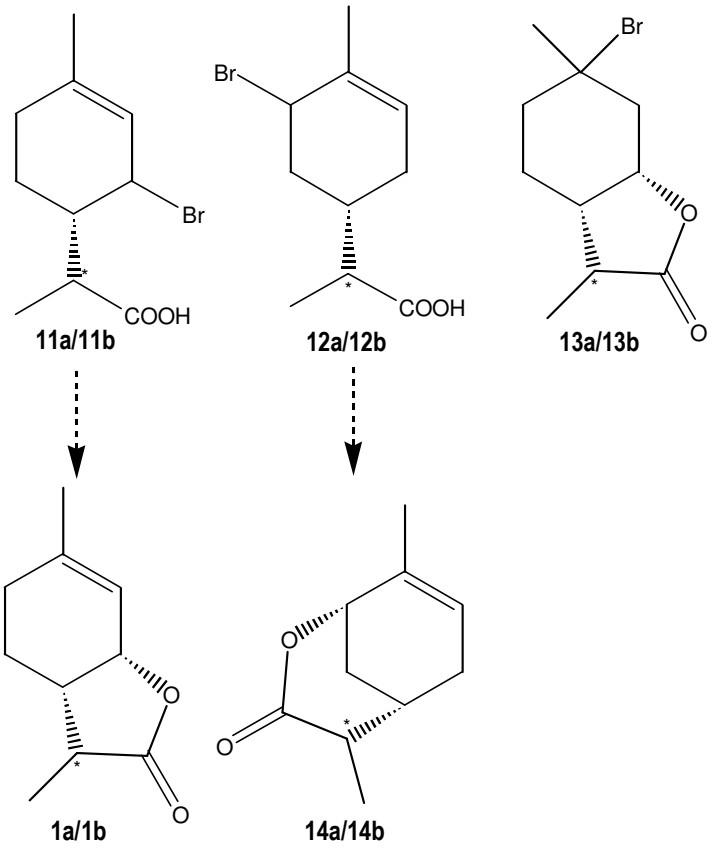
Scheme 8



The by-products from multiple batches of the radical reaction were combined and, upon silica gel chromatography, the two diastereomers of wine lactone (**1a**/**1c**) were successfully separated and enantiomerically pure wine lactone (**1a**) was isolated. This material, together with the labelled racemic wine lactone (**6a**/**6b**), was used for analytical method development.

Repeated attempts to synthesise additional diastereomeric wine lactone (**1a**/**1c**) in satisfactory yield from the acid (**10a**/**10b**) using N-bromosucinnimide (NBS) were unsuccessful (Scheme 7). Examination of the crude reaction products, by gas chromatography-mass spectrometry (GC-MS), revealed that it was a mixture of bromine-containing products, with molecular ions *m/z* 246 and 249. Additionally, small quantities of the desired wine lactone diastereomers (**1a**/**1c**) were observed among other unidentified compounds, presumably other lactones formed from competing allylic bromination products, or from hydrogen bromide addition to the desired lactone. Scheme 9 depicts some proposed bromine-containing products that correspond to the mass spectra observed. The first two compounds, **11a**/**11b** and **12a**/**12b**, which have molecular masses of 246 / 249 are products from the allylic substitution of the acid at the secondary position. There are potentially three positions where allylic bromination could take place; however, substitution at the secondary position occurs more readily than at the primary position, so it is not likely that bromination at the methyl group occurred [272]. The third product proposed (**13a**/**13b**), which also has the same molecular mass (246 / 249) is the desired lactone with the addition of HBr.

Scheme 9



The two major bromo-containing products were separated and, according to mass spectral data, were presumed to be the allylic brominated acids (**11a**/**11b**) and (**12a**/**12b**). These two compounds were found to be quite stable and no lactonisation occurred on addition of silver triflate.

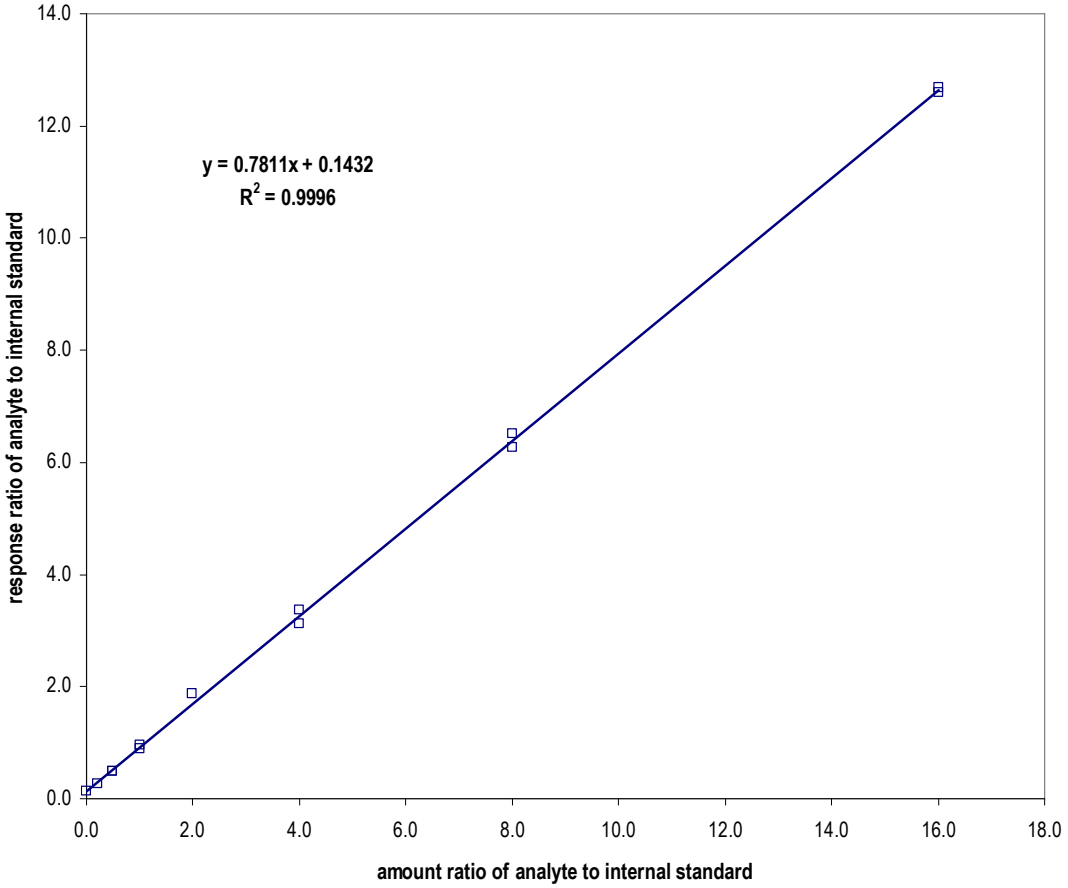
Attempts by column chromatography to separate the small amount of wine lactone (**1a**) produced in this reaction from the mixture of products generated were unsuccessful. Attempts to modify the reaction conditions to increase the amount of wine lactone formed in the reaction, also proved to be unsuccessful.

7.2.3 Analytical method development

In order to study wine lactone formation from the glucose ester (**2**) and the monoterpenoid acid (**3**), an analytical method for quantification of wine lactone was required. The method developed involved gas chromatography-mass spectrometry (GC-MS) and stable isotope dilution analysis (SIDA). Due to the difficulties of measuring wine lactone accurately at near-threshold (10 ng/L, [6]) and wine-like concentrations (100 ng/L, [6]), a higher concentration range was targeted for analytical method development and hydrolytic studies. Sample preparation, column type, oven temperatures and GC conditions were optimised for the analysis of wine lactone by GC-MS. During sample preparation, it was important to ensure

any remaining monoterpenoid acid (**3**) was not extracted. This was expected to eliminate the possibility of **3** forming wine lactone in the injector block of the GC-MS leading to elevated and misleading wine lactone concentrations. To avoid extraction of the acid (**3**) during sample preparation, the pH of the model wine was increased to above pH 7 using $NaHCO_3$ prior to solvent extraction to ensure the ionised acid remained in the aqueous layer.

Figure 7-3 Standard addition calibration function for wine lactone in model wine



concentrations plotted: 0, 100, 1000 (x 1 data point) and 250, 500, 2000, 4000, 8000 (x 2 data points) ng/L

The wine lactone standard addition curve developed for model wine (0, 100 - 8000 ng/L) is shown in Figure 7-3. The range 100 - 8000 ng/L was found to be linear and excellent repeatability was achieved at 2000 ng/L and 500 ng/L in model wine (Table 7-1).

Table 7-1 Repeatability of analysis for wine lactone in model wine

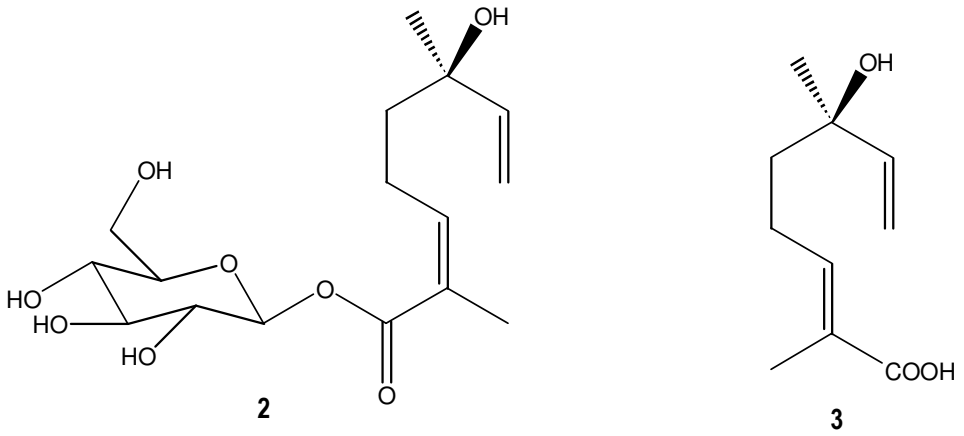
wine lactone concentration (ng/L)	repetitions	mean	standard deviation	standard deviation / mean
2000	4	1952	115	0.059
500	4	499.5	3	0.0058

7.2.4 Hydrolytic and chiral study

The rate of formation and the stereochemistry of wine lactone formed from the glucose ester (**2**) and the monoterpenoid acid (**3**) at pH values 3.0 and 3.4, and two temperatures (45°C and room temperature) was investigated.

The enantiomerically pure glucose ester of (6R)-(E)-2,6-dimethyl-6-hydroxyocta-2,7-dienoic acid (**2**) and the enantiomerically pure (6R)-(E)-2,6-dimethyl-6-hydroxyocta-2,7-dienoic acid (**3**) used in the hydrolytic study had been synthesised in this laboratory by Anders Hakansson according to published methods [273, 274] (Figure 7-4). This synthetic material was used for hydrolytic investigations.

Figure 7-4 Stereochemistry of glucose ester (2) and monoterpenoid acid (2)



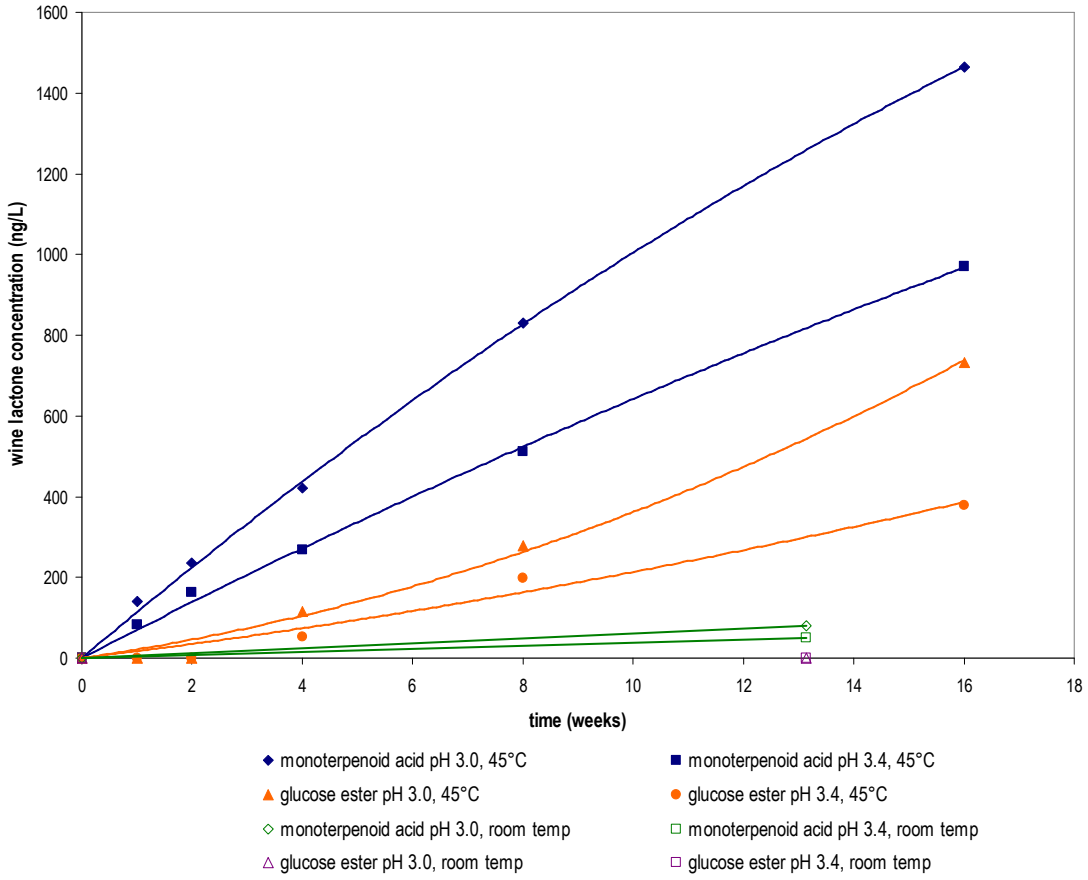
The results of the hydrolysis of the acid (**3**) and glucose ester (**2**) at room temperature and 45ºC are shown in Figure 7-5. Duplicate measurements did not vary by more than 30 ng/L. Wine lactone was formed from both the monoterpenoid acid (**3**) and the glucose ester (**2**). This is the first time that the formation of wine lactone from the glucose ester has been observed. Not surprisingly, wine lactone formed much more readily from the monoterpenoid acid (**3**) than the glucose ester (**2**). For both substrates a lower pH and higher temperature increased the rate of formation of wine lactone. At room temperature, wine lactone was just detectable after 3 months in the samples with the monoterpenoid acid (**3**) (at pH 3.0 and 3.4) but not with the glucose ester (**2**). Nevertheless, very low levels of wine lactone (**1**) might be formed after long periods of time from the glucose ester (**2**).

Given the very low reactivity of **2** and the high initial concentration of the glucose ester in this study (495 μg/L) the glucose ester is not a direct major precursor for the formation of wine lactone in wine as suggested by Winterhalter et al [270].

Low levels of wine lactone were formed at room temperature from the monoterpenoid acid (**3**) indicating the rate of formation of wine lactone from this substrate is also relatively slow

but faster than for the glucose ester (**2**). These results demonstrate that the free acid (**3**) is not likely to be an important precursor for wine lactone in young white wines. However **3** could represent an important precursor for wine lactone in white wine after storage for several years.

Figure 7-5 Hydrolytic formation of wine lactone from glucose ester (2) and monoterpenoid acid (3)



Samples from the 16 week time point of the 45°C study (both substrates, both pH's) and the 3 month time point of the room temperature (acid substrate, both pH's) study that had been prepared for analysis in the hydrolytic study were chosen for analysis by chiral GC. These samples contained the highest levels of wine lactone for each storage temperature and were most likely to give strong clear peaks by chiral GC.

Two enantiomers of wine lactone were identified by chiral GC in all samples analysed. The two unlabelled wine lactone isomers were identified as *3S,3aS,7aR* and *3R,3aR,7aS*-tetrahydro-3,6-dimethylbenzofuran-2(3H)-one (**1a** and **1b**) by comparison of retention times and mass spectral data with synthetic samples of enantiomerically pure (**1a**) and racemic (**1a**/**1b**) wine lactone. The ratios of peak areas (**1b** : **1a**) for the isomers in each sample are shown in Table 7-2. These responses have been standardised by dividing the peak area of

each unlabelled isomer by the peak area of its d_3 labelled analogue **6a**/**6b** added as internal standard. The ratios of the areas of **6a** and **6b** were in any case, close to 1 : 1.

Table 7-2 Peak area and peak height ratios in hydrolytic samples

substrate	pH	temperature	ratio of peak areas (1b : 1a)
monoterpenoid acid	3.0	45°C	1.07 : 1.00
monoterpenoid acid	3.2	45°C	0.99 : 1.00
glucose ester	3.0	45°C	0.98 : 1.00
glucose ester	3.2	45°C	1.07 : 1.00
monoterpenoid acid	3.0	room temperature	0.93 : 1.00

The ratios between isomers **1a** and **1b** for all the hydrolytic samples analysed by chiral GC were close to 1 : 1. These results show that the formation of wine lactone from both the enantiomerically pure glucose ester (**2**) and the enantiomerically pure monoterpenoid acid (**3**) is not enantioselective in wine-like conditions at either 45°C or at room temperature. Such lack of selectivity is not surprising. Enantioselective cyclisation of the acid (**3**) to a monocyclic intermediate is possible if the loss of the tertiary hydroxyl and formation of the cyclohexene ring is concerted, a process that is relatively facile and enantiospecific. Cyclisation of linalool to α-terpineol at wine pH is an example of such a reaction [275]. Formation of the lactone ring requires migration of a cation from the acid functionalised side chain into the cyclohexane ring – a process that would racemise any optically active intermediate species present. Other enantioselective pathways to **1a** are also difficult to envisage.

Since only one of the eight stereoisomers of wine lactone (**1a**) has been reported as present in young white wine [267], these results also support the conclusion that neither the glucose ester (**2**) nor the monoterpenoid acid (**3**) are significant precursors to wine lactone in young wine. Clearly other precursors must exist that are responsible for the enantiomeric enrichment of the **1a** isomer observed by Guth [267]. No other precursor for wine lactone has been reported in the literature to date.

Sensorily significant quantities of wine lactone (**1a**) could be formed, along with the relatively odourless enantiomer **1b** from the acid (**3**), and possibly also the glucose ester (**2**) over a period of several years. While the acid (**3**) has not yet been identified in grape berries or must, the formation of **3** from **2** by esterase action of wine micro-organisms (including fermentation yeasts) is feasible. Confirmation of these possibilities requires identification of **3** and **1b** as well as **1a** in older wines.

7.3 Materials and methods

7.3.1 General

All reagents used were purchased from SIGMA-Aldrich unless otherwise stated. All solvents used were HPLC grade from OmniSolv, with the exception of ethanol, which was fractionally distilled food grade ethanol. The water used was purified by a MilliQ system. Model wine was 10% ethanol in MilliQ water v/v saturated with potassium hydrogen tartrate and buffered to desired pH with tartaric acid. Positive ion electron impact (EI) mass spectra were recorded over a scan range of m/z 35 / 350 (1 second cycle time) with an Agilent 6890 gas chromatograph (GC) coupled to an Agilent 5973N mass spectrometer (MS) with a GERSTEL MPS2 Multi Purpose Sampler. ^{1}H and ^{13}C NMR spectra were recorded with a Varian Gemini Spectrometer operating at frequencies of 300 MHz and 75.5 MHz, respectively. Spectra were recorded in deuterated chloroform ($CDCl_3$). Chemical shifts (δ) are reported in parts per million (ppm) downfield. The following abbreviations are used in the assignment of ^{1}H spectra: s = singlet; d = doublet; t = triplet; q = quartet; m = multiplet; dd = doublet of doublets. Synthetic sequences were carried out by the author. Further bulk material was prepared by others and also included for use in this study. Samples of **2** and **3** were prepared by Anders Hakansson in this laboratory using published methods [273, 274].

7.3.2 Synthesis of enantiomerically pure wine lactone

Preparation of enantiomerically pure (3S,3aS,7aR) 3a,4,5,7a-tetrahydro-3,6-dimethylbenzofuran-2(3H)-one (1a), (Scheme 8)

Synthesis of (2RS)-2-((1R)-4-methylcyclohex-3-enyl)propanol (**9a**/**9b**)

(+)-(4R)-Limonene (**8**) (5.4 g, 40 mmol) was regioselectively hydroborated with a solution of 0.5 M 9-borabicyclo-[3.3.1]nonane (9-BBN) in tetrahydrofuran (THF) (80 mL, 40 mmol) according to the general procedure reported by Brown [276] and used by Guth [267]. The product was purified via silica gel chromatography (Rf 0.36, 20% ethyl acetate / petroleum spirit) followed by Kugelrohr distillation to give a 1 : 1 diastereomeric mixture of the title compound as a clear colourless oil (5.99 g) with a yield of 98% (>99% pure). MS and NMR spectra obtained of the product were in agreement with published spectra [267].

Synthesis of (2RS)-2-((1R)-4-methylcyclohex-3-enyl)propanoic acid (**10a**/**10b**) and 3S,3aS,7aR-tetrahydro-3,6-dimethylbenzofuran-2(3H)-one (**1a**)

The diastereoisomeric alcohol (**9a**/**9b**) was oxidised using the free radical 2,2,6,6-tetramethyl-1-piperidinyloxyl (TEMPO) and bis-acetoxyiodobenzene (BAIB) in aqueous acetonitrile under similar conditions to those reported by Raunkjaer et al [271]. The alcohol **9a**/**9b** (2 g, 13 mmol) was dissolved in acetonitrile (8 mL) and water (8 mL). TEMPO (0.4g, 2 mmol) and BAIB (9.6g, 30 mmol) were added and the reaction stirred at ~3ºC for five days

under nitrogen. The reaction was quenched with aqueous citric acid (5%, 20 mL) and extracted with ethyl acetate (1 x 20 mL) and diethyl ether (2 x 20 mL). The combined organic extracts were dried ($MgSO_4$) and the solvent evaporated in vacuo to give a crude oil. The combined crude products from two batches of the reaction were purified by silica gel column chromatography (Rf 0.24, 20% ethyl acetate / petroleum spirit) to give a 1 : 1 diastereomeric mixture of **10a**/**10b** as a clear colourless oil (1.24 g) with a yield of 28%. MS and NMR spectra obtained of the product were in agreement with published spectra [267]. Fractions that contained wine lactone, from multiple batches of the silica gel column chromatography purification of **10a**/**10b** above, were combined (2.36 g). This material was dissolved in diethyl ether (150 mL) and washed with saturated sodium carbonate (6 x 25 mL) and saturated sodium chloride (4 x 5 mL). The organic phase was then stirred with aqueous hydrochloric acid (0.1M, 50 mL) for 30 minutes. The aqueous phase was extracted with diethyl ether (4 x 5 mL) and the combined organics were washed with saturated sodium chloride (4 x 5 mL), dried ($NaSO_4$), and the solvent evaporated in vacuo to give crude diastereomeric wine lactone **1a**/**1c** (0.45 g). The acid starting material (**10a**/**10b**) was reclaimed from the aqueous extracts by acidification and extraction with diethyl ether. Isomers **1a** and **1c** were separated by silica gel column chromatography using ethyl acetate / hexane (1 : 4) (**1a** Rf 0.40, **1c** Rf 0.33) to give enantiomerically pure **1a** (0.085 g) enantiomerically pure **1c** (0.101 g) and an diastereomeric **1a**/**1c** (0.061 g). Enantiomerically pure **1a** was distilled by Kugelrohr (60°C, 0.2 mm Hg) to give pure **1a** (0.076 g). MS and NMR spectra obtained of the product were in agreement with published spectra [267]. Purification by chromatography was performed by Kevin Pardon in this laboratory.

Attempted synthesis of diastereoisomers (3S,3aS,7aR and 3R,3aS,7aR) of 3a,4,5,7a-tetrahydro-3,6-dimethylbenzofuran-2(3H)-one (**1a**/**1c**) by bromination

N-bromosuccinimide (0.115g, 0.65 mmol) and benzoyl peroxide (~1 mg) were added to the acid **10a**/**10b** (100 mg, 6.5 mmol) dissolved in carbon tetrachloride (7 mL). Pyridine (56 mg, 0.7 mmol) was added and the reaction mixture heated to reflux for 2 hours. The cooled reaction was quenched with water (20 mL) and extracted with dichloromethane (2 x 20 mL). The combined organic extracts were washed with hydrochloric acid (10%, 2 x 20 mL), dried ($MgSO_4$) and the solvent evaporated in vacuo to give a crude residue. Examination by GC-MS showed that it was a mixture of products with one major bromo-containing product. *m/z* 248 (M+, 1%), 246 (M+, 1%), 220 (5%), 218 (5%), 205 (4%), 203 (4%), 178 (16%), 176 (17%), 167 (25%), 139 (31%), 123 (52%), 111 (49%), 96 (100%), 95 (79%), 81 (75%), 67 (34%), 55 (51%). The crude products were separated using silica gel column chromatography (20% ethyl acetate / petroleum spirit). The two major brominated products (~10 mg each) were subsequently dissolved in dichloromethane (5 mL) and treated with

triethylamine (2 drops) and silver triflate (~2 mg). After five days at room temperature, the monitored reaction mixtures showed no change to the starting material.

7.3.3 Preparation of samples for hydrolytic study

Model wine prepared at pH 3.0 or 3.4 was measured into 2 L volumetric flasks and spiked with a stock solution (in ethanol) of either the monoterpenoid acid (**3**) or the glucose ester (**2**) to give the four solutions detailed in Table 7-3.

Table 7-3 Summary of solutions prepared

substrate	pH	solution volume	concentration of substrate
monoterpenoid acid	3.0	2 L	252 µg/L
monoterpenoid acid	3.4	2 L	252 µg/L
glucose ester	3.0	2 L	495 µg/L
glucose ester	3.4	2 L	495 µg/L

To avoid dissolving oxygen in the solutions, an anaerobic hood was used to decant the solutions into 50 mL ampoules. Ampoules were removed from the hood, sealed under nitrogen, and stored in darkness at 45°C or at room temperature. Samples were then stored at -18°C prior to analysis.

7.3.4 Analytical method for the determination of wine lactone

7.3.4.1 Preparation of samples for analysis

Each sample (50 mL) was spiked with internal standard (100 µL, d_3-wine lactone **6a**/**6b** 0.25 µg/mL in ethanol). After mixing, the sample was decanted into a 50 mL measuring cylinder (equipped with glass stopper) containing sodium hydrogen carbonate ($NaHCO_3$) (~3 g). Pentane / ethyl acetate (2 : 1, 5 mL) was added and the solution was shaken thoroughly to mix. After settling (~1 hr), the organic layer was removed and concentrated with a stream of nitrogen to approximately 0.4 mL. The concentrate was transferred into a GC-MS vial and capped for analysis.

7.3.4.2 Instrumental analysis

Samples were analysed by GC-MS. The GC was fitted with a DB-WAX fused silica capillary column (J&W, 122-7032, 30 m x 0.25 mm x 0.25 µm) for quantitation and a CycloSil-B fused silica chiral capillary column (J&W, 122-6632, 0.25 mm x 0.25 µm) for chiral analysis. The carrier gas was helium (Air Liquide or BOC gases, ultra high purity), linear velocity 39 cm/sec, flow rate 1.2 mL/min, vacuum compensated at the mass spectrometer interface. For quantitation, the oven temperature was started at 50˚C, held at this temperature for 1 min, increased to 240˚C at 10˚C/min, and held at this temperature for 10 min. For chiral analysis, the oven temperature was started at 60˚C, held at this temperature for 1 min, increased to 150˚C at 10˚C/min, then increased to 230˚C at 3˚C/min and held at this temperature for 5

min. The injector, in pulsed splitless mode, was held at 220˚C (200˚C for chiral analysis) and the transfer line at 240˚C (230˚C for chiral analysis). The splitter, at 44 : 1, was opened after 36 sec. The sample injection volume was 2 µL. The liner used was resilanised borosilicate glass, tapered, with a plug (2 - 4 mm) of resilanised glass wool near the column interface. The residence time for the needle in the injector block was 100 ms. Positive ion electron impact spectra at 70 eV were recorded in the range *m/z* 35 - 350 for scan runs. For quantification of wine lactone, mass spectra were recorded in selected ion monitoring (SIM) mode. The ions monitored for quantitation are detailed in Table 7-4 and for chiral analysis in Table 7-5.

Table 7-4 Ions monitored in analytical method used to quantify wine lactone

standard	rt (min) [a]	qt ion (*m/z*) [b]	qf ions (*m/z*) [c]	analyte	rt (min) [a]	qt ion (*m/z*) [b]	qf ions (*m/z*) [c]
d_3-wine lactone	17.18	169	154, 141, 126	wine lactone	17.20	166	151,138, 123

[a] rt: retention time of peak; [b] qt ion: ion used for quantitation; [c] qf ions: ions used for qualification

Table 7-5 Ions monitored in analytical method used to quantify wine lactone isomers

standard	rt (min) [a]	qt ion (*m/z*) [b]	qf ions (*m/z*) [c]	analyte	rt (min) [a]	qt ion (*m/z*) [b]	qf ions (*m/z*) [c]
d_3-wine lactone **6b**	13.23	154	169, 141, 126	wine lactone **1b**	13.28	151	166,138, 123
d_3-wine lactone **6a**	13.52	154	169, 141, 126	wine lactone **1a**	13.56	151	166,138, 123

[a] rt: retention time of peak; [b] qt ion: ion used for quantitation; [c] qf ions: ions used for qualification

7.3.4.3 Method validation

A calibration curve for wine lactone was obtained by spiked standard additions to model wine (pH 3.3). Enantiomerically pure wine lactone (**1a**) was added to give concentrations of 0, 100, 250, 500, 1000, 2000, 4000, 8000 ng/L. All spiked samples were prepared, extracted and analysed in duplicate as described above. Six replicates of the 2000 and 500 ng/L spiked samples were prepared, extracted and analysed to test the repeatability of the method. The calibration curve was linear throughout the concentration range with a coefficient of determination (R^2) of 0.9996 for wine lactone. When samples were analysed, they were checked against duplicate standards of 500 ng/L of **6a**/**6b** and 0 or 2000 ng/L of **1a** to adjust for response factor ratio drift.

APPENDICES

Appendix A Summary of raw data collected for Riesling wines

Table A-1 Summary of the winemaking details and routine chemical data collected for the Riesling study wines

wine code	year [a]	region [b]	varietal purity	closure [c]	retail price ($AUD)	alcohol (% v/v)	pH	titratable acidity (g/L) [d]	volatile acidity (g/L)	glucose + fructose (g/L)	SO_2 free / total (mg/L) [e]	total dry extract (g/L)	specific gravity
1	2002	Central Vic	100% Riesling	cork	$19.00	12.7	3.08	6.5	0.18	4.2	20 / 135	24	0.9927
2	2002	Clare Valley, SA		screw cap	$33.00	13.5	3.13	7.2	0.42	0.4	24 / 101	19	0.9899
3	2002	Blend, SA	100% Riesling	cork	$10.00	12.5	3.01	6.4	0.32	5.3	11 / 98	25	0.9934
4	2001	Clare valley, SA / WA	100% Riesling	screw cap	$7.00	12.5	3.05	6.9	0.40	0.9	22 / 114	21	0.9916
5	2002	ACT region, NSW	100% Riesling	screw cap	$18.00	13.7	3.05	6.6	0.62	4.0	19 / 118	22	0.9906
6	1996	Coonawarra, SA	100% Riesling	cork	$26.00	11.4	2.95	6.4	0.26	5.1	10 / 108	24	0.9940
7	2000	Blackwood Park, WA	100% Riesling	cork	$17.00	13.0	3.03	7.0	0.31	5.9	15 / 127	26	0.9932
8	2002	Barossa Valley, SA	100% Riesling	cork	$9.00	12.5	3.11	6.2	0.29	2.6	22 / 106	21	0.9917
9	1993	Eden Valley, SA		cork	$35.00	12.1	2.98	6.7	0.35	6.2	12 / 62	25	0.9936
10	2002	Northern Tas		screw cap	$25.00	12.5	3.21	6.3	0.27	9.5	20 / 115	20	0.9912
11	2002	Mount Barker, WA	100% Riesling	screw cap	$14.50	12.8	3.19	7.0	0.36	0.7	18 / 88	20	0.9911
12	2002	Blend, WA	100% Riesling	screw cap	$17.50	12.0	3.05	7.4	0.29	4.3	19 / 120	24	0.9936
13	1997	Eden Valley, SA		cork	$35.00	13.0	3.11	6.7	0.40	3.5	8 / 71	23	0.9919
14	1997	Clare Valley, SA		cork	$24.00	12.5	3.10	5.6	0.36	3.4	7 / 78	21	0.9917
15	1999	Grampians, Vic		cork	$30.00	12.6	3.06	7.4	0.31	4.5	17 / 100	23	0.9926
16	2001	Coonawarra, SA		cork	$16.00	12.2	2.94	6.2	0.43	3.7	25 / 126	22	0.9925
17	2001	Clare Valley, SA		screw cap	$20.00	10.8	2.86	6.6	0.27	3.6	24 / 84	21	0.9938
18	2002	Blend, SA		screw cap	$10.00	11.8	3.04	6.3	0.35	5.7	21 / 107	24	0.9937
19	2002	Eden Valley, SA		cork	$17.00	12.8	3.07	6.4	0.22	3.1	28 / 148	22	0.9918
20	2002	Eden Valley, SA		cork	$11.00	12.6	3.11	6.2	0.27	4.5	28 / 144	24	0.9926

[a] wine age at the time of analysis: vintage 2002 (~ 6 months), 2001 (1.5 years), 2000 (2.5 years), 1999 (3.5 years), 1997 (5.5 years), 1996 (6.5 years), 1993 (9.5 years); [b] Vic: Victoria, SA: South Australia, WA: Western Australia, NSW: New South Wales, Tas: Tasmania; [c] cork refers to natural bark cork; [d] titratable acidity as tartaric acid (at pH 8.2); [e] SO_2: sulfur dioxide

Table A-2 Mean sensory scores (aroma) collected for the Riesling study wines

wine code	*estery*	*perfumed floral*	*dried rose*	*lemon*	*grapefruit*	*lime*	*lychee*	*pineapple*	*passionfruit*	*herbaceous*	*stewed apple*	*apricot*	*honey*	*toasty*	*caramel*	*kerosene*	*rubber / plastic*
1	3.41	3.14	1.92	2.13	0.93	1.44	1.25	2.00	2.52	1.30	0.69	1.00	0.89	0.26	0.56	0.13	0.11
2	3.33	2.86	0.91	2.75	1.41	2.12	1.23	2.30	1.08	0.83	1.00	0.90	0.63	0.26	0.35	0.28	0.37
3	2.18	1.54	1.16	2.19	0.78	2.06	0.55	1.45	0.53	0.57	0.94	0.98	1.67	1.20	0.91	1.09	0.23
4	3.80	3.09	1.37	2.54	1.10	1.25	1.44	2.42	1.55	0.68	0.50	1.05	0.72	0.21	0.54	0.24	0.08
5	3.36	2.82	1.66	2.83	1.23	1.63	1.05	1.84	1.28	0.91	0.79	1.30	0.95	0.28	0.56	0.17	0.03
6	1.30	0.56	0.46	0.77	0.41	3.29	0.31	0.92	0.24	0.68	1.35	0.96	3.66	5.21	2.53	3.15	0.39
7	1.21	0.90	0.81	1.04	0.67	3.69	0.55	1.19	0.46	0.55	1.46	1.25	3.01	3.24	1.67	3.37	0.65
8	3.25	2.92	1.18	2.71	1.40	1.69	1.04	2.06	1.06	0.91	0.52	1.00	1.02	0.44	0.52	0.20	0.26
9	1.55	0.65	0.80	1.10	0.43	3.30	0.67	0.86	0.40	0.41	1.10	0.67	3.11	4.43	2.42	3.34	0.23
10	3.87	3.45	2.04	2.08	1.30	1.18	1.48	2.13	1.31	0.80	0.95	0.83	0.62	0.34	0.48	0.27	0.09
11	2.54	1.58	1.41	2.19	1.82	1.76	1.23	1.86	4.54	3.04	0.78	0.54	0.34	0.31	0.22	0.21	0.08
12	2.74	2.21	0.95	2.27	1.22	1.70	1.61	2.38	2.49	1.52	0.72	0.92	0.61	0.61	0.30	0.33	0.06
13	1.47	0.73	1.04	1.10	0.51	2.59	0.61	1.58	0.47	0.41	1.43	0.95	3.35	3.40	2.36	2.27	0.45
14	1.29	0.76	0.85	1.16	0.53	3.16	0.42	1.52	0.41	0.53	1.46	0.92	2.96	3.74	2.01	2.88	0.44
15	2.02	1.47	0.67	1.23	1.00	2.16	0.83	1.44	0.77	0.82	0.83	0.75	1.78	2.30	1.30	1.51	1.48
16	2.28	1.46	0.71	2.03	1.48	2.32	0.73	1.61	0.70	0.89	0.88	0.86	1.64	1.39	1.08	1.34	0.55
17	1.84	1.28	0.62	1.50	0.86	2.78	0.66	1.16	0.37	0.67	1.15	0.80	1.69	2.01	1.48	2.88	1.01
18	3.36	3.43	1.43	2.97	1.35	1.63	0.82	2.37	1.85	1.10	0.69	0.88	0.84	0.17	0.72	0.11	0.07
19	3.88	3.64	1.59	2.49	1.39	1.34	0.92	2.05	1.36	0.47	0.62	0.77	0.76	0.26	0.55	0.15	0.08
20	4.22	3.35	1.08	2.83	1.09	1.49	0.93	2.16	0.97	0.58	0.87	0.63	0.81	0.22	0.48	0.25	0.13

Table A-3 Mean sensory scores (flavour) collected for the Riesling study wines

wine code	*sourness*	*sweetness*	*overall flavour*	*flavour persistence*	*astringency*	*bitterness*	*taste other*
1	5.55	1.00	4.46	4.64	1.53	0.76	0.14
2	5.07	1.39	4.65	4.77	1.69	0.84	0.13
3	5.18	1.25	4.73	4.81	1.64	0.63	0.22
4	4.76	1.83	4.84	4.84	1.33	0.71	0.23
5	5.40	1.26	4.87	5.07	1.68	0.92	0.10
6	4.58	1.58	4.37	4.22	1.38	0.71	0.06
7	4.92	1.88	4.99	4.99	1.62	0.86	0.30
8	5.05	1.22	5.15	5.15	1.48	0.79	0.30
9	4.73	1.21	4.72	5.05	1.51	0.85	0.10
10	5.02	1.10	4.82	4.98	1.54	0.78	0.40
11	4.78	1.40	4.65	4.66	1.62	0.67	0.27
12	5.13	1.45	5.49	5.60	1.57	0.51	0.12
13	4.63	1.21	4.74	4.97	1.44	0.75	0.34
14	4.82	1.48	4.57	4.65	1.78	0.72	0.06
15	4.98	1.15	4.27	4.21	1.44	1.05	0.34
16	5.48	1.09	4.92	5.30	1.43	0.78	0.33
17	5.32	1.05	4.71	4.80	1.64	0.72	0.02
18	5.47	1.30	4.57	4.49	1.49	0.85	0.30
19	5.13	1.40	4.74	4.78	1.48	0.40	0.30
20	4.65	1.79	4.50	4.41	1.61	0.79	0.44

Table A-4 Mean volatile compound concentrations (µg/L) collected for the Riesling study wines

wine code	ethyl acetate	ethyl propanoate	ethyl butanoate	ethyl 2-methyl propanoate	ethyl 2-methyl butanoate	ethyl 3-methyl butanoate	ethyl hexanoate	ethyl octanoate	ethyl decanoate	ethyl dodecanoate	*trans*-ethyl cinnamate	2-methylpropyl acetate	2-methylbutyl acetate	3-methylbutyl acetate	hexyl acetate
1	55850	257	497	70	13	14	1203	2417	1123	160	0.4	58	191	2898	229
2	98710	211	577	85	7	14	1704	1640	985	35	0.3	43	100	2389	163
3	85529	204	464	102	17	31	1306	1665	528	67	0.5	11	14	208	16
4	84089	150	486	67	8	13	1489	1796	1040	95	0.3	28	71	1474	170
5	117130	192	486	53	6	12	1266	1583	919	60	0.2	32	71	1737	140
6	58706	136	438	102	38	50	1077	821	181	nd	0.8	8	nd	38	0.1
7	81947	240	520	85	23	57	1417	1427	413	6	0.4	10	nd	101	nd
8	65241	161	421	53	6	13	1471	2521	1449	103	1	40	95	2099	238
9	74519	337	305	107	30	45	955	1110	175	0	0.6	11	nd	62	nd
10	69046	154	545	53	5	8	1249	2190	637	101	1	54	163	3183	342
11	78423	154	435	47	4	9	1296	1835	830	44	1	38	114	2386	227
12	60243	109	400	84	8	15	1264	3219	1028	145	1	25	53	1181	126
13	97754	188	461	87	30	49	1372	1486	357	3	1	11	nd	88	0.8
14	82408	200	409	74	24	47	1253	1315	285	0.3	0.4	11	nd	82	0.4
15	65389	171	349	143	29	46	1211	1468	632	46	2	12	9	78	nd
16	99481	200	402	76	14	32	1373	1293	659	26	2	14	22	295	23
17	57701	261	311	74	11	26	1159	1266	560	51	0.8	7	nd	80	0.8
18	49073	192	377	48	4	10	1177	1645	570	79	1	30	59	1516	220
19	60222	164	599	47	8	12	1486	2206	871	79	0.6	43	185	3667	389
20	70788	137	573	44	7	13	1534	1877	1002	83	1	40	130	3423	411

compound not detected (nd)

Table A-4 Mean volatile compound concentrations (µg/L) collected for the Riesling study wines (continued)

wine code	2-phenylethyl acetate	2-phenyl ethanol	butanol	2-methyl propanol	2-methyl butanol	3-methyl butanol	hexanol	acetic acid [a]	2-methyl propanoic acid	3-methyl butanoic acid	hexanoic acid	octanoic acid	decanoic acid	linalool	α-terpineol	nerol	geraniol	*cis*-rose oxide
1	2059	85393	824	18784	30606	94973	1084	201168	635	460	7532	7578	2613	160	115	11	36	0.2
2	182	14765	733	10749	15190	90329	904	512292	771	307	9704	8768	2670	49	40	4	16	0.1
3	24	12412	735	10272	17068	95504	1486	369320	503	289	9959	8490	1943	11	117	19	9	0.09
4	103	10666	928	10017	15083	90152	1146	443704	487	265	9128	8514	2435	112	97	9	27	0.08
5	122	13285	927	14560	16175	94482	961	780212	473	312	5637	9572	3113	102	78	6	23	0.1
6	5	10001	729	10728	14287	86573	1777	372482	549	385	7724	8438	1647	nd	8	48	9	0.1
7	10	14250	784	11017	15689	112432	1670	413403	349	378	6912	9074	2036	3	107	22	9	0.2
8	128	11565	1473	15928	19445	105427	1849	348877	454	262	8049	8717	2968	54	51	4	19	0.1
9	8	15039	464	15746	16053	71069	2583	424909	534	315	5798	7563	1414	nd	8	27	8	0.1
10	297	14875	757	13373	29931	98022	1575	327387	572	270	7534	8116	1696	112	79	7	28	0.3
11	182	12337	980	11184	16798	100171	1322	431476	458	226	9511	9585	2827	41	39	3	16	0.2
12	94	10296	841	13030	18099	88025	1369	366196	707	302	8044	7893	2441	52	71	3	15	0.3
13	11	17321	709	15463	21052	99065	2407	509041	374	347	9046	9986	2466	0.6	39	19	9	0.1
14	9	14456	1946	13191	18229	99570	1842	418899	346	283	8409	8874	1877	0.6	51	18	10	0.07
15	18	29464	480	18268	19271	96133	1339	376299	644	316	7473	8082	1870	0.9	29	6	7	0.1
16	44	15782	928	12809	17049	104330	1805	501098	339	274	8351	9256	2723	13	99	11	9	0.1
17	9	9175	529	9390	13845	105628	1167	349987	375	221	10001	11230	3670	2	59	26	7	0.06
18	113	10927	671	11730	12511	66767	2254	425102	401	226	5324	8773	2877	55	65	5	18	0.1
19	913	56522	819	13364	20822	105404	1651	264008	431	387	6443	9912	2469	68	57	5	23	0.2
20	553	38945	833	12062	19704	98541	1738	326610	405	329	8406	10629	2977	61	50	5	23	0.2

[a] acetic acid measured as volatile acidity; compound not detected (nd)

Table A-4 Mean volatile compound concentrations (μg/L) for the Riesling study wines (continued)

wine code	TDN	TPB	β-damascenone	guaiacol	4-methyl guaiacol	4-ethyl phenol	4-ethyl guaiacol	4-vinyl guaiacol	4-vinyl phenol	*cis*-oak lactone	vanillin	diacetyl	methionol	dimethyl sulfide	carbon disulfide
1	3	0.005	6.6	0.39	0.13	0.51	0.1	0.4	0.4	2	9	19	1061	6	nd
2	2	0.003	4.6	0.25	0.03	0.19	0	0.5	0.6	0.9	nd	m	312	15	0.5
3	31	0.005	2.6	0.38	0.26	0.005	0.08	0.05	0.02	4	0.5	m	326	17	0.4
4	4	0.007	5.2	0.17	0.03	nd	0.2	0.4	0.3	2	nd	nd	392	11	0.6
5	2	0.003	6.2	0.36	0.04	nd	nd	0.3	0.3	0.9	nd	m	428	4	nd
6	93	0.03	1.2	1.96	0.67	nd	nd	0.07	0.04	0.3	nd	m	328	79	1
7	52	0.02	2.1	1.13	0.41	nd	0.07	0.06	0.04	0.2	nd	35	443	23	0.3
8	2	0.005	5.2	0.27	0.09	0.15	nd	0.2	0.5	0.4	nd	nd	390	11	0.4
9	54	0.02	0.7	1.16	0.52	0.15	0.1	0.03	0.02	4	48	m	360	81	1
10	2	0.006	2.8	0.46	0.10	1.22	0.2	0.2	0.7	0.3	0.5	30	333	12	0.3
11	1	0.006	3.1	0.24	0.04	0.14	0.005	0.3	0.9	0.4	0.6	nd	380	24	0.3
12	4	0.02	2.9	0.42	0.09	0.06	nd	0.2	0.6	0.4	0.06	m	685	19	0.6
13	37	0.02	1.4	0.83	0.34	nd	0.03	0.06	0.07	3	30	33	458	63	0.7
14	47	0.04	1.3	0.81	0.37	nd	0.03	0.05	0.04	3	29	7	278	31	0.7
15	24	0.01	1.4	0.34	0.18	0.26	nd	0.03	0.02	0.7	14	m	781	53	1
16	20	0.01	1.9	0.38	0.18	0.6	nd	0.09	0.2	1	4	m	308	18	0.4
17	56	0.03	1.6	0.65	0.29	nd	nd	0.06	0.03	0.8	nd	9	327	25	nd
18	5	0.005	4.7	0.31	0.13	nd	0.005	0.3	0.7	0.2	nd	m	285	8	0.3
19	2	0.005	3.7	0.28	0.05	0.15	nd	0.3	0.3	0.02	2	m	681	10	0.3
20	2	0.002	4.1	0.24	0.09	0.26	nd	0.3	0.4	0.07	2	11	552	22	0.5

compound not detected (nd); compound not measured indicated by missing value (m)

Appendix B Summary of raw data collected for unwooded Chardonnay wines

Table B-1 Summary of the winemaking details collected for the unwooded Chardonnay study wines

wine code	year [a]	region [b]	varietal purity	addition of oak	malolactic fermentation	yeast lees contact	closure	retail price ($AU)
1	2001	Adelaide Hills, SA	100% Chardonnay	no oak	no MLF	no contact	cork	$18.00
2	2001	Hunter Valley, NSW	100% Chardonnay	no oak	portion MLF	no contact	cork	$13.00
3	2001	McLaren Vale, SA	100% Chardonnay	no oak	no MLF	light lees contact ~ 6 months	cork	$14.00
4	2002	North-West Vic	100% Chardonnay	no oak	no MLF	no contact	cork	$9.99
5	2002	McLaren Vale, SA	92.5% Chardonnay, 5.7% Semillon, 1.8% Verdelho	no oak	no MLF	no contact	cork	$15.99
6	2002	Pipers Brook, Tas	100% Chardonnay	no oak	no MLF	no contact	cork	$20.69
7	2002	Swan Valley, WA	86% Chardonnay 13.2% Chenin Blanc 0.8% Verdello	no oak	no MLF	no contact	cork	$11.99
8	2002	Mount Barker, WA	94.05% Chardonay, 2.71% Sauvignon Blanc, 1.31% Sultana, 0.77% Chenin blanc, 0.26% Riesling, 0.45% Semillon, 0.4% Tramminer, 0.39% Verdello	no oak	no MLF	no contact	cork	$13.60
9	2002	Gingin, WA	100% Chardonnay	no oak	no MLF	no contact	cork	$9.56
10	2002	Eden Valley, SA					cork	$35.00
11	2002	Adelaide Hills, SA	>98% Chardonnay, 0.63% Riesling, 0.18% Riesling, 0.72% Semillon	no oak	no MLF	lees contact ~ 3-4 weeks	screw cap	$16.60
12	2002	Pemberton, WA	>97% Chardonnay, <3% Sauvignon Blanc	no oak	minimal if any	no contact	cork	$16.15
13	2002	McLaren Vale, SA	100% Chardonnay	no oak	no MLF	light lees contact ~ 6 months	cork	$14.00
14	2002	Limestone Coast, SA	100% Chardonnay	< 0.1 mg/L	no MLF	lees contact ~ 6 weeks	cork	$13.60
15	2002	Clare Valley / Limestone Coast, SA	85% Chardonnay, 15% Semmillon	no oak, tannin added	30% MLF	previous years wine lees added back to ferments	cork	$10.70
16	2002	Limestone Coast, SA	96% Chardonnay, 4% Riesling	no oak	no MLF	lees contact ~ 1 month	cork	$10.99
17	2002	Blend, SA					cork	$9.99
18	2002	Barossa Valley, SA					cork	$12.99
19	2002	Blend, SA	100% Chardonnay	no oak	no MLF	lees contact ~ 2 months	cork	$10.99
20	2002	Clare Valley, SA					screw cap	$14.00

[a] wine age at the time of analysis: vintage 2002 (~ 1 year), 2001 (~ 2 years); [b] Vic: Victoria, SA: South Australia, WA: Western Australia, NSW: New South Wales, Tas: Tasmania, blend: a blend of multiple regions

Table B-2 Summary of the routine chemical data collected for the unwooded Chardonnay study wines

wine code	alcohol (% v/v)	pH	titratable acidity (g/L) [a]	volatile acidity (g/L)	glucose + fructose (g/L)	SO_2 free / total (mg/L) [b]	total dry extract (g/L)	specific gravity
1	13.6	3.26	6.3	0.27	3.5	23 / 149	24	0.9916
2	12.7	3.23	6.4	0.21	3.6	24 / 122	24	0.9925
3	13.7	3.32	6.6	0.50	2.7	15 / 143	25	0.9920
4	13.4	3.39	6.6	0.37	1.6	30 / 126	24	0.9917
5	13.1	3.23	6.5	0.31	3.6	24 / 115	23	0.9918
6	14.6	3.09	8.9	0.63	7.5	9 / 53	31	0.9934
7	13.3	3.32	6.7	0.33	5.7	34 / 147	27	0.9932
8	13.4	3.42	6.1	0.32	3.5	25 / 135	25	0.9922
9	13.5	3.41	6.4	0.36	3.6	27 / 126	23	0.9915
10	13.8	3.30	6.5	0.26	3.7	24 / 122	26	0.9923
11	13.9	3.37	6.6	0.23	1.7	26 / 135	23	0.9910
12	13.4	3.17	5.6	0.35	4.6	36 / 136	27	0.9930
13	13.2	3.27	6.9	0.40	6.6	19 / 153	28	0.9938
14	13.7	3.31	6.8	0.33	5.3	31 / 132	27	0.9928
15	13.1	3.48	6.5	0.47	4.3	13 / 98	27	0.9933
16	13.9	3.21	6.5	0.37	1.9	26 / 113	21	0.9902
17	12.0	3.39	6.4	0.30	5.6	25 / 142	27	0.9945
18	12.6	3.42	6.1	0.36	3.4	27 / 109	23	0.9923
19	13.7	3.29	6.4	0.35	2.1	24 / 136	23	0.9911
20	13.8	3.19	7.1	0.56	2.8	8 / 100	24	0.9915

[a] titratable acidity (at pH 8.2); [b] SO_2: sulfur dioxide

Table B-3 Mean sensory scores (aroma) collected for the unwooded Chardonnay study wines

wine code	*estery*	*floral*	*lychee*	*citrus*	*pineapple*	*stewed apple / pear*	*stone fruit*	*passionfruit*	*herbaceous*	*sweaty*	*honey*	*butterscotch*	*woody*	*spicy*
1	2.21	1.23	1.24	1.16	1.70	1.50	2.20	0.57	0.38	0.59	1.90	1.72	2.18	1.48
2	2.25	0.95	0.82	1.89	1.58	2.00	1.41	0.76	0.35	0.62	2.60	2.15	1.95	1.21
3	2.31	1.28	1.63	1.41	1.91	1.30	1.83	0.41	0.51	0.54	2.12	1.28	1.21	1.00
4	2.61	1.60	1.08	1.70	2.48	1.93	1.39	0.61	0.44	0.33	1.73	1.00	1.18	1.42
5	2.47	1.86	0.77	1.59	2.32	1.83	1.51	0.61	0.55	0.26	1.70	0.80	1.13	0.78
6	2.30	0.99	1.53	1.11	1.84	0.60	1.27	4.56	1.85	2.87	0.71	0.50	0.48	0.71
7	2.50	1.86	0.98	1.32	2.51	1.27	1.79	1.14	0.51	0.72	1.22	0.90	1.04	0.81
8	3.25	2.09	1.21	1.63	2.02	1.53	1.85	1.13	0.43	0.88	1.43	0.63	1.23	0.79
9	2.40	1.08	1.59	1.26	1.85	0.40	1.59	5.60	2.08	2.52	0.68	0.34	0.47	0.30
10	2.57	1.70	1.43	1.51	1.91	1.53	2.06	0.55	0.48	0.92	1.54	1.05	1.36	1.05
11	3.03	1.74	2.33	1.62	2.21	1.29	2.03	3.33	1.08	1.72	1.00	0.24	0.50	0.56
12	3.39	2.17	1.34	1.79	2.82	1.82	2.15	1.37	0.59	0.95	0.76	0.65	0.69	0.76
13	2.60	1.47	1.41	1.76	2.34	1.54	1.80	1.60	0.86	1.08	1.12	0.79	0.74	0.84
14	2.91	1.79	1.07	2.02	2.12	1.57	1.81	0.93	0.46	0.50	1.35	1.25	1.55	1.39
15	2.62	1.40	1.20	1.25	2.19	1.88	2.15	0.59	0.26	0.70	2.21	1.37	0.87	0.92
16	3.00	1.65	1.45	1.37	2.63	1.05	2.12	3.09	1.12	1.36	0.98	0.33	0.65	0.47
17	2.30	1.47	0.70	1.20	1.85	1.41	1.90	0.47	0.23	0.42	1.76	1.52	2.36	1.91
18	2.33	1.43	0.81	1.67	2.43	1.60	2.12	0.41	0.31	0.69	1.70	1.50	1.44	1.07
19	2.72	1.40	1.34	1.98	2.41	1.70	2.02	0.70	0.58	0.92	1.38	0.71	1.40	0.78
20	1.93	0.75	0.96	1.32	1.79	1.52	1.80	0.40	0.49	1.24	1.74	2.34	1.71	1.21

Table B-4 Mean sensory scores (flavour) collected for the unwooded Chardonnay study wines

wine code	*sourness*	*sweetness*	*overall flavour*	*flavour persistence*	*astringency*	*bitterness*
1	4.61	1.80	4.44	4.27	1.62	1.29
2	4.75	1.29	4.17	4.40	1.93	1.28
3	4.50	1.91	4.34	4.41	1.93	1.38
4	4.41	1.53	4.14	4.29	1.63	1.19
5	4.81	1.57	4.23	4.32	1.54	1.20
6	5.12	2.28	5.41	5.53	1.69	1.09
7	4.78	1.59	4.34	4.37	1.80	1.23
8	4.63	1.87	4.24	4.40	1.69	1.37
9	4.77	2.03	5.02	5.23	1.66	1.04
10	4.68	1.85	4.41	4.50	1.71	1.41
11	4.69	1.53	4.56	4.66	1.97	1.56
12	4.41	2.12	4.40	4.53	1.56	1.27
13	4.84	1.95	4.56	4.66	1.63	1.04
14	4.56	1.88	4.49	4.34	1.56	1.07
15	4.61	1.90	4.09	4.23	1.51	1.23
16	4.97	1.70	5.01	5.33	2.10	1.01
17	4.28	1.89	4.13	4.27	1.37	1.08
18	4.31	1.90	4.13	4.18	1.47	1.03
19	4.74	1.86	4.44	4.53	1.94	1.27
20	4.85	1.45	4.43	4.71	1.61	1.51

Table B-5 Mean volatile compound concentrations (µg/L) collected for the unwooded Chardonnay study wines

wine code	ethyl acetate	ethyl propanoate	ethyl butanoate	ethyl 2-methyl propanoate	ethyl 2-methyl butanoate	ethyl 3-methyl butanoate	ethyl hexanoate	ethyl octanoate	ethyl decanoate	ethyl dodecanoate	*trans*-ethyl cinnamate	2-methylpropyl acetate	2-methylbutyl acetate	3-methylbutyl acetate	hexyl acetate
1	62710	267	527	117	19	38	1165	1490	362	97	5	11	18	574	29
2	50282	262	495	112	20	43	1398	2323	498	93	0.9	3	13	270	7
3	120171	196	433	154	15	27	1041	1308	472	202	2	18	30	513	45
4	74556	158	500	45	6	16	1270	1653	733	202	4	35	123	2516	286
5	73674	272	636	69	9	25	1463	1458	660	136	0.6	25	77	2211	227
6	212702	282	325	247	16	33	920	1249	847	812	4	50	34	1337	128
7	81121	212	522	70	8	18	1200	1261	590	64	4	29	78	1826	166
8	77057	166	459	40	6	13	1224	1507	552	146	5	29	72	2098	203
9	75345	180	612	54	6	15	1214	1207	548	264	5	27	65	1650	133
10	78541	246	727	90	15	25	1036	1223	592	273	3	51	145	3461	284
11	58440	223	529	41	7	14	1253	1610	602	109	4	43	125	3450	278
12	76607	130	448	40	5	10	1134	1637	706	148	5	80	197	4030	365
13	87529	260	732	77	10	23	1432	1478	500	110	6	51	119	3358	372
14	87220	384	1125	108	15	35	1974	2135	585	60	3	119	311	7266	598
15	100109	267	646	63	9	24	1156	1027	469	82	3	88	162	5369	301
16	84027	248	634	65	8	18	1379	1233	370	94	3	33	64	2288	248
17	60343	231	338	48	4	13	961	1349	796	262	2	52	72	1903	115
18	65882	203	309	44	3	11	1136	1872	503	74	2	28	55	1358	159
19	63701	163	533	45	8	15	1151	1609	526	174	2	32	83	2236	183
20	113678	167	441	116	14	24	1050	1206	487	110	0.7	31	66	892	136

Table B-5 Mean volatile compound concentrations (μg/L) collected for the unwooded Chardonnay study wines (continued)

wine code	2-phenylethyl acetate	2-phenyl ethanol	butanol	2-methyl propanol	2-methyl butanol	3-methyl butanol	hexanol	acetic acid [a]	2-methyl propanoic acid	3-methyl butanoic acid	hexanoic acid	octanoic acid	decanoic acid	linalool	α-terpineol	nerol	geraniol	*cis*-rose oxide
1	58	20501	365	23077	18894	131933	1976	270000	607	395	4774	6319	1413	11	32	nd	3	nd
2	33	17332	585	18958	18343	135456	939	210000	537	334	5233	11014	2386	0.6	5	nd	nd	nd
3	44	11094	589	19178	15805	98269	1876	500000	943	319	4824	7081	2012	5	16	nd	1	nd
4	219	17315	699	18625	22545	134582	2377	370000	415	301	4235	8293	2532	5	8	nd	2	nd
5	196	18510	809	17929	23484	169096	2949	310000	512	303	4936	8534	2267	3	6	nd	nd	nd
6	306	23169	944	27180	22977	170066	2719	630000	1705	451	3926	5387	2901	8	14	nd	2	nd
7	164	16099	474	20093	18791	156961	2173	330000	513	288	4957	8349	2556	4	7	nd	1	nd
8	168	11576	375	13417	12425	131218	1633	320000	386	249	4783	8495	2583	7	8	nd	2	nd
9	98	9455	353	15467	13619	95315	2141	360000	489	240	4538	6148	1733	7	8	nd	2	nd
10	395	43921	571	28744	31495	187727	4814	260000	794	599	4690	7434	2469	11	14	nd	3	nd
11	580	27902	803	15095	18768	114261	1861	230000	532	264	5974	8252	2748	12	10	nd	3	nd
12	204	13099	1286	23346	19125	128022	2542	350000	718	310	6831	9758	3120	6	4	nd	4	nd
13	176	17699	1478	25669	33011	172763	3672	400000	752	361	7599	8812	2435	5	7	nd	1	nd
14	583	30218	2122	37238	34301	187127	4416	330000	1205	609	6777	9338	2155	13	13	nd	3	nd
15	312	16206	2492	34230	31890	171055	3116	470000	884	537	5267	6315	1747	8	8	nd	3	nd
16	112	10723	872	16493	18304	118575	3164	370000	607	267	4573	7893	1693	8	7	nd	2	nd
17	144	11206	1467	22685	19242	113958	1591	300000	651	191	5695	9376	2767	4	6	nd	1	nd
18	94	8620	514	13024	13264	83686	1974	360000	483	122	6372	9431	2824	5	8	nd	1	nd
19	271	23590	1008	20808	23330	125533	2617	350000	446	269	4193	6164	1689	6	8	nd	2	nd
20	93	15971	876	17869	21147	94716	2370	560000	831	338	4169	6010	2056	6	11	nd	0.5	nd

compound not detected (nd)

Table B-5 Mean volatile compound concentrations (µg/L) collected for the unwooded Chardonnay study wines (continued)

wine code	TDN	TPB	β-damascenone	guaiacol	4-methyl guaiacol	4-ethyl phenol	4-ethyl guaiacol	4-vinyl guaiacol	4-vinyl phenol	*cis*-oak lactone	vanillin	diacetyl	methionol	dimethyl sulfide	carbon disulfide
1	4	0.02	2	1	0.07	nd	0.7	0.08	0.2	10	33	16	638	195	3
2	2	0.01	0.8	3	1	nd	0.4	0.03	0.09	25	20	47	385	132	1
3	3	0.01	3	0.3	nd	nd	0.4	0.04	0.1	8	10	7	482	149	4
4	1	0.006	2	2	0.3	nd	0.3	0.02	0.09	17	12	29	353	168	4
5	1	0.01	1	0.4	nd	nd	0.06	0.005	0.02	11	15	1	285	115	2
6	1	0.01	4	0.6	nd	nd	0.03	0.02	0.07	8	18	3	653	350	17
7	1	0.007	0.8	0.3	nd	nd	0.08	0.03	0.2	12	4	55	399	107	3
8	1	0.009	2	0.9	nd	nd	0.05	0.08	0.4	11	10	9	359	123	2
9	0.9	0.006	1	0.4	nd	nd	nd	0.05	0.2	8	3	66	275	95	2
10	2	0.01	3	0.7	0.4	nd	0.6	0.08	0.4	14	9	133	1010	139	2
11	0.7	0.008	3	0.3	nd	nd	nd	0.1	1	11	3	55	833	243	5
12	0.3	0.005	1	1	nd	nd	0.2	0.09	0.4	11	11	30	359	48	1
13	1	0.005	2	0.3	nd	nd	0.07	0.04	0.3	10	6	110	548	116	10
14	1	0.006	2	2	2	nd	0.2	0.05	0.4	36	25	48	338	142	1
15	1	0.008	4	0.4	nd	nd	nd	0.05	0.2	8	7	111	494	96	2
16	0.3	0.004	1	0.2	nd	nd	0.05	0.04	0.3	3	6	nd	301	35	1
17	1	0.003	2	2	3	nd	0.4	0.05	0.2	28	81	57	261	31	0.7
18	1	0.005	1	0.4	nd	nd	nd	0.04	0.2	15	4	39	258	71	3
19	1	m	3	0.2	nd	nd	0.06	0.07	0.3	12	6	41	638	105	3
20	3	m	4	0.9	0.3	nd	0.2	0.06	0.1	20	13	233	476	73	0.9

compound not detected (nd); compound not measured indicated by missing value (m)

Appendix C Riesling PLS model results using all variables

Table C-1 PLS model results for the prediction of Riesling aroma attribute scores using 27 *x*-variables

attribute	R^2	RMSECV	F value	C_{opt}	*x*-var
estery	0.61	0.47	0 ^{ns}	1	27
perfumed floral	0.77	0.51	-1 ^{ns}	1	27
dried rose	0.48	0.31	0 ^{ns}	1	27
lemon	0.81	0.31	-1 ^{ns}	3	27
grapefruit	0.74	0.2	-1 ^{ns}	2	27
lime	0.74	0.38	-1 ^{ns}	1	27
lychee	0.55	0.24	0 ^{ns}	1	27
pineapple	0.67	0.28	-1 ^{ns}	1	27
passionfruit	0.23	0.88	0 ^{ns}	1	27
stewed apple	0.04	0.58	0 ^{ns}	1	27
honey	0.49	0.21	0 ^{ns}	1	27
toasty	0.88	0.36	-2 ^{ns}	2	27
caramel	0.96	0.32	-7 ^{ns}	4	27
kerosene	0.92	0.23	-3 ^{ns}	2	27
rubber / plastic	0.86	0.45	-2 ^{ns}	2	27

R^2: coefficient of determination; RMSECV: root mean square error of cross validation; C_{opt}: optimal number of components used in the PLS model; *x*-var: number of *x*-variables used in the model; ^{ns} not significant.

Appendix D Unwooded Chardonnay PLS model results using all variables

Table D-1 PLS model results for the prediction of unwooded Chardonnay aroma attribute scores using 24 *x*-variables

attribute	R^2	RMSECV	F value	C_{opt}	*x*-var
estery	0.59	0.24	0^{ns}	2	24
floral	0.28	0.32	0 ^{ns}	2	24
lychee	0.37	0.30	0 ^{ns}	2	24
citrus	0.12	0.27	0 ^{ns}	1	24
pineapple	0.29	0.28	0 ^{ns}	1	24
stewed apple / pear	0.01	0.42	0 ^{ns}	1	24
herbaceous	0.00	1.61	0 ^{ns}	1	24
passionfruit	0.01	0.52	0 ^{ns}	1	24
sweaty	0.02	0.71	0 ^{ns}	1	24
honey	0.13	0.49	0 ^{ns}	1	24
butterscotch	0.67	0.33	0 ^{ns}	2	24
woody	0.65	0.33	0 ^{ns}	2	24
spicy	0.47	0.28	0 ^{ns}	2	24

R^2: coefficient of determination; RMSECV: root mean square error of cross validation; C_{opt}: optimal number of components used in the PLS model; *x*-var: number of *x*-variables used in the model; ^{ns} not significant.

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